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1. Biodecolorization of Orange G Dye by Indigenous Diazobacteria

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Abstract

The problem of environmental pollution is continuously increasing due to release of different xenobiotic substances in water. With increasing demand, textile industries and their wastewater have increased proportionally and become main contributors to pollution. Recently, industries have significantly increased the use of synthetic dyes especially azo dyes with increasing population and their demand. Many of these synthetic dyes especially azo dyes threaten the life due to its mutagenic and carcinogenic nature. The available traditional methods for wastewater treatment and Physico-chemical methods are unable to address this issue due to their limitations. The alternate solution is Bioremediation of dye effluent using bacteria is being a much more advantageous over other methods. For present studies, Orange G dye as a model azo dye and free living indigenous diazobacteria isolates were used. The indigenous diazobacteria were acclimatized with increased concentration of Orange G dye, enriched and isolated using Burk's Nitrogen Free media. Decolorization ability of isolates was checked using Mineral salt media containing dye. Isolates were screened for their ability to utilize dye as a carbon source and tolerance with luxuriant growth at higher dye concentration. The potent dye decolorizing diazotroph isolate was identified as *Klebsiella oxytoca* based on morphology, cultural and biochemical utilization tests and finally 16S rRNA gene sequencing. The change in visible spectra of dye during biodecolorization also suggests the possibility of dye degradation. The further studies are going on for parameters optimization controlling biodecolorization, identification of intermediates / products of dye degradation to elucidate the pathway followed during biodecolorization and for toxicity study.

Key words: Azo dyes, Biodecolorization, Diazobacteria, Orange G dye

Introduction

The decline in the environmental quality as a consequence of pollution is evidenced by loss of vegetation cover and for biological diversity. The excessive concentration of harmful chemicals in the environment is responsible for growing risks of threat to life support system.
This problem of environmental pollution is continuously increasing due to release of different xenobiotic substances into water, soil and air. Among all types of pollution, water pollution needs to be addressed extensively (Narendran N., 2014). With increasing demand, textile industries and their wastewater have increased proportionally and become main contributors to pollution. Moreover, to consider all pollutants of textile industry viz. oils, dyes, resins and other chemicals below one roof is not advisable (Prasad Uday U. et al., 2017). Many industries did not fulfil the prescribed pollution standards given by Pollution Control Board even after having Effluent Treatment Plants (ETPs). For small scale industries, it is uneconomical to have ETPs of their own, and in turn they become major contributors of water pollution in developing countries (Murty M.N. and Kumar S., 2011).

Based on the chemical structure of the chromophore group, the synthetic dyes are classified as azo dyes, nitroso dyes, triphenylmethane dyes, indigoid dyes, nitro dyes and anthraquinone dyes. Recently, industries have significantly increased the use of synthetic dyes especially azo dyes with increasing population and their demand. It has been observed that around 12% of these synthetic dyes are lost while manufacturing and processing. From this, around 20% enters the environment through effluents and become environmental threat (Prasad Uday U. et al., 2017). These industrial effluents are the most problematic wastewaters to be treated because of toxic metals, high COD and BOD, suspended solids, turbidity, and for colour, which is the first contaminant discernible by the human eye. Dyes reduced the light penetration and affect the photosynthetic activity and toxic to biological life in water, Many of these synthetic dyes especially azo dyes are mutagenic and carcinogenic (Kant R., 2012 , Banat et al., 1996).

The traditional technologies used for wastewater treatment viz. sedimentation, filtration, etc. are ineffective to treat textile synthetic dye wastewater (Forgacs et al., 2004). The available physico- chemical methods are not 100% efficient in pollutant removal because of their limitations (Yagub M.T. et al., 2014). The alternative solution to this problem can be biological treatment of wastewater. The bioremediation of dyes using bacteria carries merits like eco-

friendly, cheap, easy to grow, no sludge generation etc. (Khandare R.V. and Govindwar S.P., 2015). Indigenous diazobacteria are well documented for their role as bio fertilizer in maintaining soil sustainability and in bioremediation of herbicides and tannery effluents (Jasim S. M. and Abdul-Adel E., 2015, Kannan V. et al., 2012).

For current study, Orange G dye as a model ago dye and free living indigenous diazobacteria isolates were used. Hence, the attempts were made to exploit their potential for selected azo dye remediation.

Materials and Methods

All the chemicals used for study were of AR grade and Orange G dye was purchased from Hi- media Ltd. Mumbai.

i. Dye and Effluent Sample - For current study, Orange G dye was used as a model azo dye. The dye effluent sample was collected from Ulhas nallah, Ulhasnagar of Dist. Thane.

ii. Dye Acclimatization of Microorganisms - The micro floras present in collected dye effluent sample were dye acclimatized using 50 mg/lit. of Orange G dye and Mineral Salt media for 15 days. The 20% of dye concentration was increased at interval of 5 days for 15 days.

iii. Enrichment and Isolation of Free Living Diazobacteria - For enrichment of free living diazobacteria, sterile Burk's Nitrogen Free media was used for three enrichments at interval of 5 days. Initially, 3% of dye acclimatized micro flora effluent was used as inoculum followed by enriched culture for subsequent enrichments. After, every enrichment the isolates were plated on same solid media. To check selectivity of the media, *E.coli* was used as test culture. All the isolates were preserved and checked for their Orange G dye decolorization ability.

iv. Decolorization Assay - To assess the decolorization ability of diazobacteria isolates, sterile Mineral Salt liquid media containing 50mg/lit. Orange G dye and supplemented with 0.2% Yeast extract and 0.1% Glucose was used. All the isolates were checked for dye decolonization qualitatively and quantitatively after incubation at Room Temperature (30^0 C) for 48 hrs. under static conditions. The decolonization assay was carried out by centrifuging the bio decolorized broth at 10,000 rpm for 10 minutes. The

clear supernatant was read using spectrophotometric at λmax of dye. The percentage decolonization was calculated using formula –

%ge Decolonization = OD @ 0 hrs. – OD @ time 't' hrs. X 100 / OD @ 0 hrs.

Where, OD @ 0 hrs.is absorbance of dye taken at starting of decolonization (0 hrs.) OD @ t hrs.is absorbance of dye taken after decolonization period of 't' hrs.

v. Screening of Dye Decolorizing Diazobacteria Isolates - The Primary screening of decolorizing isolates was carried out with respect to their utilization of dye as carbon source during growth. For study, sterile Mineral Salt liquid media with 50 mg / lit. Orange G dye was used with 3% inoculum and incubated the flask at 30^0 C for 48 hrs. under static conditions. The dye utilization as carbon source was checked in terms of dye decolonization.

vi. The isolates using dye as carbon source from Primary screening were further screened for their tolerance and luxuriant growth at higher dye concentration. In Secondary screening, dye tolerance (in terms of decolonization) at 100 – 500 mg/lit. of Orange G was checked using decolonization assay. Isolates showing decolonization at higher concentration were further checked for their luxuriant growth at that dye concentration. The luxuriant growth of decolorizing isolate was assessed with respect to its viable count at different time intervals (Growth Curve).

vii. Identification of Potent Dye Decolorizing Diazobacteria - The identification of potent decolorizing diazobacteria was carried out for its morphology, cultural and biochemical utilization, using Bergey's Manual of Determinative Bacteriology 9th ed. (2000). The potent decolorizer was also identified using 16 S rRNA gene sequencing. Further studies were carried out with this isolate.

viii. Analysis of Biodecolorization using Spectrophotometer - Dyes, as colorants absorb light in the visible region of the spectra and depending on its visible color each one has a maximum absorbance at specific wavelength. The easiest way to monitor dye decolonization is by means of spectrophotometry (Pereira L. and Alves M., 2012). The analysis of biodecolorization of Orange G was carried out using sterile Mineral Salt liquid media with 3% inoculum. The flask was incubated at 30^0 C for 48 hrs. under static conditions. The control flask was also maintained in parallel devoid of inoculum. The change in visible spectra of Orange G dye was checked at 0, 24 and 48 hrs. by

scanning clear supernatant of decolorized broth in visible range using spectrophotometer.

Results and Discussion

Orange G dye is the orange microcrystals of powder, CAS Number 1936 -15-8, Color Index Number-16230 and chemical formula of dye is $C_{16}H_{10}N_2Na_2O_7S_2$. The molecular weight of dye is 452.39 gm/mole. The synonyms of dye are Acid orange 10, Wool orange 2G, 7-Hydroxy-8- phenylazo-1,3-naphthalenedisulphonic acid disodium salt, 1- Phenylazo – 2-naphtha- 6, 8- disulphonic acid disodium salt.

A total of 24 samples were collected from different sites of Ulhās mullah in triplicate using stoppered sterile wide mouth glass bottles. The temperature of effluent was 32.7^0 C ± 0.2 checked on site using calibrated thermometer and pH was found slightly alkaline around 7.5 using pH paper strips and Universal indicator.

All the collected samples were mixed and allowed to settle for 3 - 4 hrs. under static conditions to allow settling of all suspended solids present in effluent. This dye effluent sample was acclimatized for 15 days with increasing concentration of Orange G dye. Because of dye acclimatization, micro floras present in effluent were adapted for dye toxicity. Kalme S.D. et al., (2006) carried out the studies on biodegradation of benzidine based dye. They acclimatized *Pseudomonas desmolyticum* NCIM 2112 with Direct Blue 6 by increasing dye concentration to 100 – 300 mg/lit. during studies.

For enrichment of diazobacteria, sterile Burk's Nitrogen free media was used which supports only growth of free living diazobacteria. No growth of *E.coli* on media confirmed its selectivity for atmospheric molecular nitrogen fixing free living bacteria. Total 32 diazobacteria were selected based on their different cultural morphology and designated as DA1, DA2, DA3 and so on. Kannan V. et al., (2012) isolated the diazotrophs from tannery effluent and used them for its bioremediation. Jasim S.M. and Abdul-Adel E. (2015) used soil indigenous diazotroph bacteria for herbicide degradation.

Out of 32 diazobacteria isolates, 17 were able to decolorize the Orange G dye (Table -1). These 17 isolates were further screened for their ability to utilize Orange G dye as carbon source.

Table 1- Decolonization Ability of Diazobacteria Isolates

Sr. No.	Isolate	% ge Decolor-ization	Sr. No.	Isolate	% ge Decolor-ization	Sr. No.	Isolate	% ge Decolor-ization
1.	DA 2	45.61	7.	DA13	11.89	13.	DA24	41.11
2.	DA3	28.13	8.	DA17	60.14	14.	DA26	76.23
3.	DA6	54.19	9.	DA19	27.97	15.	DA29	27.01
4.	DA8	20.89	10.	DA20	56.34	16.	DA30	33.09
5.	DA10	42.22	11.	DA23	25.41	17.	DA31	40.62
6.	DA11	19.45	12.	DA24	38.29			

Generally the dye effluents are deficient in excessive carbon source. Hence, in current study the trials were carried out to assess the ability of isolates to utilize the dye as a carbon source during growth. The seven isolates viz. DA3, DA8, DA11, DA17, DA19, DA26, and DA30 were able to utilize / decolorize the Orange G dye in broth (Figure -1) as a sole source of carbon.

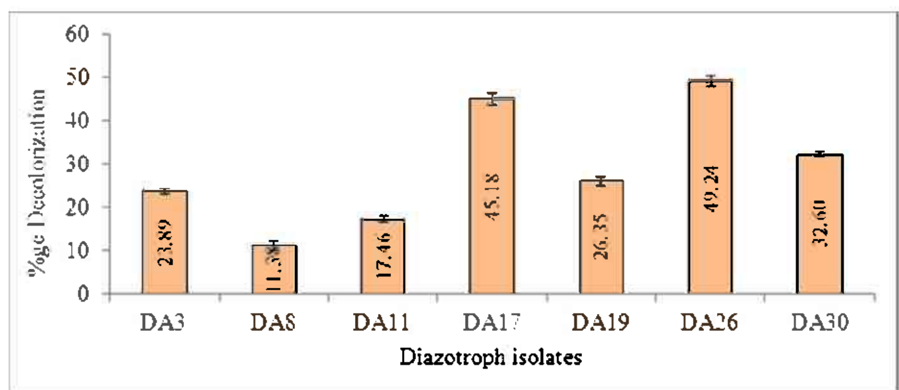

Figure 1 – Dye Utilization by Isolates as their Carbon Source (Primary Screening)

Alalewi A. and Jiang C. (2012) used dyes as a sole carbon source during their studies on bacterial treatment for textile effluent. Similarly, Bheemaraddi M.C. et al., (2013) also reported use of Reactive red 2 as a carbon source for dye degrading bacteria screening from dye contaminated soil.

In secondary screening, the isolates utilizing dye as carbon source were further checked for their decolorization at higher dye concentration ranging from 100-500 mg/lit. The decrease in decolorization was seen with isolates DA17 and DA26 for increasing dye concentration from 100 – 500 mg/lit. The isolate DA26 showed higher decolorization at 200 mg/lit. of dye

comparatively followed by diazotroph isolate DA17. Whereas, remaining isolates of primary screening viz. DA3, DA8, DA11, DA19 and DA30 had not shown decolorization at 100 mg/lit. of Orange G dye (Figure-2).

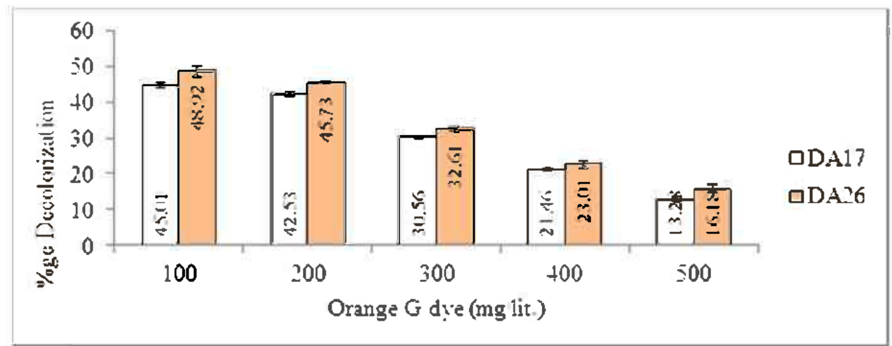

Figure 2 – Biodecolorization of Orange G Dye at higher concentration (Secondary Screening)

The isolates DA17 and DA26 were further checked for their luxuriant growth at 200 mg/lit. of Orange G dye. The luxuriant growth was assessed in terms of effect of dye toxicity on isolate growth (Figure -3). The isolate DA17 showed growth with initial lag period of 4 -5 hrs. and comparatively less growth in terms of viable count in presence of 200 mg/lit. of Orange G dye.

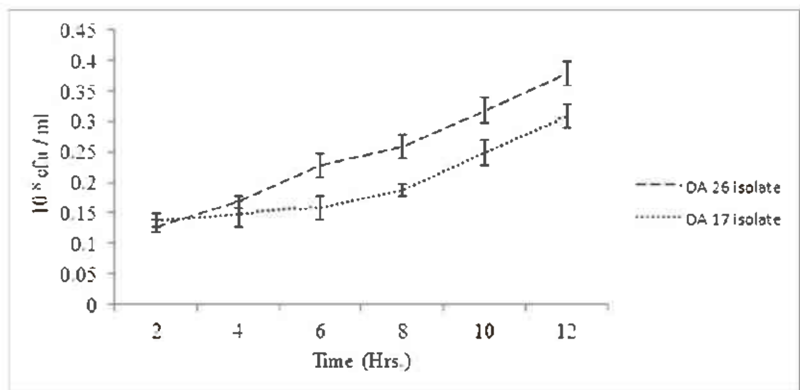

Figure 3 – Effect of Orange G dye concentration on luxuriant growth of Diazotroph Isolates (Secondary Screening)

The isolate DA 26 showed luxuriant growth in terms of higher viable count at same concentration of dye. Hence, this potent decolorizing diazotroph isolate was identified with reference to Bergey's Manual of Determinative Bacteriology 9th ed. (2000) and 16S rRNA gene sequencing (Figure -4) as *Klebsiella oxytoca*.

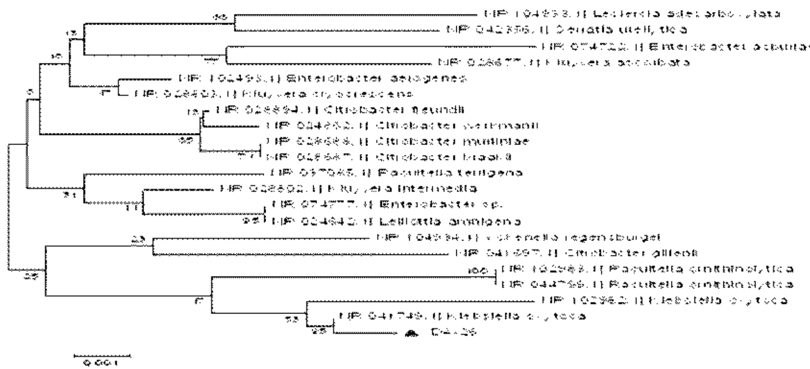

Figure 4 - Phylogenetic Tree for Isolate using 16S rRNA gene Sequence

Change in visible spectra of Orange G dye during decolorization by isolate *Klebsiella oxytoca* was observed at different time intervals. The results of same are illustrated in Figure - 5.

Figure 5 – Change in Visible Spectra of orange G dye during Biodecolorization by *Klebsiella oxytoca*

The λmax of Orange G dye observed at 480 nm, was completely disappeared within 24 hrs.of biodecolorization. Also disappearance of λmax and appearance of new peaks were observed at 24 and 48 hrs.of biodecolorization by potent decolorizing diazotroph isolate.

According to literature, fate of dye during biodecolorization is either it undergoes biodegradation or biosorption for its removal. The decrease in visible spectral peaks in proportion to each other is seen in case of bioadsorption of dyes, whereas in biodegradation of dye, either the λmax peak disappears completely and / or new peaks appears (Asad et al., 2007 and Chen K. et al., 1999).

Conclusion

The role of diazobacteria as Plant Growth Promoting Rhizobacterias (PGPRs) is well documented for increasing soil fertility and its sustainability. Also many studies shown their role

in bioremediation of tannery effluent and herbicide degradation.In current studies, efforts were made to isolate and assess the decolorization ability of indigenous diazobacteria using Orange G dye as model azo dye.

The indigenous decolorizing diazobacteria isolate was identified as *Klebsiella oxytoca.* The isolate was able to utilize the dye as carbon source and also showed luxuriant growth at higher dye concentration in terms of their growth curve without any lag period. On assessing the change in visible spectra of dye during biodecolorization, disappearance of λmax and formation of new peaks at different time intervals supports the possibility of dye degradation. The further studies are going on for optimization of parameters controlling biodecolorization, identification of the intermediates / products of dye degradation to elucidate the pathway followed during biodecolorization and also for toxicity studies.

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2. Isolation, Identification and Optimization of Amylase Producer *Bacillus Subtilis* from Municipal Wastewater

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Abstract

Amylases are one of the most important industrial enzymes represent approximately 30% of the world's enzyme production and have a wide variety of applications in many industrial processes. In present study, amylase producing bacterial species was isolated from municipal wastewater on nutrient agar medium. This isolate was screened for its amylolytic ability on starch agar medium. Based on the morphological, cultural and biochemical tests, the selected isolate was identified as Bacillus subtilis. The results showed that optimum pH, temperature and incubation time for highest amylase activity was found to be 7.5, 370C and 48 h respectively whereas the highest activity was observed with sucrose and yeast extract as a carbon and nitrogen source respectively. Similarly, Bacillus subtilis also showed bioremediation potential as it grows on wastewater agar medium which was devoid of any external nutrients in the medium.

Keywords: Amylase, Bacillus Subtilis, bioremediation, municipal wastewater.

Introduction

The α-amylases are enzymes that hydrolyze starch molecules to generate progressively smaller polymers such as glucose, maltose and maltotriose units (Rajagopalan and Krishnan 2008). They can be obtained from several sources such as plants, animals and microorganisms. However, microbial amylase is more beneficial than other sources because it is economical as well as production rate is high and can be engineered to obtain enzymes of desired characteristics (Panneerselvam and Elavarasi, 2015). The α-amylases have many potential applications in a wide number of industrial processes such as food, fermentation, textile, paper, detergent and pharmaceutical industries. However, with the advances in biotechnology, the amylase

application has expanded in many fields such as clinical, medicinal and analytical chemistry (Saravanane et al. 2001).

Municipal wastewater is generated by residential, institutional, commercial and industrial establishments and includes household liquid wastes from baths, toilets, kitchens and sinks that are disposed off via sewers (Pandey et al. 2000; Sonune and Garode, 2015). It has intolerable odour, irritating colour and high organic material. One of the major components of organic material is polysaccharide. Bacteria present in wastewater have potential to produce the enzymes required for hydrolysis of these polysaccharides and used them as a food or nutrients. Such microbes may be used for amylase production (Rajannan and Oblisami, 1979). Hence, aim of present study was to isolate, characterize and optimize amylolytic bacterial isolate from municipal wastewater.

Materials and Method

Sample Collection and Bacteria Isolation: Municipal wastewater sample was collected from Dhamangaon city, district Amravati (M.S.), India, in pre-sterilized bottle by standard procedure given in American Public Health Association (APHA, 2005) and transferred immediately to the laboratory. The sample was subjected to serial dilution on the nutrient agar medium by spread plate method and incubated at 370C for 24 h. After incubation, morphologically different colonies were isolated and maintained at 40C on nutrient agar slants in refrigerator.

Screening and Identification Bacterial Isolates: Primary screening of isolates for ά amylase production was done on starch agar medium. Bacterial isolates from nutrient agar slant were streaked on the starch agar plate and incubated at 370C up to 72 h. After incubation, iodine solution was flooded with dropper for 30 seconds on the starch agar plate. The clear zone of hydrolysis around the colony indicates positive result. This isolate was further used for identification, production and optimization study. Bacterial isolate was identified on the basis of morphological, cultural and biochemical tests based on Bergey's Manual of Determinative Bacteriology 9th ed. (Holt 1994).

Inoculum Preparation and Enzyme Production: The nutrient broth was inoculated with a single isolated colony and incubated for 24 h at 370C with continuous shaking on a rotary shaker (REMI, CIS-24BL) at 150 rpm. From this, 1 ml (1%) of inoculum with optical density 0.5 at 600 nm was transferred to 100 ml sterile production medium containing 1% starch, 0.6%

peptone, 0.05% KCl, 0.05% MgSO4 and pH 7 in 250 ml Erlenmeyer flask and incubated on rotary shaker at 150 rpm, 370C for 48 h. The bacterial cells were removed by centrifugation at 8000 rpm for 15 minutes at 40C in cold centrifuge (REMI, C-24BL) and the supernatants were used as crude enzyme for determination of enzyme activity. The enzyme activity was determined according to Miller method (1959).

Optimization of Cultural Condition: The amylase activity was optimized for various conditions such as pH, temperature, incubation time, carbon and nitrogen sources. The effect of pH for amylase production was determined by culturing the bacterial isolate in the production media with different pH such as 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5. The enzyme assay was carried out after 48 hours of incubation. The effect of temperature on amylase production was studied by incubating the culture media at various temperatures such as 25, 30, 37, 45 and 550C. The enzyme assay was carried out after 48 hours of incubation. The incubation time was optimized by inoculating bacterial isolate into production medium and incubated for 24, 48, 72 and 96 h at 370C and at 150 rpm. The enzyme assay was carried out after every 24 h. The effect of different carbon sources such as glucose, lactose, fructose and sucrose were studied by replacing the original carbon source of the medium with equivalent carbon amount (2%) of each of the tested carbon sources. Similarly, different organic (yeast extract, tryptone and beef extract) and inorganic (potassium nitrate) nitrogen sources were studied by replacing the original nitrogen source of the medium with equivalent nitrogen amount (0.6%) of each of the tested nitrogen sources.

Bioremediation Efficiency of Bacteria: It was carried out by inoculating bacterial isolate on wastewater agar medium containing 100 ml sterilized wastewater and 2% agar. There were no any external nutrients added in the medium. The plate was incubated for 48 h at 370C. Similarly, agar degradation study of this isolate was also carried out.

Statistical Analysis: All the experiments were carried out in triplicates. The results are presented as mean± Standard deviation of triplicates (n=3).

Results and Discussion

Isolation, Identification and Screening of Amylolytic Bacteria: In present study, bacterial isolate was isolated and purified on nutrient agar medium from municipal wastewater. This isolate showed starch hydrolysis zone after 24, 48 and 72 h incubation on starch agar medium (Fig. 1) and hence, it was selected for further studies. Based on the morphological, cultural and

biochemical characters, the isolate was identified as Bacillus subtilis (Table 1 and Table 2). Many studies reported isolation of bacillus sp. from municipal wastewater (Garode A.M. and Sonune N.A, 2013; Sonune and Garode, 2018: Rakaz et al., 2021).

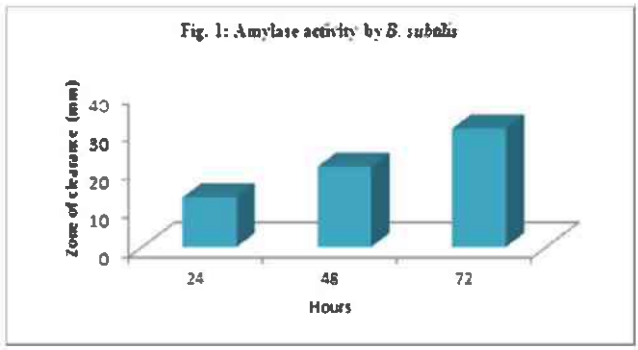

Table 1: Morphological characterization of the selected bacterial isolate	
Characteristics	Result
Shape	Circular
Pigment	White
Opacity	Opaque
Gram Staining	Positive rods
Motility	Motile
Endospore	Positive

Table 2: Biochemical characterization of the selected bacterial isolate	
Biochemical Tests	Result
Catalase	+
Oxidase	+
Indole production	-
Methyl Red test	+
Voges Proskauer test	+
Citrate utilization test	-
Nitrate reduction	+
H2S production	-

Starch hydrolysis	+
Sugar fermentation	
Glucose	+
Lactose	+
Mannitol	+
Sucrose	+
Trehalose	+
+= positive, - = negative	

Optimization of Cultural Condition: Optimization of culture conditions is very important for maximum enzyme production by bacteria.

Effect of pH: Among the physical parameters, pH of the growth medium plays an important role by inducing physiological changes in microbes and their enzyme secretion. The present results (Fig. 3) demonstrated that though amylase activity was detected over broad pH range from 5.5 to 8.5, highest amylase activity (36.99 U/ml) was noted at slightly alkaline pH (pH 7.5). When pH is altered below or above the optimum, activity decreased due to denaturation of proteins (Hayashi et al. 1988). Our results were comparable with previous reports (Mishra et al. 2014; Raul et al. 2014).

Effect of Incubation Temperature: The results showed that amylase activity was found in the temperature ranged 250C to 550C. However, the maximum activity was observed at 370C (74.79 U/ml) whereas it was declined below and above 370C. At temperature 25, 30, 45 and 550C, the activities were 46.91, 57.25, 72.02 and 43.03 U/ml respectively (Fig. 4). Temperature is one of the most important factors that controls the growth and production of metabolites by microorganisms. Enzyme activity was low at high temperature because it inactivates the expression of gene responsible for the starch degrading (Aiba, 1983). Our results were similar with previous studies (Raul et al. 2014; Mendu et al. 2005; Das et al. 2004).

Effect of Incubation Time: The present results revealed that highest amylase activity was observed after 48 h incubation (38.66 U/ml) and after that it was declined (Fig. 5), this could be due to the depletion of nutrients in the medium or release of toxic substances (Sundarram and Krishna Murthy 2014). The earlier studies reported that highest amylase activity was found to be

at 48 h incubation for Bacillus sp. (Mishra et al. 2014), Bacillus amyloliquefaciens P-001(Deb et al. 2013) and B. subtilis (Vijayalakshmi et al. 2012). Our results were similar to these studies.

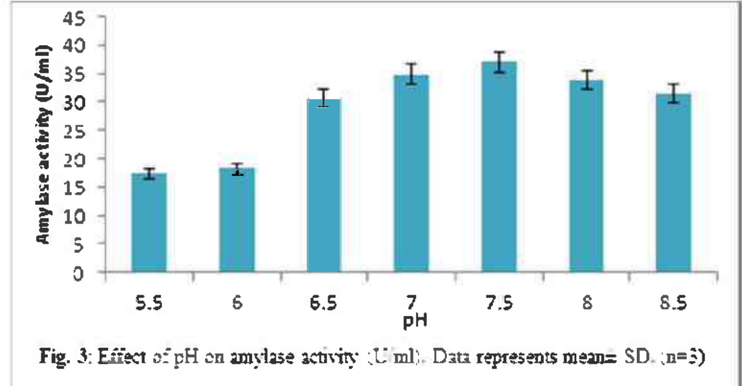

Fig. 3: Effect of pH on amylase activity (U/ml). Data represents mean± SD. (n=3)

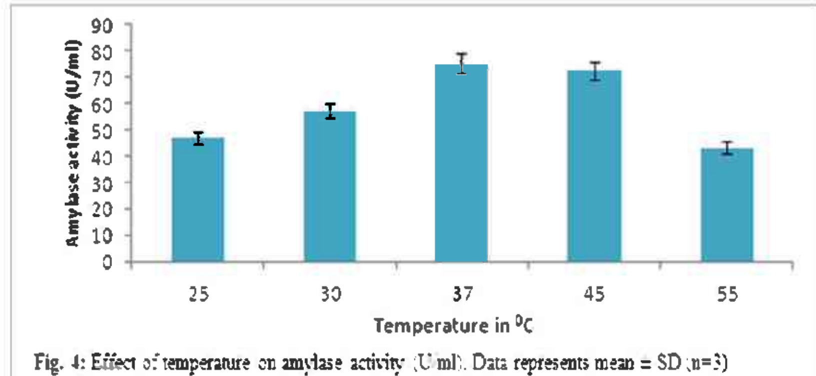

Fig. 4: Effect of temperature on amylase activity (U/ml). Data represents mean ± SD (n=3)

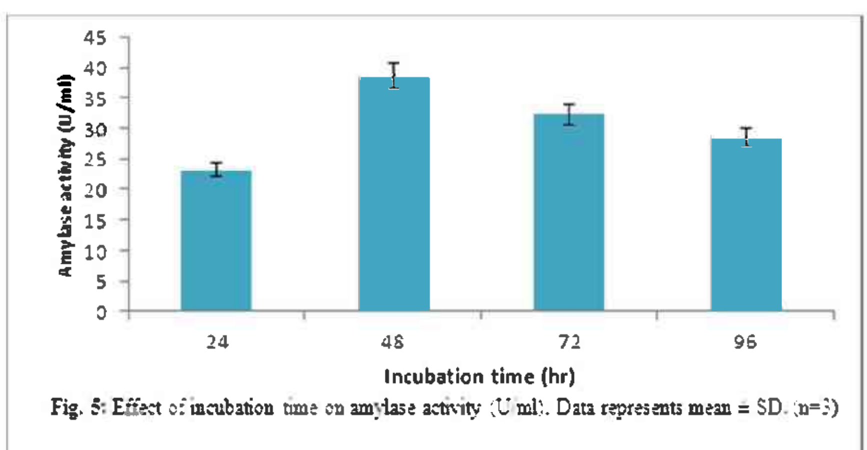

Fig. 5: Effect of incubation time on amylase activity (U/ml). Data represents mean ± SD. (n=3)

Effect of Carbon Sources: Production of amylase by B. subtilis was affected by various carbon sources (Prakash et al. 2009). In this study, sucrose induced maximum amylase activity (51.87 U/ml), followed by fructose (45.82 U/ml) and glucose (45.75 U/ml), whereas lactose

induced low activity (44.24 U/ml). However, there is no statistically significant variation in enzyme production among the carbon sources used in our study except sucrose (Fig. 6). Our present results were different from the earlier report (Aiyer 2004).

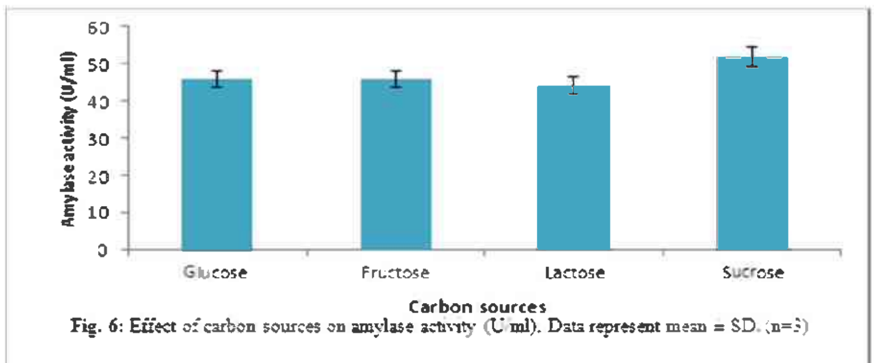

Fig. 6: Effect of carbon sources on amylase activity (U/ml). Data represent mean ± SD. (n=3)

Effect of Nitrogen Sources: In our study highest amylase activity (64.17 U/ml) was observed with yeast extract as nitrogen source (Fig. 7). This was followed by tryptone (50.28 U/ml) and beef extract (44.73 U/ml) whereas KNO3 showed minimum activity (36.82 U/ml). Many studies reported that yeast extract stimulate the amylase production (Thippeswamy et al. 2006; Sharma et al. 2012). Thus, our results were in agreement with these studies.

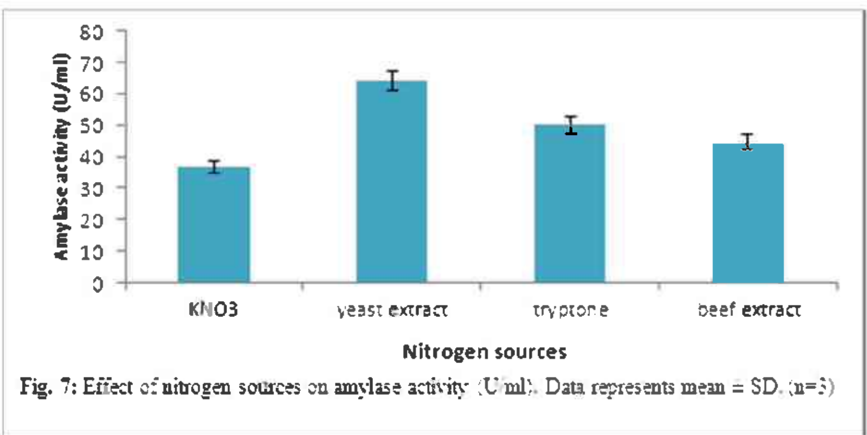

Fig. 7: Effect of nitrogen sources on amylase activity (U/ml). Data represents mean ± SD. (n=3)

Bioremediation Efficiency of Bacteria: In present study, B. subtilis showed growth on wastewater agar medium without addition of any external nutrients. It proved that the isolate has ability to utilize the components from wastewater as nutrients for their growth as wastewater contains carbohydrates, proteins, lipids etc. which was released from household activities. Similarly, this isolate was unable to utilise agar as carbon source. Hence, our isolate may be used for bioremediation of municipal wastewater. There was previous report on growth of B.

licheniformis on wastewater agar medium, isolated from municipal wastewater (Sonune and Garode, 2015).

Conclusion

The result of the present study elucidated that municipal wastewater can be a very good source for isolating amylolytic bacteria. Such bacteria can innately tolerant to adverse environment present in municipal wastewater. In present study Bacillus subtilis was isolated and optimized for various parameters. The highest activity of amylase was found to be at pH 7.5, 370C temperature, 48 h incubation time, sucrose and yeast extract as carbon and nitrogen source respectively. Similarly, this isolate also showed bioremediation potential of municipal wastewater. Hence, this isolate may further exploit for amylase production as well as bioremediation of municipal wastewater.

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3. Diversity of Cyanobacteria from the Paddy Fields of Lanja Taluka, Ratnagiri District

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Abstract

The present research article deals with the Cyanobacterial diversity of Lanja Tehsil. Lanja is situated in Ratnagiri district with unique geographical conditions and paddy as main crop. Paddy crop is flooded in water and is associated with many species of Cyanobacteria in positive co-relation.

The sample from field collected and microscopically observed in laboratory for the identification of the Cyanobacterial species. The current study specifies the number of Cyanobacterial species lives in association with the paddy fields of Lanja Taluka.

Key words: Cyanobacteria, Lanja, Paddy.

1. Introduction

Blue Green Algae (Cyanobacteria) are one of the major components of the nitrogen fixing biomass in paddy fields. Blue Green Algae play an important role in maintenance and build up of soil fertility, consequently increasing rice growth and yield as a natural biofertilizer. Increase in water- holding capacity through their jelly structure. Increase in soil biomass after their death and decomposition. Preventing weeds growth. BGA fix nitrogen under anaerobic conditions in specialized cells called heterocyst which comprises 5 to 10% of cells in a filament.

The growth of BGA contributes significantly to spontaneous fertility of paddy soils (Singh 1961, Venkataraman 1972). Because there are about 100 million hectares of paddy fields in the world, considerable research is focused on the use of BGA in agriculture. Blue-green algae are distributed world-wide and contribute to the fertility, either as free-living organisms or in symbiotic association with the water-fern Azolla. The abundance of blue-green algae in rice fields has been reported in numerous papers since Fritsch's accounts. Many rice fields show visually obvious growths of blue-green algae, although eukaryotic green algae may be more

abundant where high quantities of nitrogenous fertilizer have been added. Reports from many countries indicate that the blue-green algal flora is often rich in species.

Cyanobacteria thrive in favourable growth conditions in fresh water and wetland ecosystems including lowland paddy fields, which are manmade wetland ecosystems. They live as free-living organisms (e.g. Aphanocapsa, Gloeocapsa and Merismopedia) and in symbiotic associations (e.g. Anabaena sp. with Azolla) (Kulasooriya, 2011). Cyanobacteria are a morphologically diverse group. They can be grouped into uni-cells (e.g. Synechocystis), colonies of individual cells (e.g. Aphanothece), unbranched filaments (e.g. Lyngbya), aggregations of multiple filaments in a common sheath (e.g. Microcoleus), false-branched forms (e.g. Scytonema) and true branched forms (e.g. Stygonema), and those forming baeocytes (endospores) (e.g. Myxosarcina) or forming exospores (e.g. Chamaesiphon) (Wehr and Sheath, 2002). Cyanobacteria are major nitrogen (N2) fixing prokaryotic organisms in the paddy field water and the uppermost soil layer (Roger, 1996). Their diversity in the rice paddies and nitrogen supplying potentials vary according to the growth stage of the rice plant and physico-chemical environment of the paddy soil (Prasanna et al., 2009; Song et al., 2005).

Cyanobacteria have been used in formulations of biofertilizers especially for rice cultivation (Kulasooriya and Magana-Arachchi, 2016). In addition to N2 fixation, cyanobacteria stabilize soil surface and increase water holding capacity. Some cyanobacteria excrete plant growth promoting substances such as growth hormones, vitamins, amino acids, and organic acids, and suppress weed growth, increase available phosphorous level in soil and decrease the effects from soil salinity (Saadatnia and Riahi, 2009). Soil pore structure is improved by filamentous growth and secretion of adhesive substances. Endosymbiosis of some cyanobacteria with aquatic flora (e.g. Azolla), (Kneip et al., 2008) found to be more successful in fixing nitrogen compared to free-living cyanobacteria. Azolla are widely used in China, certain parts in Philippines and Vietnam as a fertilizer (Kulasooriya, 2011).

Here we present the cyanobacterial diversity that exists in the paddy fields from diverse habitats of Lanja Taluka, Ratnagiri. Lanja is a census town in Ratnagiri district in the Western Indian state of Maharashtra. About 50 km from the district headquarters. Lanja is well known for its climatic variability as it has ample rains as well as good enough winters followed by heating summers. Lanja is located at 16.85°N 73.55°E. It has an average elevation of 166 meters (544 feet). It's a Taluka head in Ratnagiri district. National highway popularly known as

Mumbai-Goa Highway passes through it. The cropping system in this area is only paddy in rainy season. Rice is the staple food in this area. There is little information available on the cyanobacteria from Ratnagiri district.

2. Material and Methods

I. Area of Collection

Cyanobacterial samples were collected from different rain-fed areas of Lanja Taluka Ratnagiri. Samples from ten locations were collected during rainy season from June to September 2019. Name of the sampling sites were mentioned in table 1. The collected samples were observed under the microscope. The physico-chemical properties of the water studied from each sampling site.

Site.	Location	Global Positioning System
1	Kurne	16.910N, 73.500E
2	Agargaon	16.870N, 73.520E
3	Zarye	16.760N, 73.740E
4	Shiposhi	16.920N, 73.630E
5	Kante	16.860N, 73.480E
6	Korninko	16.860N, 73.720E
7	Bhambed	16.830N, 73.650E
8	Khanvali	16.830N, 73.400E

Table No. 1: Details of the Collection sites of Cyanobacterial Algae

II. Morphological Classification

Morphological classification of cyanobacteria will base on characters observable under a microscope. Identification of specimens will carry out using the taxonomic publications.

i. Physicochemical Properties of the soil: PH, temperature, salinity, and dissolved oxygen of the soil of the paddy field were measured. The temperature was measured with the help of soil thermometer and PH measured with the help of soil PH meter. The physiochemical properties were not observed much variations.

3. Results and Discussion

i. Distribution of Cyanobacteria

The distribution results and morphological characteristics are shown in table 2. Ten genera of cyanobacteria were collected from rice paddy fields at eight different locations in Lanja. Nostoc and Gloeocapsa sp. were commonly distributed almost in all paddy fields. The unicellular algae and diatoms also found during the observation. The cyanobactria are

microscopic. Paddy fields are favorable for the growth of Cyanobacteria by providing suitable ecological conditions. The blue-green algae which were found in paddy fields are Anabaena sp., Spirulina sp., Oscillatoria sp., Gloeothece sp., Tolypothrix sp., Scytonema sp., Rivularia sp. and Microcystis Sp. Different types of colony were observed like unicellular, with and without heterocystous cyanobacteria were found. After comparing cyanobacteria populations at the different sampling sites, no statistically significant differences were found among the sampling sites, Therefore, it appeared that the cyanobacteria grew rapidly in the rice fields that contained ample organic matters in the soil and water as well as conditions such as pH, temperature, organic sources, etc. that allowed propagation. The optimum pH for the growth of cyanobacteria was ranges from 6.5 to 7.

Cyanobacterial diversity from the paddy Fields of Lanja Taluka of Ratnagiri District (Table No. 2).

S. N.	Cyanobacteria	Site-1	Site-2	Site-3	Site-4	Site-5	Site-6	Site-7	Site-8
1	Nostoc sp.	+	+	+	+	+	+	+	+
2	Anabaena sp..	+	-	+	-	+	+	+	+
3	Spirulina sp.	+	-	+	+	+	+	-	-
4	Oscillatoria sp.	+	+	-	+	+	+	-	+
5	Gloeothece sp..	-	+	-	+	-	+	+	+
6	Gloeocapsa sp.	+	+	+	+	-	+	+	+
7	Tolypothrix sp.	-	+	+	+	-	+	+	+
8	Scytonema sp..	+	-	+	-	+	-	+	+
9	Rivularia sp.	+	-	+	+	+	+	+	+
10	Microcystis Sp.	+	-	+	-	-	+	-	+

Table No. 2: Diversity of Cyanobacterial Algae found in Lanja Taluka.

ii. Diversity of Heterocystous Filamentous Cyanobacteria

To investigate the diversity and populations of heterocystous filamentous cyanobacteria in rice fields in Lanja, Nostoc, Anabaena and Tolypothrix were isolates. Nostoc (100%), Anabaena (75%) and Tolypothrix (75%) were isolates. Non-heterocystous cyanobacteria belonging to the genus Gloethece, were also observed. The major filamentous heterocystous cyanobacteria, Nostoc, was somewhat expected, as Nostoc can withstand desiccation. In fact, Nostoc spp. has already been found to be the predominant cyanobacteria in most soil samples from rice fields.

4. Conclusion

From the present study, it could be concluded that rice field soil harbors the impressive cyanobacterial diversity in different sites within study area. Further studies are necessary for culturing and biochemical characterization of these species. The prevalence of different types of cyanobacteria is higher in paddy fields, the diversity of cyanobacteria is high in the regions with high diversity of paddy cultivated environments. Molecular analysis for further identification of cyanobacteria isolates is prospected. In the present scenario, the use of cyanobacterial biofertilizers has much reduced mainly as a consequence of their poor establishment patterns in different soil types or ecologies. For wider exploitation and success of these biofertilizer technology in agriculture, coordinated strategic research efforts in the laboratory and at field level are highly essential.

Acknowledgment

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4. Gut Microbiome - A Companion or Foe to Health?

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Abstract

Human body is colonized by numerous microorganisms collectively referred to as Microbiome with broad diversity distribution from the surface of the skin to the internal organs in a specific range. Microbiome acts as a defense mechanism against the exposure to dietary proteins, vitamins, antibiotics, artificial food coloring and smoking etc. The xenobiotic activities of these compounds, affects directly or indirectly, which disturbs the normal distribution of microbiome, especially in gut and oral cavity and also affects the vital organs. Abnormality in distribution, can lead to diseases associated with liver, kidney (Hotspot of metabolism), heart (cardiovascular disease), gut (obesity), brain (Neurological disorders), cancer, autoimmune disorders and so on. Hence there is scope in analyzing the presence and distribution of microorganisms (individual or combination) responsible for provoking xenobiotic metabolism, biotransformation etc.

Keywords: Microbiome, Microbiota, Xenobiotic, Toxic metabolites, Biotransformation, Gut Dysbiosis

Introduction

The interests of studying microorganisms have been increased over the years by human beings. All these started when Anton von Leeuwenhoek coined the term ‘Animalcule’ with his own microscope. Later many scientists joined the timeline with their inventions, postulates and theories. The word Microbiota expresses the association between human and microorganisms from Greek origin micro- small, biota- life. The term Microbiome slightly varies as they are associated with the humans at gene level (Marchesi, et al., 2015).

The microbial community can be classified based on their interactions: beneficial (mutualism, synergism, or commensalism), pathogenic (Amensalism: antagonism or predation), and neutral (Lederberg et al., 2001; Ho et al., 2017). These interactions were observed when

microbes sustained for survival based on the availability of nutrients in the presence of different environment and microbial distribution. The scope of this review is mainly to understand how the diversity of microbes is necessary for good health and its associated clinical conditions like organ based diseases, metabolic disorder, autoimmune disorder and other life threatening diseases including, irritable bowel syndrome & cancer (Zmora et al., 2019; Jobin, 2018).

Diversity of Microbiome

Microorganisms that particularly harbor the habitat of host are predominantly bacterial groups. Other than bacteria, Archaea, Viruses, fungi and protozoan were also collectively called as Microbiota (association occurring at gene level known as Microbiome) (Jandhyala et al., 2015). The distribution of Microflora will vary in each healthy individual. Part of the habitat varies with different groups of organisms at distinct range. The Researchers shows interest in microbial study of gut mainly, because it is the entry point of almost all the compounds which causes either direct or indirect effects in host.

Based on Metagenomic studies dominantly two phyla Bacteroidetes and Firmicutes were observed in the gut (Thursby, 2017). Adult gut averagely contains trillions of microorganisms, which holds more genes in number than human genome (Falony et al., 2016; Zhernakova et al., 2016).The colonization begins at the birth of an infant through the transfer of microbial community from mother's vagina or through c- section.

When the alternation of Microbiome takes place by many factors as well as at different range of distribution, the host immune mechanism will try its best to replace the lost organisms or at least brings up the sub class of the lost Microbiota. By doing this they themselves try to reach homeostasis (Shade et al., 2012; Toju et al., 2018; Cernava et al., 2019; Shi et al., 2018).

The Problem begins when this Stabilization Fails to Occur Due to following Reasons

I. The effect of medication leads to complete loss of certain organisms, which fails to get recognize and stabilize by host defense mechanism

II. Secondarily, dormant state organisms will take advantage of this downfall of health, which affects normal diet, capability of immune system and mental health.

Human body will always try to bring back the homeostatic condition, even by reviving back original microbiota otherwise it will try to bring the balance by replacing microorganisms which carries similar physio- chemical properties like alpha, β- diversity.

Factors influencing the Dysbiosis of Microbiome

A. Age

The colonization of gut takes place even before the birth of infant in utero (Dominguez-Bello et al., 2010) and later gets developed depending on the birth process (normal delivery or c-section), modified through diet (breast feed or formula milk), around the age of 2 to 3 years and stabilization of gut composition depicts like adult gut with 40- 60% (Yatsunenko et al., 2012). Based on the observations from 16s rRNA maternal vaginal microorganisms like Enterococcus, Leuconostoc, Lactococcus, Escherichia, Shigella belongs to the family of Lactobacillus and Prevotella were observed initially. But in cesarean delivery, Streptococcus, Corynebacterium and Propionibacterium were dominant (Dominguez-Bello et al., 2010; Gosalbes et al., 2013).

B. Diet

Diet and hygiene is the deciding factor of the capability of human immune system and in combination of microbiome gene susceptibility will cause dysbiosis and diseases. Next level of stabilization of gut solely depends on the pre-existing microbiota from dietary milk source (breast- fed Vs formula milk). Naturally human microbiota remains stable for longer period of time but changes. In diet will affect and vary from mild to severe depending on the individuals food habit. For example, Intake of high amount of western diet which is rich in refined sugars, legumes, nuts, olive oil, refined grains, low consumption red and processed meat, beverages with lot of carbon dioxide gas and additive, more spicy and oily foods will eventually be associated with numerous immune- mediated inflammatory diseases (IMIDs) – psoriasis, rheumatoid arthritis. Obesity further follows up with several chronic disorders (Bander et al., 2020; Noble et al., 2017).

C. Xenobiotic Metabolism

Any substances that are foreign to human body enters, especially chemical compounds like drugs are known as xenobiotic compounds will cause adverse effects on host health. When the exogenous molecules, entered into host it will first gets adsorbed by gut, later gets reaches to the targeted site of necessary dosage. During this process the changes of metabolism can happen because of poor adsorption by gut & providing chances for the gut microbiome to react directly or indirectly leads to the condition called biotransformation.When there is loss of biodiversity this will directly cause hepatic diseases, along with access of these pathogens to other organs.

Thus the change in microbial metabolite activity related to stress- related psychiatric disorders results in depression and carbohydrate craving (Voigt et al., 2016; Johnson et al., 2017; Otte et al., 2016).

D. Antibiotic Activity

Antibiotics can be classified as narrow and broad spectrum with different modes of administration. Aftereffects of the antibiotic will leave short and long term implications in health. One of the important properties of gut microbiota is that, it will follow the competitive exclusive mechanism in presence of pathogens. For example, long term effects of broad spectrum will disturb the interspecies level of interaction, later to no possible recovery of important phylum like Bacteroides will allow pathogens such as Salmonella phimurium and Clostridium difficile. Clarithromycin is used to eradicate the infection of Helicobacter pylori. In return the phyla of Actinobacter get tremendously diminished from gut with spread of resistance gene erm B (Ng et al., 2013; Jernberg et al., 2007; Jakobsson et al., 2010).

E. Environmental Effects

Waste from different sources of manufacturing industries such as untreated effluents; burning off tyres, plastics, smoking, fumes ashes from mills; chemical explosives, powders – crackers; smoke from gasoline toxic metabolites will have effect from the entry point of host till the gut which in-turn gain access to brain affecting the mental health either directly or indirectly. For example, exposure of heavy metals to gut causes mental diseases. Smoking and prolonged secondary smoking alters Bacteroides and Prevotella species. Silver nanoparticles in food industry will produce environmental stressors which highly affects the diversity of gut microbiome (Benjamin et al., 2012; Duan et al., 2020).

Factors Affecting Gut Microbiome

Figure 1: Factors affecting Gut Microbiome

(Source: Adapted from Clarke et al., 2019)

Gut Dysbiosis – Diseases and Disorders

I. Irritable Bowel Syndrome

IBS is one of the gastrointestinal disorders where reoccurrences are common in chronic persistence. Based on the food habits, way of defecation and its consistency, IBS will affect the individual both mentally and physically. It becomes difficult to live normal routine cycle. Cases of getting post infection related to IBS are observed in current decade which also alters the microbiome diversity of gut.

Subtypes of IBS are classified based on the symptoms standardized by Rome criteria (Drossman et al., 2005; Tillisch et al., 2005; Guilera et al., 2005; Mearin et al., 2003)

1. IBS and constipation (IBS-C)—Passing painful hard stools.
2. IBS and diarrhea (IBS-D)—Free watery stools.
3. Mixed IBS (IBS-M)—Combination of hard and loose stools.
4. Unsubtyped IBS—Not enough data to meet criteria for IBS-C, D, or M.

Factors Influencing the Recurrence of IBS

People were not able to distinguish between diarrhea and dysentery, along with the lack of attention to study their bowel movements, food habits, consuming medicine without doctor's prescription and not openly consulting with doctor.

II. Obesity

Many chronic, metabolic disorder conditions have been increased worldwide the main reason behind is that, individual being overweight. This may be due to metabolic disorders factors such as high calorie intake, carbohydrate, sugars, salt and sour, oily food items. The problem is entirely not only the food habits of what a person but also absence of physical activity. Once the BMI of a person increases will follow many disorders. Thus, the weight of the pregnant women is always monitored closely to maintain the normal range of blood pressure and glucose level, to avoid antibiotic consumption which will affect the microbial distribution of gut and causes other chronic conditions like gestational diabetes, other metabolic disorders such as artherosclerosis.

III. Diabetes: Type 2 Diabetes Mellitus

Type 2 Diabetes Mellitus is one of the metabolic disorders caused by imbalance of blood glucose level, altered lipid profile. Intake of antibiotics is also one of the additional factors that disrupt the gut profile.

IV. Non- Alcoholic Fatty Liver Diseases

The reason behind today's prevalence of chronic liver diseases is NAFLD. The extended access from gut – liver access increased other clinical conditions such as obesity and Type 2 Diabetes Mellitus.

S.N0	Clinical conditions	Alternation of Microbiome	Reference
1	IBS (110) Control (39)	Bacteroides ↑ Prevotella ↓ Methanobacteriales ↓ No difference of alpha and beta diversity in IBS as well as control	Tap et. al.,2016
2	Obesity Western diet	Firmicutes↑ Enterobacteriaceae↑	Simpson et al., 2015
	Vegetarian diet	Bacteroidetes↑ Bacteroides fragilis↓	Baothman et al., 2016
3	Type 2 diabetes : antibiotic exposure - Streptomycin	Ruminococcaceae↓ Bacteroidaceae↑	Lichtman et al., 2016
4	Non- Alcoholic Fatty Liver Diseases	α diversity ↓ Prevotella copri ↑	Schwimmer et al., 2019

Note: ↑ Increase; ↓ Decrease

Table 1: Clinical Conditions Related To Gut Dysbiosis

The above table (Table 1) represents the summary various experiments performed with human study to observe the homeostatic as well as other clinical conditions causing dysbiosis of microbiota

VI. Lung Diseases

Increase in number of respiratory diseases like cystic fibrosis, asthma, chronic obstructive pulmonary disease (COPD) and their symptoms are related to gastrointestinal disturbance like irritable bowel syndrome (IBS), reflux between esophageal and stomach (Labarca et al., 2019; Vutcovici et al., 2016; Wang et al., 2014).

The compositions of gut and lung microbiome vary but their epithelial cells are generated from common embryonic stem cell structure shows the possible way for gut- lung access (Bingula et al., 2017; Frati et al., 2019; Marsland et al., 2015).

The below Table (Table 2) Shows the Factor Affecting different types of Lung Disease.

No	Lung Disease	Change in diversity of Gut Microbiome	Available Treatment	Reference
1	Asthma Adults	Clostridium↑ Eggerthellalenta, ↑ Bifidobacterium↓ Faecalibacterium↓	Oral administration of Faecalibacterium, Veillnella and Rothia FMT	Wang et al., 2018 Fujimura et al., 2016
	Children	Candida ↑ Rhodotorula↑		
2	COPD	Acinitobacter↑ Firmicutes↑ Proteobacteria↓ Bacteroidetes↓	Lactobacillus rhamnosus, Bifidobacteriumbreve anti-inflammatory against smoke	Biedermann et al., 2013 Mortaz et al., 2015
3	Cystic Fibrosis	Streptococcus↑ Staphylococcus ↑ Bifidobacterium adolescentis↓ Faecalibacterium prausnitzii↓	Administration of Lactobacillus	Enaud et al., 2019 del Campo et al., 2014
4	Lung Cancer	Enterococcus ↑ Actinobacter↓ Bifidobacterium↓	FMT – under study	Zhuang et al., 2019 Routy et al.,

				2018
5	TB	Acinitobacter↑ Proteobacteria↑ Bacteroidetes↓	Lactobacillus- under study	Luo et al., 2017 Negi et al., 2019

Note: Increase↑; Decrease↓

Table 2: Types of Lung Diseases Associated with Shift in Microbiome

VII. Relation of Dysbiosis with Current Pandemic of COVID - 19

All strains of respiratory infection causing corona (SARS COV- 1 -2002; MERS COV - 2012 and SARS COV-2 – current) resulted in pandemic at different years with various complications and symptoms. Gut Microbiome is the host defense mechanism, against the pathogens that gains access to other organs with their bi-directional pathway. This is the main reason of increase in susceptibility and difficulty in treating people contain, health issues in heart, diabetic and other respiratory illness.

Dysbiosis of gut in Covid - 19

Homeostasis

Commensals

COVID 19

Figure 2: Gut Dysbiosis in COVID-19

(Source: Adapted from Zuo et al., 2020)

Strategic development of Probiotics against COVID-19 Patients with Gastrointestinal Problems

IBS: Diarrhea and other gastrointestinal symptoms

30% of gastrointestinal tract symptoms are observed in the patients who are primarily infected by respiratory infections. These criteria will fit in the current situation. Effects of

patient's psychology shows increase in more IBS cases with other gastrointestinal discomforts (An et al., 2020).

Toxins

Fungal dysbiosis increases the severity of COVID19. The treatment of probiotic exhibiting the antifungal property slows the infection (Tkhruni et al., 2020; Bartkiene et al., 2020).

ACE2 Receptor

B38- CAP is the bacterial enzyme similar to ACE2 receptor that works as the suppressor for hypertension, tissue injury and cardiac arrests. The function of B38 – CAP is acts as receptor for SARS- COV 2 to lower its pathogenecity (Minato et al., 2020).

Treatment: Microbiome in Duty to Reverse the Clinical Conditions

Due to imbalance of microbiome the condition called dysbiosis in gut is not developed it affects the gut and also gains access to parts of the body and results in many chronic, metabolic, immunity based disorders. Chances of reversal of this imbalanced biodiversity of microbiome can be regulated to some extent. Though many research experiments were conducted to study about disease implications as well as trying to solve the current diseases prevalent all over the world, the interest of providing treatment for diseases through microbiome is currently in study models such as rat, mice, human with or without administrational activity of pre and probiotics.

Administration of Probiotics

Nowadays, consumption of probiotics has been globally increased in various forms and easy to consume. The main group behind bring ease to bowel belongs to Lactobacillus, Bifidobacterium, Lactococcus, Bacillus etc. Probiotics like Fructo-oligosaccharides (FOS), trans-galacto oligosaccharide (TOS) are given, shows significant improvement but not in all clinical conditions (Tanner et al., 2015).

Fecal Microbiota Transplant (FMT)

Dysbiosis in gut affects both gastrointestinal tract as well as systemic infections like obesity, irritable bowel syndrome, and extra intestinal disorders. FMT is one the treatment method used to control these implications in health. It is otherwise known as stool transplantation.

Conclusion

Co- evolution between human and microbiome have their own benefits as well as limitation. The remarkable benefit is that the external organelles like mitochondria were identified to be in association with environment. Later the evolution, co- existence produced cell with mitochondria as their integral component. But the same evolution has also paved the way for transferring disease causing, resistance gene from one generation to next. This is one of the main reasons for drug resistance along with other factor.

Attention must be paid to what kind of food we are consuming because, if the food is high in sugar, salt, spicy, additives, preservatives, low fiber diet, high fat content will definitely create pressure on the metabolism of host and disturbs the resident microbial community. Automatically when BMI of a person gets increased and it will provide chances for chronic disease development. These chronic conditions will not end here itself, by gaining access to other regions such as gut- skin access; gut- lung access; gut- brain access; gut- liver access etc. after its entry the changes in the microbial environment will affect host normal activity either directly or indirectly.

The treatment of antibiotics works in bidirectional way because it will suppress or spikes more immune responses. Challenges of this treatment can be included such as there is no accurate representation or significant biomarkers that are not available for every clinical condition, depends on host immune response against treatment, complications, diet and performing experiments without errors when using study model and computational data are much more difficult because every individual will possess their own microbiome. So comparison of controls and positive patients is not perfectly possible. This clearly states that despite of the advantages, for successful treatment, overcoming the challenges is more important.

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    Zuo et al.,. (2020). Alterations in Gut Microbiota of Patients With COVID-19 During Time of Hospitalization. Gastroenterology , 159:944–955.

5. Comparative Study of Some Conventional Indian Spices against Pathogenic Bacteria

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Abstract

The present work was designed to evaluate the comparison of five Indian spices using crude ethanol extracts, namely Trachyspermum ammi L. (Ajwain), Laurus nobilis L. (Bay Leaf), Myristica fragrans Houtt. (Nutmeg), Coriandrum sativum L. (Coriander seeds) and Syzygium aromaticum L. (Clove) were examined for their antimicrobial activity against pathogenic bacteria like Escherichia coli, Salmonella typhi, Salmonella paratyphi A and Salmonella paratyphi B using Agar well diffusion method as primary screening. All these conventional Indian spices are traditionally used not only to create a flavours or aromas, but are also valued for their nutritional and medicinal properties which are rooted in Ayurveda or folk medicines. The present study reveals among all crude ethanol extracts; Syzygium aromaticum extract had the high inhibitory effect on the growth of Escherichia coli, Salmonella para typhi A, Salmonella para typhi B strains. Trachyspermum ammi extract were effective against Salmonella para typhi A. Myristica fragrans extract had the high inhibitory effect on the Salmonella typhi. The crude ethanol extract of the spices showed more inhibition on Salmonella typhi.

Keywords: Bacterial Pathogens, Crude Ethanol Extract, Antimicrobial Activity

Introduction

Nature has been an origin of many Medicative agents for a century and since the increasing of mankind. Extraction of bioactive compounds from medicinal plants has been approved for their physiological activity by medicinal researcher. A medicinal plant represents major sources of variety primary and secondary metabolites that measures bioactive compounds of great therapeutic value. (Evans et al, 2002). Therefore, there is need to search plants with

medicinally importance and magnificent extracts from wide varied substrates. Spices are for not solely flavor and aroma of the foods however conjointly provide antimicrobial properties (Nanasombat et al., 2002). Additionally these spices are foremost used as natural antimicrobial agents in foods. A number of the natural compounds found in numerous spices possess antimicrobial activity.

Therapeutic uses of T. ammi fruits include; stomachic, carminative (Chialva et al., 1993) and expectorant, antiseptic (Choudhury et al., 1998), antimicrobial; it is also used as antipyretic, febrifugal and in the treatment of typhoid fever (Umadevi et al.,1990 and Rao et al., 1994). The leaves of L. nobilis L. are effectively used orally to treat the symptoms of gastrointestinal problems, such as epigastric bloating, impaired digestion, eructation, and flatulence (Kivcak and Mert, 2002). Syzygium aromaticum commonly known as clove is a median size tree. Clove is the key vegetal source of phenoplast compounds like flavonoids, hydroxybenzoic acids, hydroxycinnamic acids and hydroxy phenylpropenes'. The foremost bioactive compound of clove is Eugenol. This plant has been used for hundreds of years as food preservatives and as medicinal plants mainly as antioxidant agents and has antimicrobial activities. Recently, several reports confirmed the antibacterial, antifungal, antiviral and anticarcinogenic properties of this plant (Shan et al., 2005). The foremost common use of Myristica fragnans is they are employed as a carminative, anti-inflammatory, analgesic, diuretic, anti-pyretic, hypolipidemic, aphrodisiac, antiulcerogenic, anti-oxidant, and as a stimulant agent. It enhances the digestion. It is used as remedy for nausea, stomach ache, head ache, vomiting, diarrhoea and symptoms related to infectious diseases like cholera. It is useful in treating the issues related to spleen, liver, and eyes. It considerably enhances the blood circulation, boosts up the brain functioning (Sharma et al., 2000, Lokhande et al., 2010, Thakur et al., 2018 and Trivedi 2020). In folk medicine, coriander is used against enteric parasites. (Wichtl, 1994). From current pharmaceutical studies, extra pharmaceutical applications of C. sativum have revealed antibacterial (Silva et al., 2011a), antifungal (Silva et al., 2011b), antioxidant (Wangensteen et al., 2004), protection of gastric mucosal damage (Al-Mofleh et al., 2006) blood pressure lowering, and diuretic (Jabeen et al., 2009) activities among others.

The present study was aimed towards studing the antimicrobial activity of Trachyspermum ammi L. (Ajwain), Laurus nobilis L. (Bay Leaf), Myristica fragrans Houtt. (Nutmeg), Coriandrum sativum L. (Coriander seeds) and Syzygium aromaticum L. (Clove)

against pathogenic bacteria like Escherichia coli, Salmonella typhi, Salmonella paratyphi A and Salmonella paratyphi B.

Materials and Methods

Preparation of Crude Ethanol Extracts of the Spices

The following spices were processed for crude ethanol extract. Spices used were Trachyspermum ammi L. (Ajwain), Laurus nobilis L. (Bay Leaf), Myristica fragrans Houtt. (Nutmeg), Coriandrum sativum L. (Coriander seeds) and Syzygium aromaticum L. (Clove) were washed individually with clean sterile water and oven-dried for one hour at 800C. Each were blended in fine powder and used for extraction. The respective plant material was processed for the cold extract, for 24 hrs. at 25 ° C. 3 grams of each spices were mixed in 30 ml ethanol. The extracts were concentrated using vacuum evaporator. The extracts obtained were tested for antimicrobial activity against pathogenic bacteria (Kaoutar et al., 2010).

Test Organisms

The pathogenic bacteria were brought from Pathological Laboratory and pure culture was maintained in Zoology Laboratory of G.M.Momin Women's College, Bhiwandi. The different pathogenic strains used in the present study are Escherichia coli, Salmonella typhi, Salmonella paratyphi A, Salmonella paratyphi B. All the test cultures were maintained on the blood agar to maintain the pathogenicity.

Antibiotic Sensitivity Assay

Agar well diffusion method (Schillinger and Lucke 1989) was followed for primary screening. Respective cultures of pathogenic bacteria were swabbed on Muller Hilton Agar plates. Wells were made using sterile cork borer (7 mm in diameter) and loaded with crude ethanol extracts of Trachyspermum ammi L. (Ajwain), Laurus nobilis L. (Bay Leaf), Myristica fragrans Houtt. (Nutmeg), Coriandrum sativum L. (Coriander seeds) and Syzygium aromaticum L. (Clove). All the plates were incubated at 370 C for 24 hrs. Zone of inhibitions were observed around the wells after 24 hrs. The control used was 80% Ethanol for the assay.

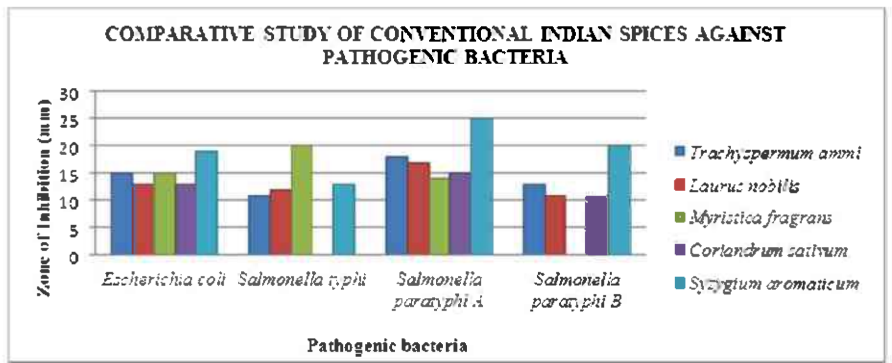
Results and Discussion

Spices are well known for their preservative and medicinal value in households. It is however in recent years that the spices have drawn the attention of researchers due to increasing resistance against antibiotics amongst pathogens (Uraih 2004, Souza et al., 2005 and

Voravuthikunchai et al., 2004). Five different spices that were tested, exhibited antimicrobial activity. The results of the antibacterial activities have been given in the Table 1.

Sr. No.	SPICES USED (80ug/mL)	ZONE OF INHIBITION (mm)			
		Escherichia coli	Salmonella typhi	Salmonella paratyphi A	Salmonella paratyphi B
1	Trachyspermum ammi L. (Ajwain)	15	11	18	13
2	Laurus nobilis L. (Bay Leaf)	13	12	17	11
3	Myristica fragrans Houtt. (Nutmeg)	15	20	14	-
4	Coriandrum sativum L. (Coriander seeds)	13	-	15	11
5	Syzygium aromaticum L. (Clove)	19	13	25	20
6	Control (Ethanol)	11	-	12	10
8	Remarks (Effectiveness)	Syzygium aromaticum	Myristica fragrans	Syzygium aromaticum , Trachyspermum ammi	Syzygium aromaticum

Table 1: Comparative Study of Conventional Indian spices against pathogenic bacteria



Among the five spices crude ethanol extract, Syzygium aromaticum showed the inhibition for all four test pathogens. Highest inhibitory effect was shown on Salmonella paratyphi A with 25 mm zone of inhibition. It has been found that Syzygium aromaticum can be used as a potent inhibitor of Escherichia coli, Salmonella paratyphi A, Salmonella paratyphi B. Myristica fragrans extract had the high inhibitory effect on the Salmonella typhi with 20 mm zone of inhibition.

Conclusion

From this study, it may be concluded that the ethanolic extract of spices may be used as a potential source of natural antimicrobial compound against pathogenic bacteria. This preliminary

study can be further extended in determinant of active compounds of the spices so that effective medicinal preparations may be created. The employment of plant extracts and phytochemicals, each with known antimicrobial properties, may be of great significance in therapeutic treatments (Messina et al., 1994 and Locatelli et al., 2010).

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6. Physiochemical Evaluation of Brahmi Ghrita: A Polyherbal Formulation

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Abstract

Ayurveda has been a cornerstone of Indian medicine since ancient times. Mehya drugs which are considered to enhance cognitive potential; Brahmi is one of the Medhya drug reported in literature. The present study emphasizes on preparation of a polyherbal formulation consisting Brahmi as its major constituent. A ghee based polyherbal formulation Brahmi Ghrita was prepared as per Ayurvedic literature and standardization study was done. Acid value, saponification value and iodine value were the parameters considered for standardization and they showed significant values. The GC/MS analysis was done to study the fatty acid profile of Brahmi Ghrita and it has shown the increase the number of unsaturated fatty acids as compared with plain ghee/ghrita. This significant rise helps in drug delivery to brain cells and thus may progress in cognition and learning efficacy.

Keywords: Brahmi Ghrita, GC/MS, Standardization

1. Introduction

Ayurveda is an ancient Indian medical system. Ayurveda is part of the 'Vedas,' particularly the 'Atharvaveda,' which is written in Sanskrit. Because of its effectiveness and safety, there has been a worldwide movement in recent years to accept this comprehensive science. The growing global demand has necessitated the standardisation of natural medications. Medication standardizations are first addressed in Ayurvedic classics under the specialties of Bhaishajya Kalpana and Rasa Shastra, which deal exclusively with drug formulation and production.[3] The majority of the tests recorded in ancient literature appear to be observational and subjective, with little scientific backing.[3,15] As a result, standardisation and the establishment of dependable quality processes are critical.

Medhya Rasayanas are a set of medicinal herbs listed in Ayurveda that have a variety of uses, including improving memory and intellect by being nootropic. Continuous usage of the Medhya medication nourishes and improves memory, immunity, and ultimately longevity. Mandukaparni (Centella asiatica Linn.), Yastimadhu (Glycyrrhiza glabra Linn.), Guduchi (Tinospora cordifolia Miers.) and Shankhapushpi (Convolvulus pleuricaulis Chois or Evolvulus alsinoides) are the four medications that make up the Medhya Rasayana and can be used individually or in combination.[1] However, in practise, a small number of additional medications used as a Medhya drug are reported in Ayurveda classic textbooks. They are Brahmi (Bacopa monniera), Jyothishmati (Celastrus panniculata), Kushmanda (Benincasa hispida), Vacha (Acorus calamus) and Jatamamsi (Nardostachys jatamamsi).[10] Medhya Rasayana can be used alone or in combination with other herbs as polyherbal formulation.

Brahmi is an example of a Medhya Rasayana that is widely used by all Ayurvedic practitioners.[2] Both Bacopa monnieri and Centella asiatica are known as Brahmi. In North India, Centella asiatica is known as Brahmi. Bacopa monnieri is a creeping perennial herb that grows in wetlands and muddy shorelines. Brahmi constitutes a vast number of phytochemicals and Bacoside A is one of the major phytoconstituents responsible for neuro pharmacological effects and the nootropic action. It can also be used for treating various mental conditions such as anxiety, poor cognition, and amnesia. [6]

Ghee or ghrita has long been regarded by Ayurveda as the healthiest source of edible fat, due to its numerous health benefits. Ghee promotes longevity and protects the body from different ailments, according to Ayurveda. It promotes absorption and assimilation of meals by increasing the digestive heat. [4,12] All of the body's tissues are nourished by it. It enhances the brain and nerve system while also improving memory. It also makes the body more flexible by lubricating the connective tissues.[9] Ghrita is employed because it transports the medicinal components of plants to all tissues of the body and it's been a good vehicle for delivering herbs to the body's deeper tissue layers including brain. [11]As the cell membrane also contains lipid, ghee's lipophilic activity enhances transport to a target organ and final administration inside the cell. [9]

Brahmi Ghrita (BG) is a formulation which includes cow's ghee, Bacopa monneri, Piper longum, Piper nigrum, Acacia concinna, Embelia ribes. Brahmi or Bacopa monnieri is the major constituent of Brahmi ghrita. This formulation has traditionally been used as a learning and

memory booster, for reducing symptoms of anxiety and as an anticonvulsant agent[8] and used for the treatment of various mental disorder. 'Murcchana' samskara is the process by which ghee is processed to improve the medicinal potency of it and to remove foul odour and rancidity. [5,7] A Murcchana ghee enhances shelf life of ghrita with herbs. In current study, a polyherbal preparation of plant/herbal components with cognitive capabilities was attempted using cow ghee.

Materials and Method

2.1. Procurement and Processing of Plant Materials

Research plants/herbal ingredients viz. Bramhi (Bacopa monnieri), Shunti (Zingiber officinale), Marich (Piper nigrum), Pippali (Piper longum), Trivrut(Nishottar) and Shweta trivrut (Operculina turpethum), Danti (Baliospermum montanum), Shankhapushpi (Convolvulus prostrates) , Aragvadha (Cassia fistula), Saptala (Euphorbia dracunculoides Lam.), Vidanga (Embelia ribes) were procured from local authenticated ayurvedic shop and were of pharmacopoeial quality. Fresh Brahmi plant/ herb was washed with water and grinded, filtered through muslin cloth to obtain Brahmi Swarasa. Rest all herbs were dried and pulverized to get coarse powder and were passed through sieve number 40 then stored in airtight containers separately until further use. This powder form of drug is known as Kalka.

2.2 Formulation of Polyherbal Brahmi Ghrita

The authentic ghee was procured for the preparation of polyherbal formulation. 'Ghrita Paka Kalpana', a traditional Ayurvedic method, was used to make a polyherbal ghrita formulation. The quantities of all ingredients were estimated according to Ayurvedic scriptures.[14] Briefly, the specified amount of ghee (Sneha Dravya) was placed into a large stainless steel vessel and melted over a low flame. Furthermore, equal amounts of Brahmi Swarasa, water (Drava Dravya), and molten ghee (Sneha Dravya) were added in the ratio of 1:16:4 and boiling was started until all moisture had evaporated and the distinguishing qualities of Brahmi Ghrita had appeared. The entire 'Ghrita Paka Kalpana' process was carried out on a low to medium flame. Then it was allowed to cool, filtered and stored in airtight container.

2.3 Analytical Study of Brahmi Ghrita

Analytical approaches were used to assure reproudicibility of the Brahmi Ghrita. The analytical parameters viz., iodine value, acid value and saponification value were calculated as per mentioned in standard Ayurvedic procedures.[16]

2.4. Esterification and Gas Chromatography Analysis

Ghrita is a fatty acid combination that combines both saturated and unsaturated fatty acids. Unsaturated fatty acids (HDL) are beneficial fatty acids that play an important role in brain construction and function, as well as the activity of neurons and astrocytes. Saturated fatty acids clog the arteries, whereas omega-3 and omega-6 fatty acids are beneficial. During the processing of Brahmi Ghrita, some saturated fats are broken, and the unprocessed part of the ghrita is removed, allowing it to be easily digestible and perform its function more effectively. (API) The total saturated and unsaturated fatty acid profile was studied by gas chromatoghraphy (GC)

Transesterification of fatty acids was done by dissolving molten ghrita in methanolic HCl in screw cap test tubes. The tubes were vortexed and heated up in water bath for 2 hours at 80°C. After cooling at room temperature, sample was kept on ice for 30 minutes and samples were extracted with hexane for extraction of fatty acid methyl esters (FAME). [13] FAME was evaporated by passage of Argon gas, the remaining FAME sample was resuspended in chloroform. 1ml from this was injected into GC unit and was analyzed using GC Shimadzu TQ8030 GC-MS instrument.

3. Results and Discussion

In current study, an attempt was made to make a cow ghee-based Polyherbal Ghrita formulation according to Ayurvedic literature. Brahmi is Medhya drug and a major constituent of Brahmi Ghrita. [2,6] Other plants/herbs shows synergistic effect in this nootropic formulation. This formulation is memory and cognition enhancer also possesses anti-convulsant activity. Because probable fatty acid decomposition during storage can cause foul odour and rancidity, which can damage ghrita's stability and shelf life, the prepared polyherbal ghrita formulation was treated using ancient Ayurvedic processes.

The prepared Brahmi Ghrita formulation was standardized on the basis of qualitative and physiochemical analysis. The cow ghee used for the preparation of Brahmi Ghrita was golden yellow, granular and with characteristic taste and odour and hence the formulation also retained the characteristic odour and consistency. The resultant formulation was green coloured viscous fluid and after cooling it was granular as like ghee. The acid value is a metric for determining the amount of free fatty acids in the body. Triglycerides are transformed into fatty acids and glycerol as oil and fats begin to rancidify during storage, raising the acid value. The lower the acid value, the less likely to decompose ghrita formulation, extending its life and enhancing its therapeutic

effectiveness. The resultant Acid value was 3.478%. The saponification value indicates the number of fatty acids in the ghrita formulations as well as their average molecular weight. The higher the fatty acid content or carboxylic functional group per unit mass, the greater the risk of rancidity, and the shorter the shelf life and therapeutic value. The saponification value of Brahmi Ghrita was observed as 178.3 %.

The iodine value represents the amount of iodine absorbed at unsaturation, indicating the degree of unsaturation in ghrita formulations. The formulation with a higher iodine content is more reactive and oxidation-prone. The iodine value was 22.99% which shows it is compiling with standards of Ayurvedic Pharmacology of India.

Gas chromatography is a high-throughput technology for determining a compound's fatty acid profile. During preparation of ghee, some unprocessed fatty acids remain in saturated form which can be harmful for body. When ghee is processed by adding kalka dravya and heated for longer time, the unprocessed fatty acids may change into unsaturated fatty acids which body can digest easily, and they are required for important organ such as brain. The GC/MS analysis of plain ghrita and Brahmi Ghrita was done to analyze fatty acid profile. The total number of fatty acid components in plain ghrita was 23 and Brahmi Ghrita was 22. The number of unsaturated fatty acids was 5 and rest were saturated one. The major saturated fatty acid in both samples were caproic, caprylic, caoric, lauric, tridecanoic, myristic, pentadecanoic, palmitic, stearic and arachidic and unsaturated fatty acids were linoleic, linolenic, eicosadienoic. Total polyunsaturated fatty acids were increased from 21.10% of plain ghrita to 24.90% of Brahmi Ghrita. The total saturated fatty acids were decreased from 73.20% to 68.10%. The unsaturated fatty acids such as linoelic acid, omega-3 are major components of brain, during gas chromatography analysis it has been observed that the level of unsaturated fatty acid has been increased due to processing of ghee. This processed Brahmi Ghrita can be used to deliver Medhya drugs to brain and helps in enhancing cognition.

4. Conclusion

The goal of this study was to create and test a ghee based polyherbal Brahmi Ghrita formulation using Ayurvedic process. As it contains the Medhya drug, this can be utilized to improve learning behaviour and cognition. The increased number of unsaturated fatty acids helps in delivering the drug after the processing of ghrita and addition of herbs. The Medhya activity potential of prepared polyherbal formulation needs further evaluation by in-vivo methods.

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7. Evaluation of *Vitis Vinifera* Seed Power as a Bioenhancer: Expedite Anti-Diabetic and Anti-Bacterial Potential

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Abstract

The seed power extract of Vitis Vinifera (grape) is a potent anti-diabetic and antibacterial agent. It shows a viable wound healing agent for diabetes sufferers. The Antidiabetic and antibacterial activities of Vitis vinifera (grape) seeds were evaluated in this study. The mature vitis vinifera (grape) seeds are powered and then tested for antibacterial activity using antibacterial assay techniques against Escherichia coli, Pseudomonas Aeruginosa, Staphylococcus Aureus, and Streptococcus Mutans, as well as Antidiabetic activity using Invitro-glycosidase and -amylase inhibition assays. According to this research, grape seeds perform as Bioenhancers for Antidiabetic investigations by suppressing carbohydrate hydrolyzing enzymes and having antibacterial properties. The seeds of Vitis Vinifera (grape) will be used in the pharmaceutical industry due to their Theranostic applications.

Keywords: Vitis Vinifera seeds, antibacterial, Antidiabetic activity.

Introduction

Vitis vinifera (grapes) are the most valuable conventional fruit in the world and have also received much interest.12 Grape seeds have been shown in several studies to have disease-preventive and health-promoting properties. It contains a high concentration of biotherapeutics. This therapeutics has been linked to a reduced risk of diseases. Grape seeds have phenolic compounds like proanthocyanidin and also contain monomeric phenolic compounds such as (+)-catechins, (-)-epicatechin, and (-)-epicatechin-3-O-gallate, which serve as anti-diabetic and antibacterial agents.5,3

Diabetes mellitus is a metabolic disorder defined by high blood sugar levels. Diabetes affects a huge number of people all around the world 8. Different drugs with multiple modes of action would be required for these people. In several studies, plant components have been demonstrated to have an essential role in boosting glucose metabolism and decreasing the risk of diabetes in several ways. Sometimes, diabetes may lead to chronic complications due to improper management of this metabolic conditions.9 Five synthetic drugs like alpha-glucosidase inhibitors, biguanides, dopamine agonists, DPP-4inhibitors, glucagon-like peptides, meglitinides, sodium glucose transporter (SGLT) 2 inhibitors, sulfonylureas, and thiazolidinediones are currently used to control diabetes.1 These diabetic control drugs are more molecularly effective, but some of the challenges are due to undesirable adverse effects.7 The medical histories of some of the patients show that better molecular compounds containing diabetic medicine have the demand for a safer treatment for complications. The plants are a rich source of several bioactive compounds that are good sources for treating various diseases like diabetes and acting as therapeutic agents.2

Several well-studied pathogens, such as Staphylococcus species, Pseudomonas aeruginosa, and Escherichia coli, as well as Streptococcus, were identified from the wounds saw in the diabetic patient's foot ulcers.4 Diabetic foot ulcers that lead to infection and limb loss have both been linked to an increased incidence of diabetes as people become older.

Materials and Strategies

Collection and Preparation of Plant Material

Parshwa Enterprises, Pune, India, collected healthy, ripe, and fresh Vitis vinifera (common grape) from the Vitaceae family. The seeds were washed with double distilled water to remove any unwanted contaminants or particle suspensions, and then the colour was dried and grinded with the use of a digital blender. For future investigation, the powder should be kept dry and airtight. (Fig.1)

Antidiabetic Analysis

Invitro A- Amylase Inhibition Assay

For α -amylase inhibition experiment, sample was prepared in various concentrations. Starting with a stock concentration of 5mg/mL, prepare various concentrations using a 20mM (pH 6.9) phosphate buffer and makeup to 100μl, and then incubate at 25°C for 10 minutes. Following the pre-incubation, 20mM phosphate buffer (pH 6.9) and 20 μl starch solution were

added, followed by 10 min incubation at 25°C. To prevent the process, colours such as 3, 5, and dinitrosalicylic acid were used. After cooling the microplate to room temperature, place it in a hot water bath for 5 minutes. The absorbance at 540nm was measured using a microplate reader (Erba, Lisascan); acarbose was used as a standard, and the formula was used to calculate it.

$$\% \text{ inhibition} = \frac{\text{Control} - \text{test}}{\text{Control}} \times 100 \quad (1)$$

Invitro α- Glycosidase Inhibition Assay

For the preparation of different concentrations, prepare a 10mg/mL stock concentration of sample and incubate for 5 minutes. Sucrose (37 mM) and 1 mL of phosphate buffer (0.1 M) were mixed as the final reaction mixture and incubated at 37 ∘C for 30 minutes before starting the reaction with substrates. The reaction was ended after incubation by boiling the mixture in a water bath for 2 minutes. The controls were phosphate buffer and enzyme, while the standard was acarbose. Incubate for 10 minutes after adding 200μL of glucose reagent. After that, measure absorbance at 510 nm with a microplate reader (Erba, Lisascan) and determine using the formula

$$\% \text{ inhibition} = \frac{\text{Control} - \text{test}}{\text{Control}} \times 100 \quad (2)$$

Antibacterial Assay Activity

The antibacterial activity of grape seed powder of varied concentrations was examined using a growth kinetics assay.

Bacterial growth is measured kinetically. After 30 seconds of agitation, samples were taken every 2 hours to analyze bacterial concentrations in the presence of grape seed powder extracts. A graph of contact time against OD600 was used to evaluate antibacterial kinetics.

Data analysis: Growth of Inhibition (%)

The growth of inhibition (%) was calculated to assess antibacterial activity using the formula:

$$I\ \% = \frac{(C18 - C0) - (T18 - T0)}{(C18 - C0)} \times 100$$

Where I = The percentage of inhibition of growth

- C18 = The positive control of the organism's blank corrected optical density at 600 nm (OD600) at 18 hours

- C0 = The blank-compensated OD600 of the organism's positive control at 0 h
- T18= The organism's negative control-compensated OD600 in the presence of the test sample at 18 hours
- T0= The organism's OD600 in the presence of the test material, adjusted for the negative control at 0 hr

Result and Discussion

Invitro Carbohydrate-Hydrolyzing Enzyme Assays

Carbohydrate-hydrolyzing enzymes like α -amylase and α -glucosidase efficiently inhibit grape seeds. The level of enzymatic activity is influenced by the sample concentration. Grape seeds had IC50 values of 68.5 and 67.7 lg/mL for α -amylase and α -glucosidase, respectively (Fig 2a, b).

Both carbohydrate-hydrolyzing enzymes, α -amylase and α -glucosidase, are found in the digestive system and control absorption by breaking down carbohydrates into monosaccharides. The digestive enzymes are inhibited by grape seeds. The grape seed includes photochemicals such as proanthocynidine, saponins, phenols, and others that have excellent anti-diabetic action mechanisms. 11–10 Some of the Diabetes patients have severe complications like high cardiovascular risk, inflammation, glycemia etc. According to the findings of this study, grape seed extract may have a therapeutic effect in lowering cardiovascular risk 6 as well as acting as an anti-inflammatory and glycemic biomarker.

Antibacterial Assay Activity

The antibacterial activity of grape seed powder extract improves as the concentration increases. The total growth kinetics of four microorganisms was studied in this study (Staphylococcus aureus, Streptococcus mutans, Escherichia coli, and Pseudomonas aeroginosa). At 90g/mL, the sample inhibits the growth of Staphylococcus aureus, Streptococcus mutans, and Escherichia coli by up to 70%, 50%, and 90%, respectively, while at 100g/mL, the sample inhibits the growth of all three bacteria. (Fig.3) On the other hand, grape seed power extract, inhibits the growth of Pseudomonas aeruginosa in both dosages. As a conclusion, the most effective way of assessing the antibacterial activity of grape seeds was discovered to be the growth kinetics of bacteria.

Grape seed extract has the ability to speed up the healing of wounds, which might lead to the spread of germs. Proanthocyanidin, found in grape seeds, provides a faster healing effect.

The use of grape seed powder extract to enhance cutaneous wound healing is a viable and effective option. This chemical was discovered to boost the synthesis of vascular endothelial growth factor, which is essential for wound healing.

Conclusion

Grape seeds are used to treat wounds and are also used to treat diabetes by hydrolyzing carbohydrates and releasing phytopharmaceuticals. The antibacterial properties of grape seeds were used to demonstrate remarkable wound healing activity against diabetic wound-causing microorganisms in this study. The antibacterial, angiogenesis, and necrotic effects of grape seeds, which are strengthened by phytochemicals like proanthocynidine, are thought to be responsible for the accelerated rate of diabetic wound healing. The grape seeds' cellular penetration characteristics worked as bio-enhancers, increasing the bioavailability of the biomolecule. The therapeutic potential of grape seeds in pharmacological research has now been scaled up into a commercially viable topical treatment, according to this study.

Acknowledgments

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Conflict of Interest

The authors declare that they have no conflicts of interest to disclose.

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(a)

(b)

Fig.1. The visual observation of Vitis vinifera (Grape) seeds (a), Fresh Powder form of grape seed (b).

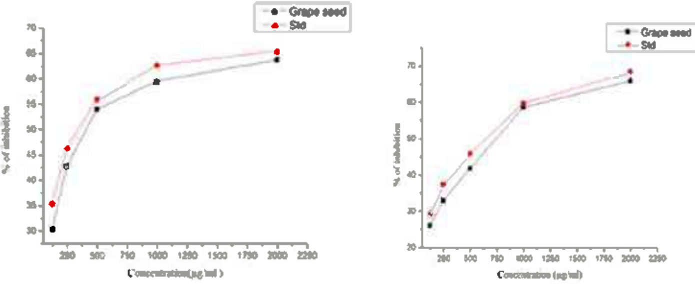

Fig.2.Graph represents carbohydrate hydrolyzing enzymes (antidiabetic) activity (a) Invitro α- amylase inhibition assay by grape seed powder extract and standard acarbose. (b) α-glucosidase inhibition assay by grape seed and standard acarbose.

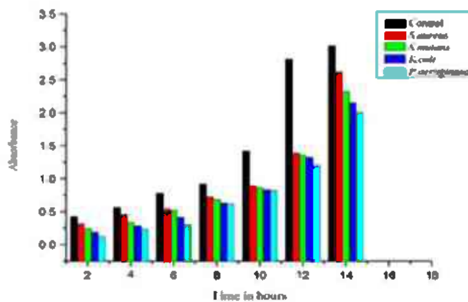

Fig.3. The Graph represents antibacterial activity of Vitis vinifera (Grape) seed powder extracts against (a) Staphylococcus aureus, (b) Streptococcus mutans, (c) Pseudomonas aeroginosa, (d) Escherichia. Coli.

8. Review: Applications of Probiotics

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Abstract

The aim of this study is well explicit to deal with in recent advances on probiotics within the past fifty years. Probiotics area unit the living cell organisms. It's a presence in human and animal gut. The probiotic organisms area unit natural antibiotics within the human-animal gut. This reviews the most role mechanisms of action in probiotics like probiotics area unit antibiotics, probiotics in medication activity, probiotic action in fish gut microorganism, dietary supplements of probiotics, microbiota of fishes, microorganism community of the epithelial duct of fish, bioactive compounds from probiotic microorganism, probiotic uses in cancer therapeutic applications, and applications of probiotics. the foremost common probiotics area unit utilized in many ways like boosting the system, inhibition of infective organism, interference of cancer, reduction of inflammatory gut unwellness, reducing sterol level. Nowadays, food merchandise area unit in the main concerned in chemical preservatives. These preservative agents area unit inflicting the unwellness to have an effect on the human health. The probiotic food merchandise area unit wont to the event of human-animal health.

Keywords: Antibiotic resistance, antimicrobial activity, bioactive compounds, gut microbes, probiotic, and therapeutic applications

1. Introduction

Probiotics

The probiotic suggests that merely life for originating from the Greek words "pro" and "bios."(Gismondo MR, et al., 1999) the foremost normally quote that means was created by Fuller (1989). The probiotics area unit live microorganism feed supplement that beneficially affects the host animal by rising its enteric balance. This correct definition continues to be normally noted, despite continual competition. Today, probiotics area unit quite a day in health-

promoting "functional foods" for humans, additionally as therapeutic, prophylactic growth supplements in animal production human health.(Mombelli B, Gismondo MR, 2000; Ouwehand AC, et al.,1999) alternative normally studied probiotics embody the spore-forming LAB spp. Yeasts. LAB spp. are shown to possess adhesion skills, manufacture bio activated molecules give immunostimulation.(Sullivan A, Nord CE, 2000: Cherif A, et al., 2001) LAB spp. hold an extra interest in probiotics as they will be unbroken within the reproductive structure type and so keep indefinitely on the shelf.(Barbosa TM, et al., 2005) it's usually reportable that a probiotic should be adherent and colonize at intervals the epithelial duct (GIT), it should replicate to high numbers, it should manufacture antimicrobial substances, and it should face up to the acidic setting of the scum bag.(Hong P, et al., 2005) Lactic acid microorganism (LAB) are wide used and researched for human terrestrial animal purposes; research laboratory are identified to be gift within the internal organ of healthy fish.

The most normally used probiotic is research laboratory, namely, lactobacilli sp. area unit typically characterised by gram-positive, nonmotile, nonsporulating microorganism that manufacture carboxylic acid as their main byproduct thanks to fermentation. The utilization of probiotics for growth promoter in aquatic animals is increasing with the demand for environment-friendly property cultivation.(Gibson GR, Roberfroid MB 1995) Probiotics area unit reportable to reinforce by stimulating vegetative cell activity, complement-mediated microorganism killing immune serum globulin production.(Ringø E, Gatesoupe FJ, 1998) the utilization of antibiotics to cure microorganism infection stop fish mortality in cultivation is changing into restricted as pathogens develop resistance to medicine.

In the past decade, many scientists rigorously examined the role effects of probiotics in cultivation as another to antimicrobial. Effects of Lactic acid microorganism area in milk process and naturally enrich soured with a good vary of bioactive metabolites drugs, demonstrating positive effects on fish survival, growth, stress resistance, system improvement, finally general welfare. The importance of probiotics in human-animal nutrition is widely known. The role of probiotics in nutrition health of sure cultivation species has conjointly been investigated by Fuller, Balcázar et al., Rinkinen et al., and Lara-Flores and Olvera-Novoa.

Mechanisms of Action of Probiotics

Probiotic microorganism will inhibit pathogens by the assembly of antagonistic compounds/by competitive exclusion (competition for nutrients attachment sites). Probiotic

microorganism directly take up or decompose the organic matter associate degreed improve the water quality of an aquatic scheme. Helpful microorganism cultures manufacture a spread of exoenzymes like enzyme, protease, and lipase, that facilitate to degrade the unconsumed feed body waste within the lake, additionally to the potential role of those enzymes within the nutrition of the animals by rising feed edibleness and feed utilization. Among all the microorganism interventions to enhance the assembly, use of probiotics is within the central dogma. The modes of action of probiotics embody the inhibition of a microorganism through the assembly of bacteriocin-like compounds, competition for attachment sites, competition for nutrients (particularly iron in marine microbes), alteration of the accelerator activity of pathogens, immunostimulatory functions, and organic process edges like rising feed edibleness and utilization.(Demain AL, Sanchez S, 2009)

Probiotics in Antibiotics

Probiotics have conjointly been reportable to stimulate the immunologic system, counteract allergies decrease sterol level. Microorganism medicine area unit creating their continuous influence as cancer chemotherapeutical agents. The invention of antibiotic drug has junction rectifier to venture into a microorganism world within the search antineoplastic compounds. Among the approved merchandise meriting special attention area unit antibiotic drug D, anthracycline, bleomycin, (mithramycin, streptozotocin, and pentostatin), calicheamicin, and taxol epothilones. Antibiotic drug, associate degree antibiotic isolated from actinomycete antibiotics, has served well against Wilms tumour in youngsters (Rengpipat S. et al., 1998).

Probiotics in Medication Activity

The helpful effects of probiotics are attributed to their ability to push the immunologic and no immunological defense barrier within the gut; standardization of augmented enteric permeableness altered gut microflora. 12 totally different enteric microorganism colonies were isolated from black tiger shrimp (Peanuts monodon). Among them, a microorganism, Bacillus strain was studied and characterised thanks to antagonistic properties against 3 target infective microorganism strains of eubacteria alginolyticus, eubacteria Harvey, and eubacteria vulnificus (Mahakam GB, et al., 2011)

Several microorganism are reportable as infective to fish. Among them, six gram-negative rods (Aeromonas, Proteus, Citrobacter, bacteria genus, and Flavobacterium

Chromobacterium) and 3 gram-positive cocci (Micrococcus and true bacteria Staphylococcus) are reportable for his or her pathogenicity.

Probiotic Action in Fish Gut Microorganism

The use of probiotic for aquatic animals is increasing with the for environment-friendly property cultivation. The gut micro biota of aquatic animals is maybe well-grooved by autochthonic microbiota collectively with unnaturally high levels of microorganisms therefore maintained by their constant bodily process from the encircling water. Probiotic strain redoubled the survival of larvae of the crab and Portunus trituberculatus conjointly reduced the number of vibrion sp. within the water accustomed rear the larvae. Afterward, it's been reportable that microorganism strains related to enteric skin secretion of adult marine turbot (Scophthalmus maximus) dab (Lima lima), suppressed the expansion of the fish infective agent V. anguillarum.(Verschuere L, 2000)

The overall completed study discovered that the isolated Bacilli spp. fulfill the desired criteria for probiotics like acid tolerance, salt tolerance, auto aggregation, antibiotic resistance, and antimicrobial activities to harsh conditions; it will be created bacteriocin extracellular that inhibits infective organisms. These isolates were used for potential probiotics.

Dietary Supplements of Probiotics

Dietary supplements like probiotics originally outlined as live microorganism feed supplements that beneficially have an effect on the enteric microorganism balance of the host organism and have received heightened attention in cultivation over the past many years. The dependent and infective organisms are full of prebiotics are compete necessary roles in many processes likewise as growth, digestion, and immunity unwellness resistance of the host organism as incontestable in poultry, alternative terrestrial eutherian companion animals, likewise as in humans. At this point, the appliance of prebiotics in cultivation has been rather restricted however holds substantial potential. However, to effectively apply prebiotics or probiotics in cultivation, the microorganism community of finfishes must be higher characterised understood (Elrod-Erickson M, 2000)

2. Conclusion

The probiotics are most commonly used in many ways such as foods and drugs scientific research etc. and the probiotics' main important role is the development of the human animal health. Now, a day's probiotics are classified to uses of human health; the world health

organization approved to uses of probiotics in human animals these are generally recognized as safe. In this review, probiotic and its secondary metabolites are used to the development of aquaculture therapeutic applications.

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9. Isolation and Identification of Microorganisms from Various Drinking Sources

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1. Abstract

The recitation of fecal coliform specifically of Escherichia coli (index of fecal impurity) from mortal as well as animal sources per 100 ml of external water used for drinking purposes has been recommended by World Health Organization (WHO) worldwide to cover the quality of drinking water. According to World Health Organization (WHO) guideline norms for drinking water aggregate and fecal coliform the index of fecal impurity mustn't be Sensible in any 100 ml samples. From the research carried out, it was cited that Escherichia coli, Kleibsella Pneumoniae, Pseudomonas Aeruginosa and Staphlococcus Aureus were seen in sample 1 taken from railway station contained the highest no. of colonies of Microorganism contamination with sample taken from local snacks corner showed 2nd highest contamination followed by sample taken from Bus Depot and college canteen, with water taken from home's RO purifier didn't show any contamination. This study helped us to get a basic idea on water quality in Mumbai. From this study it is very important to note that we should check and verify the source before consuming water from it. Water supplying authorities should take account of this situation and take measures for the provision of impurity free drinking water to help waterborne complaint outbreaks by bacterial pathogens.

Key words: Fecal Coliform, Waterborne and Contamination.

2. Introduction

Everywhere we go our eyes catches the banner of Drinking water on water coolers, filters and place with large 'drinking water' banners. So the question arises what is drinking water? This made me question, how safe drinking water is and what organism will be present not only

organism but also heavy metals and minerals which are really dangerous and lethal to our health and can cause disease like dysentery, Hepatitis A and typhoid if we drink from any sources, it is also our birthright to access to safe and drinking water. The major threat arises from water we consume that comes from local municipal office. The question made me realize how important it is to check the purity of the water we drink and I thought I could take this topic for my research project. It is, thus, absolutely necessary that the monitoring and assessment of drinking water quality should be done regularly in order to gain the position of water impurity as well as to understand the impact of mortal conditioning on the state of water coffers for policymaking, planning, and conservation measures.

Access to safe drinkable drinking water is one the introductory amenities of humankind, especially in civic areas of the world because of the high-consumption pattern of the large population inhabiting these civic areas. Among the colorful sources of water, groundwater is considered to be the safest source of drinking water in civic as well as pastoral areas (Simpi etal., 2011). The need for stoner involvement in maintaining water quality and assessing other aspects, similar as hygiene, environmental sanitation, storehouse, and disposal are critical rudiments to maintain the quality of water coffers (Khare, Lall, Bharose, & Kumar, 2013).

As water is a universal detergent it dissolves mariners, inorganic and organic composites and feasts that take part in metabolic responses, maintain the macromolecular frame, transport nutrients, thermoregulation, stabilize tube membrane, and maintain hemostasis and body volume/ weight. (Armstrong, L.E.; Buyckx M.; Campbell S. and Fulgoni V. 2007)

According to the presented reports of the World Health Organization (WHO), about 80 percent of all conditions in the world are directly or laterally affiliated to the impurity of water. Water in its natural state is tintless, odorless, and free from pathogens. The pH of water ranges from 6.5 to 8.5. This water is nominated as 'drinkable water' or 'potable water'. (Roohi Rawat and A. R. Siddiqui. 2019)

3. International View on Drinking Water

In around 2017, around 71 percent of the global population (5.3 billion people globally) used a safely managed drinking water service that is, one located on demesne, available when demanded, and free from impurity.

90 percent of the global population (6.8 billion people) used at least a introductory service. A introductory service is an advanced drinking-water source within a round trip of 30 minutes to collect water.

785 million people warrant indeed a introductory drinking-water service, including 144 million people who are dependent on face water.

Encyclopedically it is found that, at least 2 billion people use a drinking water source defiled with feaces.

Defiled water can transmit conditions similar diarrhoea, cholera, dysentery, typhoid, and polio. Defiled drinking water is estimated to beget 485 000 diarrhoeal deaths each time.

It is estimated that around by 2025, half of the world's population will be living in water-stressed areas and will see it's effect in future.

In least developed countries it is observed that, 21 with no sanitation service, around 22 of health care installations have no water service, and around 22 with no waste operation service. (World Health Organizaion. 2019)

It's estimated that waterborne conditions have an profitable burden of roughly USD 600 million a time in India. This is specially true for failure-and deluge-prone areas, which affected over a third of the nation in the once couple of times.

Lower than around 50 per cent of the population in India has basic access to safely managed safe and drinking water. Chemical impurity of water, substantially through fluoride and arsenic, is present in1.96 million residences.

Redundant fluoride in India may be affecting knockouts of millions of people across 19 countries, while inversely worryingly, redundant arsenic may affect up to 15 million people in West Bengal.

Also, around two-thirds of India's 718 sections are affected heavily by extreme water reduction, and the current lack of planning for water safety and security is a major as well as threat and concern which needs to covered and should be worked. One of the challenges is the fast rate of groundwater reduction in India, which is known as the world's topmost stoner of this source due to the proliferation of drilling over the formerly multitudinous decades. Groundwater from over about 30 million access points is supplied to about 85 percent of drinking water in pastoral areas and about 48 percent of water conditions in collaborative areas. (United Nations Children's Fund, UNICEF. 2017)

4. Water Quality in Mumbai

Mumbai being one of the major metropolitan city of India and financial capital of India, contributes majorly in country's every aspect from financial to stable economic conditions.
In 2019, Mumbai topped the ranking released by Bureau of Indian Standards (BIS) for quality of tap water, conveyed by our respected Union Minister Ram Vilas Paswan with over several other major cities of India falling apart and lagging behind from Mumbai, this test was conducted in order to get a basic idea of drinking water available in majorly populated cities. (The Print. 2019)

BMC collected water samples from April 2018 to March 2019. And, they've plant that only 0.7 of it has examined positive for coliform bacteria. Way better than the quality standard set by the World Health Organization limit for it's 5. As a result, the communities need to sustain the cleanliness and sanity of the tanks and storehouse. On their observation, BMC's Master Balance Reservoir present in Bhandup has one of the purest water in the whole world. But as it goes by the distribution system, its quality degrades, and the end consumer gets weakened water, which is unsafe for consumption without filtering. (Tap Safe., May 14 2021)

But as of the September 17, 2021 Dadar, Parel and Dharavi are the areas that saw rise in water contamination in 2020-21 in Mumbai according to BMC.

Officers said that out of the total water samples collected for testing, 275 showed the presence of E-coli bacteria, which can beget conditions similar as diarrhoea and dysentery. Areas such as Dharavi, Dadar, Parel, Goregaon, Byculla and Mulund have reported an increase in contamination of drinking water in the last one year, according to data from the Brihanmumbai Municipal Corporation's (BMC) annual Environment Status Report (ESR) for 2020-21. Of the total 29,051 water samples that were checked by the civic body for the period, 275 around 0.94 per cent of the total samples were found contaminated.

The ESR for 2020-21, which was participated with corporators on Thursday, showed that the loftiest quantum of impurity in water samples of about3.4 per cent was detected in the G-north ward (Dadar, Dharavi), followed by2.4 percent P-south (Goregaon),2.3 per cent in T ward (Mulund) and2.2 in F-north (Sion, Matunga).

The testing of samples was carried out at BMC's laboratory at Dadar. According to the officials, the contamination could have been due to damaged or old water supply pipelines. "," An official from the Hydraulic Engineer department said in the last five years, they have replaced old and eroded pipelines across the city. However, some are yet to be changed. They

ensure that water from reservoirs are 100% pure, and the contamination could be during the transmission to users, he also conveye that they collected sample from random places with varying from 200 to 250 samples.

The BMC inventories million litre water daily to the megacity. There are 27 service budgets from which the water is supplied to casing societies, marketable establishments and slums. Mumbai has approximately more than 4 lakh metered water connections overall the city.

This March, the BMC had bagged the Jal Nirmalata Award for 2019-20 from the Ministry of Consumer Affairs for purity of water for their remarkable work. The civic body is also constructing water tunnels from Powai to Veravali, Powai to Ghatkopar, and Amar Mahal to Trombay Reservoirs in a bid to prevent water contamination. (Hindustan Times. 2019)(The Indian Express. 2021)

5. Drinking Water Source of Mumbai

Lakes That Supply Or Acts As A Source Of Drinking Water Are As Follow

a. Vihar Lake
b. Tulsi Lake
c. Modak Sagar
d. Tansa Dam
e. Bhatsa
f. Middle Vaitarna
g. Upper Vaitarna

The seven lakes that give water to Mumbai megacity are now about 99.2 percent full and this is 1 percent further than a time ago and 1.4 percent further than two times agone. Water reserves near-full capacity means there will be sufficient amounts to meet the megacity's water force conditions throughout the time and residers are doubtful to face any water- cuts. (Mumbai live. 2019)(Hindustan Times. 2021)

6. Are Bottled Water Safe?

A new study has found 90 per cent of bottled water companies globally to be contaminated with plastic particles including top brands like Bisleri and Aquafina leading WHO to launch a review to look into the same. (India Today. 2018)

The research was led by microplastic researcher Sherri Mason of the State University of New York at Fredonia, according to a summary released by Orb Media, a US-based non-profit

media collective. The study claimed that 90 per cent of bottled water samples were found to be containing traces of plastic.

Sherri Mason et.al., conducted a 3 month research in which she took about sample from 250 bottles from over 11 brands in 9 countries from Brazil, India, Indonesia, Kenya, Lebanon, Mexico Thailand and The US.

Sample included brands being sold in India like Evian, Aquafian and Bisleri, also from India 19 locations in Mumbai, Delhi and Chennai. She used a technique to view microplastic particles by staining them using specific dyes like Nile red dye, which makes plastic particles fluorescent when showered or irradiated with blue light.

After 3 month research results were quite shocking, smaller particles were common with averaging from about 325 per litre, even a Bisleri sample is known to contain a concentration of about 5,000 microplastic particles, and as for other brands range reached a limit of upto 10,000. Also, many other carcinogenic substances were found like bromate, that too 4 times the limit of WHO guidelines. Polypropylene used in cap making of water bottle was found in many samples, with Nylon being the second most abundantly found product in sample, with 93 percent of sample containing plastic particles, with many samples containing polyethylene terephthalate (PET) and with only 17 bottles out of 259 bottles didn't contain microplastic. (Sherri Mason et.al. 2018)(India Today. 2018)(mint. 2018)

Many Big companies like coca cola, Nestle and PepsiCo criticized the research work carried out bu Sherri Mason.

Similarly, a new study by scientists at the Bhabha Atomic Research Centre (BARC) in Mumbai has revealed the presence of a carcinogenic element bromate in packaged drinking water in the city. Bromate is basically produced as a by-product when water containing bromide is treated with ozone.

Bromate is linked as a "possible carcinogen" by the World Health Organization's (WHO's) International Agency for Research on Cancer (IARC). While bromate is said to be mutagenic (an agent that causes mutation of inheritable material), its implicit carcinogenicity is still inconclusive. The study was published in January 2015 in the journal Current Science by a platoon of scientists led by G G Pandit from the Environmental Monitoring and Assessment Section at BARC. They tested for disinfection by- products (DBPs), including bromate, in 18

different marketable brands of packaged drinking water attained from original requests of Mumbai.

Bromate situations detected in the samples varied from below discovery limit to 43 µg/ l (micrograms per litre), with an normal of 10.7 µg/l. Twenty-seven per cent of the samples contained bromate situations that were over 10 µg/ l, the safe limit defined by the WHO. (DownToEarth. 2015)(The Logical Indian. 2015)(Bhabha Atomic Research Centre (BARC). 2015)

7. Indicator Organisms

Since it would be virtually insolvable to test water for each of the wide variety of pathogens that may be present, microbiological water quality monitoring is primarily grounded on the tests for index organisms. There's no single index organism that can widely be used for all purposes of water quality surveillance. Each of the wide variety of index available for this purpose has its own advantages and disadvantages, and the challenge is to elect the applicable index, or combination of pointers, for each and every particular purpose of water quality assessment. Indicators are most generally used are of faecal or sewage origin and the following are some of the most important conditions of similar indicators:

1. Should be present whenever pathogens are there.
2. Should be present in more or same numbers as of pathogens.
3. Should be specific for sewage or faecal pollution.
4. At least as resistant as pathogens to conditions in natural water environment, and water purification and disinfection processes.
5. Basically Non pathogenic
6. It's Detection Should be easy, rapid (DesalegnAmenu et.al. 2013)
    a. Following are some commonly found microorganisms
    b. Coliform Bacteria
    c. Salmonella
    d. Escherichia coli
    e. Klebsiella pneumonia
    f. Vibrio cholera

7. Methodology

7. a Collection of Samples

5 samples were collected from 5 different locations, Sample 1 was collected from railway station, sample 2 was collected from a local snacks corner, Sample 3 was collected from bus depot, Sample 4 was collected from college canteen and sample 5 was collected from home's water filter.

7.b Isolation and Enrichment of Samples

0.1 ml of sample has added in different 10 ml broths name as Nutrient broth, EMB agar, MacConkey's Agar, Mannitol Salt Agar and Centrimide agar. We carried the dilution techque for getting isolated colonies

After 24 hours several colonies were spotted and streaked on different medias such as EMB agar, MacConkey's Agar, Mannitol Salt Agar and Centrimide agar.

7.c Identification of Microorganism by Gram nature and Biochemical technique

Identification Of microorganism which highest colonies found in water samples The bacterial strain was identified on the basis of Cultural, "Morphological and Biochemical

Characteristics as given in the Bergey's Manual of Synematic Bacteriology vol. 2 (Holt et al., 1984).

Morphological and Cultural Identification

Strain showing highest antibacterial activity was Gram stained and stained microscopically for cellular morphology and Gram stain phenotype. Colonies developed on Certrimide agar were characterise by observing various parameters viz, shape, size, colour, elevation, margin, surface etc. and recorded in the table no.3.

Biochemical Characterization

Catalase test was performed by inserting loop full colony into tube containing 3% hydrogen peroxide. A citrate utilization test was carried out by Koser citrate Agar (Schillinger, 1996) and assay for nitrate reduction were performed (Harrigan, 1998).

IMViC tests was also performed and ability to ferment various carbohydrates were evaluated using Sugar fermentation medium broth supplemented with filter sterilized sugar solutions to a final concentration of w/v and 0.004% Phenol red (Schillinger, 1996).

Result and Discussion

1. Collection of Sample

The ph of samples has shown in table no. 1

Table no. 1

Sr. No.	Location	PH
1.	Railway Station	7.0
2.	Local Snacks Corner	7.0
3.	Bus Depot	6.8
4.	College Canteen	7.0
5.	House's RO Filter	7.0

2. Isolation and Enrichment of Water Sample

Total 108 different colonies were obtained on Nutrient agar plates from different samples.

Then Out of 108 bacterial isolates, 35 isolates found on EMB agar, 14 on Mannitol Salt agar, 40 on MacConkey agar and 19 on Cetrimide agar. Table no. 1 and photoplate 1, has shown the growth of microorganisms in different medias

The Colony Growth on Each Nutrient Media Shown in Table no. 2

Table no. 2

Water Sample	MacConkey agar	Mannitol Salt agar	Cetrimide agar	EMB agar	Total
Railway Station	17	5	19	11	52
Local snacks corner	11	4	9	4	28
Bus depot	4	3	7	2	16
College Canteen	3	2	5	2	12
RO water purifier	0	0	0	0	0
Total	34	14	40	19	108

From above result it is confirmed that there can be contamination even in labelled sources as drinking water and should be careful before consuming it. Roohi Rawat et.al and A. R. Siddiqui et.al conducted similar experiment in Allahabad with over 20 samples and found out all were well within the guidelines and parameters set by WHO and BIS, but the one collected from Beniganj, Rajapur and Krishna nagar were slightly above the guidelines set by WHO and BIS, but can be consumed by filtering or by boiling prior to consumption, so it is safe to assume that Allahabad's water can be used for household and irrigation purpose. (Roohi Rawat and A. R. Siddiqui. 2019)

Similarly Sherri Mason et.al. 2018 conducted research on bottled water being sold in India in Major cities like Mumbai, Chennai and Delhi. She found that it contained harmful polymers like polymer Polypropylene and Nylon.

Similar experiments were carried out by Laís Anversa et.al 2019 in Dois Córregos, Itapuí and Pirajuí – in the midwest of São Paulo State, Brazil, and over 251 samples were taken into consideration and out of which 7.6% that is 19 samples showed the presence of P.aeruginosa.with testing over three district of municipalities.

3. Identification of Microbe

Gram of the Microbe has been shown in Table No. 3

Table no. 3

Colony Characteristics	Colony 1
Size	0.5 to 0.8 µm
Colour	Green color
Shape	Circular
Margin	Entire
Opacity	Opaque
Consistency	Butyrous
Elevation	Low convex
Gram nature	Gram negative
Morphology	Short rods

Biochemical Testing of Bacteria Shown in Table No. 4

Table no. 4:

Characteristics	**Result**	**Characteristics**	**Result**
	Morphological Characteristics		
Shape	Long rod-shaped	Gram Staining	Gram Negative
Size	0.5 to 0.8 µm	Motility Test	motile
Colour	green color	Spore	Non-sporulating
	Decomposition/Enzymatic studies		
Oxidase Test	Positive	Gelatine Hydrolysis Test	Positive
Catalase Test	Positive	H2S production test	Negative
Nitrate Reduction Test	Positive	Urease Test	Negative

	IMViC Set		
Indole test	Negative	Methyl- red test	Negative
Voges-Proskauer Test	Negative	Citrate Utilization test	Positive

From above Table we can conclude that microorganism obtained is Pseudomonas Aeruginosa

Conclusion

According to present result, the Pseudomonas aeruginosa found in all water sources.

Precautions to be taken before Drinking

Keeping in mind the above results following precautions should be taken care of:

i. It should be properly boiled before drinking
ii. Government should keep an regular check on water being supplied to source's.
iii. There should be proper addition of chlorine as disinfectant in water source.
iv. Water quality should be in proper guidelines as stated by WHO.
v. There should not be any animal or contaminating agent near the source of water.
vi. The basic hygiene and cleanliness of main water storage must be maintained.
vii. There should be proper treatment of sewage before disposing it into water bodies. (WHO. 2019)(Health Unit. 2021)

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10. Isolation and Identification of *Lactococcus SP* from Vegetable Samples

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Abstract

The antimicrobial activity of plants and its extracts has been recognized for several years. Lactococcus spp. that is taken into account is Generally Recognized as Safe (GRAS), helpful to regulate the quick development of pathogens and spoiling microbes in food and feed. Lactococcus spp. was isolated from vegetable sample like Cabbage, Cluster beans (Guar), Yam, Bitter gourd, Beans, and Cucumber by the employment of selective media like De Man, Rogosa and Sharpe (MRS) Agar media. Gram staining and colony morphology were used to identify the morphology. In the present study Lactococcus spp, was isolated from different vegetable samples. Highest number of microorganisms was found in cabbage sample (42x107) followed by the guar sample (26 ×107) and minimum number of colonies was found in bitter gourd sample (14 x 105).

1. Introduction

Lactococcus is a genus of lactic acid bacteria (LAB), members of this genus are Gram-positive cocci that can appear ovoid depending on growth conditions and are typically 0.5–1.5mm in size. They don't produce spores and aren't mobile. Lactococcus species grow in pairs or small chains, unlike many Streptococcus species, and do not form large chains (Batt, 2014). They can be found in a wide range of environments, including plants and gastrointestinal tracts (Mansour et al., 2011). They have a fermentative metabolism and create a lot of lactic acid, as one would anticipate from lactic acid bacteria. They are auxotrophic for a number of amino acids

and vitamins; they have complex nutritional requirements. They can grow at temperatures as low as 10°C but not at 45°C, and their ideal growth temperature is 30° C.

Natural (wild) L. lactis strains can be found in raw cow, ewe, or goat milk, as well as artisanal cheeses and other traditional dairy dishes, where they often outnumber the microbiota of other lactic acid bacteria (LAB) (Parapouli et al., 2013). Despite its common link with dairy products, Lactococcus lactis was first extracted from plants where it was thought to be inactive, and only became lively and flourished in the presence of milk or in the gastrointestinal tract after being consumed by ruminants (Bolotin et al., [n.d.])

Lactic acid bacteria are found in abundance in green plant material (Vandenbergh, 1993). However, just a few investigations have been done on isolating and characterising lactococcus strains from plant leaf surfaces (Leifert et al., n.d.). While certain studies have established the presence of lactobacillus strains on plant surface in very large numbers (Daeschel, Andersson, and Fleming 1987). Others have discovered lactic acid bacteria in dead organic matter, as well as in a wide range of biological products (silage raw materials like cabbage, olives, carrots, and beets) and fruits, suggesting that plants could be a source of Lactococcus spp. (Peres et al. 2012).

Hence, the objectives of this research were to isolate and identify of Lactococcus sp. from vegetable samples. Lactococcus sp. strains were screened from leaf surface of cabbage and antibacterial activities against food pathogens were tested.

2. Material and Methods

2.1 Collection of Samples

The fresh vegetable samples like Cabbage, Cluster beans (Guar), Yam, Bitter gourd, Beans, and Cucumber were collected from the nearby market. The edible part of the selected vegetables was used for the isolation of LAB.

2.2 Raw Material Extraction

Vegetable residue samples (1.0 g) were blended with 90 mL of sterilized water and serially diluted (10−6 to 10−8) in sterilized water. The numbers of LAB were measured by plate count on De Man, Rogosa, Sharpe agar (MRS) incubated at 30°C for 48 h under anaerobic conditions. Bacilli and aerobic bacteria were distinguished by colony shape and counted on nutrient agar incubated for 24h at 30°C under aerobic conditions. Colonies were counted as viable numbers of microorganisms in colony-forming units. Each LAB colony was purified twice by streaking on MRS agar.

2.3 Enrichment and Isolation of Lactococcus Strains

In enrichment method, 1.0g of vegetable minced into small pieces was suspended in flask containing 9mL MRS broth. This broth was anaerobically incubated at 37°C for 48h. The appropriate dilution was plated on MRS agar and incubated under same conditions as above.

Morphologically distinct colonies were further streak on another media plate and selected for biochemical characterization and the colonies were observed under the microscope for gram staining.

3. Results and Discussion

3.1 Isolation and Identification of LAB

Lactic acid bacteria (LAB) like Lactococcus, Lactobacillus, Leuconostoc, and Streptococcus play an essential role in the dairy industry due to the high levels of human consumption of several dairy products. Present study was carried out to isolate and identify bacteriocin producing LAB from different vegetable samples such as Cabbage, Cluster beans (Guar), Yam, Bitter gourd, Beans, and Cucumber. In the present study Lactococcus spp, was isolated from different vegetable samples. Highest number of microorganisms was found in cabbage sample (42x107) this was closely followed by the guar sample (26 ×107) and minimum number was found in bitter gourd sample (14 x 105). The counts of viable microorganisms are shown in table 1, and cultural, morphological and biochemical characteristics of bacterial isolates is shown in table 2. The Lactococcus sp. grew well on the MRS agar Plate (fig. 1).

The presence of different Lactococci spp. in vegetables were characterized by their morphology using Gram's staining method. The colonies were found to be of gram-positive nature with cells being in ovoid shape and in short clusters or chains.

Gram staining of LAB and morphological characteristics were determined after 24 h of incubation on MRS agar. The activity of catalase and the production of gas from glucose were determined. Lactococcus sp. It's morphological, physiological, and biochemical properties were determined.

Cell structure and its characteristic patterns were isolated from vegetable extracts. All the isolates were found to be gram-positive and catalase-negative bacteria. According to the morphological, physiological, and biochemical properties, these strains were divided on the basis of strains were able to grow at the lowest pH (3.5) and produced the highest lactic acid content (1.4%) in MRS broth compared with other groups.

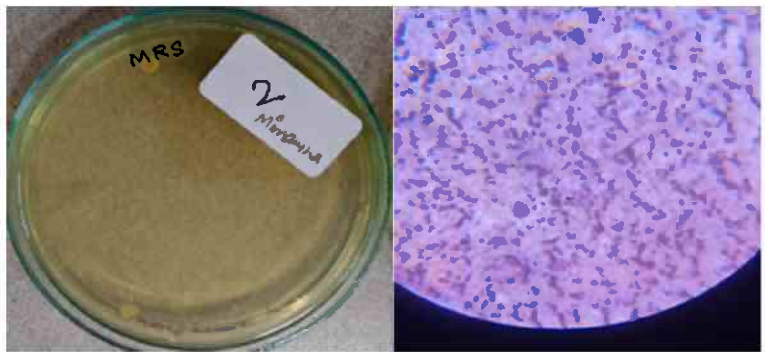

Fig. 1: Growth of Lactococcus sp. on MRS agar

Fig. 2: Gram Nature of Lactococcus sp.

Table 1. The Counts of Viable Microorganisms, Chemical Composition, and Silage Fermentation of Vegetable Residues are Shown

Sr, No	Vegetables	Total Colony Count
1.	Cabbage	42x107
2.	Cluster beans Guar)	26 ×107
3.	Yam	9 x 105
4.	Bitter gourd	14 x 105
5.	Beans	12 x 105
6.	Cucumber	9 x 105

Table 2. Cultural, Morphological and Biochemical Characteristics of Bacterial Isolates.

Vegetables	Colony Morphology					Microscopic observation	Catalase test	Motility test
	Form	Colour	Elevation	Margin	Surface			
Cabbage	Circular	white	Convex	Entire	Smooth	Cocci, single, in pair or short chain	-	-
Cluster beans Guar)	Circular	white	Convex	Entire	Smooth	Cocci, single, in pair or short chain	-	-
Yam	Circular	white	Convex	Entire	Smooth	Cocci, single, in pair or short chain	-	-
Bitter gourd	Circular	white	Flat	Undulated	Smooth	Cocci, single, in pair or short chain	-	-

Beans	Circular	grey	Convex	Entire	Smooth	Cocci, single, in pair or long chains	-	-
Cucumber	Circular	white	Convex	Entire	Smooth	Cocci, single, in pair or short chain	-	-

4. Conclusion

The results obtained in this study revealed the presence of a wide variety of LAB from vegetable samples. Some of the isolates obtained were discovered to be of LAB species and they have excellent lactic acid production ability. The results of this study the results of this study showed that the highest colonies of LAB in cabbage vegetables is Lactococcus sp. Moreover, Lactococcus sp. was recognized as a probiotic species vegetables. The raw data obtained from this study can be used in to improve the nutritional value of cabbage.

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11. Impact of Tulsi *(Ocimum Sanctum Linn)* Leaf Extract on Pathogenic Organisms

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Abstract

In present study, ethanolic extract of Tulsi was ready by cold extraction technique. Extract was diluted with organic solvent, alcohol and water, to get six totally different concentrations (1%, 3%, 5%, 7%, 9% and 10%). Absolute lcohol was used as management. The extract and controls were subjected to the microbiological investigation against E. coli, S. aureus, P. aregunosa , S. typhi. Agar well diffusion technique was used to see the concentration at that Tulsi gave inhibition zone, the same as alcohol. Knowledge were analyzed victimization a way analysis of variance. At 9 % and 10 % concentrations, Tulsi extracts incontestable antimicrobial activity against S. aureus, P. aeruginosa , the same as alcohol with similar inhibition zones ($P > 0.05$). However, exhibited resistance to Tulsi extract that showed considerably smaller inhibition zones ($P < 0.05$). Tulsi incontestable effective antimicrobial property against S. aureus, P. aeruginosa , suggesting its attainable use as a good and cheap "adjunct" together with the quality care within the management of periodontics conditions. The utilization of tulsi in daily rituals may be a testament to Ayurvedic knowledge associate degreed provides an example of ancient data providing solutions to trendy issues. However, additional analysis assessing the toxicity, durability, and different assessments followed by clinical trials is critical to explore the potential of Tulsi in combating oral conditions

Keywords: Ocimum Sanctum, E. coli, S. aureus, P. aeruginosa, S. typhi, medicament activity

1. Introduction

The use of healthful plants in ancient medication has been delineated in literature qualitative analysis back many one thousand years (Chang et al., 2016). Books on Ayurvedic medication, written within the Vedic amount (3500–1600 B.C.) describe practices, as well as the utilization of healthful plants, that shaped the idea of all different medical sciences developed on the Indian landmass (Pattanayak et al., 2010). In trendy complementary and different practice, plants are the first supply of medicine and every a part of the plant, as well as the seeds, root, stem, leaves, and fruit, probably contains bioactive parts (Jiang et al., 2014, 2015; Mandave et al., 2014; Sun et al., 2014). The most bioactive parts in healthful plants are thought-about to be combos of secondary metabolites (Singh et al., 2010; Chinese et al., 2016). There are several blessings and edges related to the utilization of healthful plants, the most ones being their cost-effectiveness and world convenience. Their safety compared to different healthful product and also the lack of major side-effects are different clear blessings (Niu et al., 2011). However, plant metabolism is incredibly variable and before healthful plant extracts or product are approved for primary health care, they have to be standardized, subjected to rigorous internal control and assessed to confirm their safety (Mantri et al., 2012; Olarte et al., 2013).

Among the healthful plants, aromatic herbs are a chip supply of biologically active compounds helpful each in agriculture and medication (Mathela, 1991; monger and monger, 1999). Of these, Ocimum tenuiflorum, conjointly called Ocimum sanctum, Tulsi, or Holy Basil from the family Labiatae has been delineated because the “Queen of plants” and also the “mother medication of nature” because of its perceived healthful qualities (Singh et al., 2010). It has been one among of the foremost valued and holistic herbs used over years in ancient medication in Bharat and nearly every a part of the plant has been found to possess therapeutic properties (Singh et al., 2010). Historically, Tulsi is employed in several forms; binary compound extracts from the leaves (fresh or dried as powder) are employed in flavorer teas or mixed with different herbs or honey to boost the healthful price. ancient uses of Tulsi binary compound extracts embrace the treatment of various kinds of poisoning, stomach-ache, common colds, headaches, malaria, inflammation, and heart condition (Pattanayak et al., 2010). Oils extracted from the leaves and inflorescence of Tulsi are claimed to possess various helpful properties, as well as expectorants, analgesics, anti-emetics, and antipyretics; stress reducers and inflammation relievers; and as anti-asthmatic, hypoglycaemic,hepatoprotective, hypotensive,

hypolipidemic, and immunomodulatory agents (Singh et al., 2010). many scientists have examined medicine effects of Tulsi product obtained by totally different extraction strategies, like steam distillation, benzene extraction and fossil fuel extraction. Prakash and Gupta (2005), reviewed all the scientific studies of the therapeutic significance of Tulsi and eugenol, a serious part of Tulsi. These medicine studies could also be useful to ascertain a scientific basis for the therapeutic use of this plant. In this study, we tend to study on medicament activity of leaf extract on pathogens and phytochemical characters.

2. Material and Method

2.1 Collection of Tulsi

Tulsi leaves were obtained from courtyards and gardens in Mumbai town. Specimens were known by a biologist and a pharmacognosist for his or her credibleness. Leaves were separated from the stem, washed in clear water and dried till they were adequately dry to be ground (dried for seven days). Dried leaves were pulverised singly in an electrical grinder till a consistent powder was obtained. A complete of 250 g of finely pulverised Tulsi was macerated with 100 % alcohol for three days. The alcoholic boiling was subjected to filtration with Whatmann filter paper no. 1 paper to get a transparent filtrate. The filtrate so obtained was reduced at an occasional temperature of powder dissolved in 1 L of alcohol, just about 18g of solid residue (extract) was obtained. 1 gm of this extract was dissolved in 10ml of abs. alcohol to get a tenth concentration of extract. Similarly, concentrations of 1%, 3%, 5%, 7%, 9% and 10% of Tulsi extract were obtained by diluting with acceptable amounts of solvent. These extracts were collected in sterile containers and transported for microbiological assays. DFuring this study, alcohol was used as an effect, and the solvent employed in the extract preparation.

2.2 Agar Well Method

Microbiological assay Agar well diffusion methodology was accustomed confirm the antimicrobial activity of Tulsi leaves extract in vitro. Nutrient agar was accustomed culture completely different micro-organisms examined during this study. Colonies of microorganisms were transferred to the agar plates employing a swab, and their cloudiness was visually adjusted with the broth to equal that of a 0.5 ml McFarland cloudiness normal that had been vortexed (Fig. 1). Among 15 min of adjusting the matter to a McFarland 0.5 ml cloudiness normal, a sterile cotton swab was swaybacked into the matter and turned against the wall of the tube higher than the liquid to get rid of excess matter. the whole surface of agar plate was then swabbed three

times with the cotton swab, transferring the matter, whereas the plates were turned by just about 60° between streaks to make sure even distribution. The general procedure of matter preparation and immunisation of culture media remained identical for all four bacterium.

Each microorganism was inoculated on 5 agar plates for 5 several concentrations (1%, 3%, 5%, 7%, 9 % and 10%) of the Tulsi extract. Therefore, a complete plates were inoculated to check all the infective bacterium. The inoculated plate was allowed to minimum of 3min however now not than 15min, before creating wells for various compounds to be tested. A hollow tube of 5mm diameter was heated and ironed higher than the inoculated agar plates. It had been removed instantly by creating a well within the plate; likewise, 5 wells on every plate were created, one every for management and Tulsi extract. Every well received 5 µl of several compound appointed for it. Plates were incubated at 37°C in associate degree setup among fifteen min of compound application. Incubation was incubated at 370C for 48h. When the period of time, plates were browse providing the field of growth was convergent or nearly convergent. The diameter of inhibition zone was measured to the closest whole metric linear unit. The microbiological procedure was continual three times for every microorganism. The values therefore obtained were compared among the cluster (same concentration of extract) and with completely different teams (different concentrations of extract) and conjointly with the management for various bacterium. Statistical analysis was done victimization the computer code MINITAB 14. Information were analysed victimization unidirectional analysis of variance (ANOVA), for comparison among the cluster and with completely different teams. Statistical significance level was established at P < zero.05

2.3 Confirm Phytochemical Activity

Qualitative Phytochemical Analysis

All extracts of tree leaves were subjected to qualitative phytochemical analysis to spot presence of assorted phytochemicals viz. alkaloids, glycosides, proteins, reducing sugar, tannins, resins, sterols, phenoplast compounds and saponins as per the strategy delineated by Rosenthaler (1930).

2.3.a Detection of Alkaloids

A tiny low quantity of extract was taken in tube and another with 5 ml of 1.5 N HCl (v/v) so filtered. Some drops of every of the subsequent reagents were another to the Filtrate and

mixed well, look of cloudiness or any changes in colour to the take a look at indicates the presence of alkaloids.

Wagner's chemical Agent Test

The chemical agent was ready by dissolving iodine 1.27 gm and 2gm of iodide in 5 ml of H2O and diluted to a100 ml. The microscopic quantity of the higher than extract (filtrate) was another to the current chemical agent, look of brown to soft precipitation discovered the presence of organic compound.

2.3.b.Test for Detection of Glycoside

a. Benedict's chemical agent test: Equal amount of each the extract and benedict's chemical agent was another and heated to boil for 2 minutes, look of brown to red colour indicate presence of organic compound.
b. Folin's copper chemical agent test: A touch quantity of extract was another to few drops of folin's reagent, the event of red colour provides positive reaction for organic compound.

2.3.c Detection of Proteins

a. Biuret test: Few amounts of the extracts were another to four-dimensional hydrated oxide answer followed by a drop of I Chronicles cupric sulphate answer, the event of violet to pink color indicates presence of proteins.

2.3.d Detection of Reducing Sugar

a. Benedict's chemical agent test: The extract was another with benedict's chemical agent in equal quantity and mixture was heated for two minutes, look of brown to red colour indicates presence of reducing sugar.
b. Folin's copper chemical agent test: A touch quantity of extract was another to few drops of folin's reagent, the event of red colour provides positive reaction for organic compound.

2.3.e Detection of Tannins

A little amount of alcohol extract taken during a tube was warm and filtered. The filtrate was accustomed do the tests.

a. Dye test: Few drops of fifty dye answer were another to the filtrate, formation of precipitation indicates the presence of tannins.

b. Metal chloride test: Few drops of Metal chloride were another to the miscroscopic quantity of the filtrate, development of inexperienced colour discovered presence of tannins.

2.3.f. detection of Phytosterols

a. Salkowski reaction: A tiny low quantity of extract was another with 2 ml H_2SO_4 and was mixed well, the event of red or brown colour indicates the presence of sterols.

2.3.g.Test for Detection of Phenoplast Compounds

A tiny low quantity of extract was treated with a pair of millilitre of metal chloride. The looks of pale brown colour to the take a look at indicates presence of phenoplast compounds.

2.3.h. Look at for Saponins

a. Foam test: A tiny low quantity of extract was treated with 2ml of Sodium bi-carbonate and another with H_2O, the mixture jolted smartly. The event of froth to the take a look at indicates presence of saponins.

Fig. 1: Tulsi leaf and Methanol extract of Tulsi leaf

3. Result and Discussion

3.1 Evaluation of the antibacterial activity of Tulsi (Ocinum tenuiflorum)

Zones of inhibition displayed by Tulsi extract (at totally different concentrations), controls against E. coli, S. aureus, P. aeruginosa, S. typhi area unit compiled in Table 1. The smallest amount zones of inhibition were displayed by the management and exhibited the widest zones of inhibition against all the bacterium. Tulsi leaves' extract showed increasing zones of inhibition with increasing concentration against all the bacterium. Mean zone of inhibition for every concentration and every bacteria was calculated for analysis. All the concentrations of Tulsi and fuel showed marginal repressing zones against E. coli and S. typhi. Tulsi leaves extract

at a level of 1 %, 3%, 5%, 7 % showed a marginal zone of inhibition. However, Tulsi displayed wide inhibition zones at 9% and 10 percent. All the bacterium tested showed condition to leaf extract explained by the wide inhibition zones displayed (Table 1). Unidirectional multivariate analysis disclosed a rise within the mean zones of inhibition against all the bacterium with increasing concentration of Tulsi, that was statistically important ($P < 0.001$). Though several previous studies like those conducted by Agarwal et al., Rathod, Shah of Iran et al. and Prasannabalaji have all shown the antimicrobial properties of Tulsi against totally different organism

Table 1: Antibacterial Activity of Tulsi

Sr. No.	Conc. of plant extract	1%	3%	5%	7%	9%	10%
		Zone of Inhibition (mm)					
1	E. coli	-	9.5	11	12.5	12.5	14
2	S. aureus	9	12	13.5	14	17.5	19
3	P. aereuginosa	10.5	13	15.5	16	18	21.5
4	S. typhi	-	8	10.5	12	13.5	15
5	Methanol (Control)	9	12	12	15	17	19

3.2 Phytochemical Analysis

The alkaloids, phenoplast compounds, saponin, tannin and phytosterols were gift during a leaf extract. The healthful values of the secondary metabolites area unit thanks to the presence of chemical substances that created by the plant extracts against specific organism manufacture an explicit physiological action on the body. (Susmitha et al., 2013).

Table No. 2: Phytochemical analysis of Tulsi Leaf Extract

Sr. no	Phytochemical tests	Name of test	Extracts of Tulsi	
			Methanol	Water
1	Alkaloides	Dragner's Test	+	+
		Wagoner's Test	+	+
2	Glycolides	Benedict's test	-	-
		Fehling's test	-	-
3	Reducing sugar test	Benedict's test	-	-
		Fehling's test	-	-
4	Tannin	Lead acetate Test	+	+

		Ferric Chloride test	+	+
5	Phenolic Compounds	Phenolic Comound Test	+	+
6	Phytosterol	Salkowksi Test	+	++
7	Saponine	Foam Test	+	+
8	Protein	Ninhydrin test	-	--
		Biuret test	-	-

3. Conclusion

In summary, the extracted from genus Ocimum tenuiflorum showed antimicrobial activity against S. aureus (including MRSA) and P. aeruginosa, however was less active against E. coli and S. typhi.

Modern day research project into Tulsi demonstrates the numerous psychological and physiological edges from overwhelming Tulsi and provides a testament to the knowledge inherent in Hinduism and written material, that celebrates Tulsi as a plant which will be loved, ingested, created into tea and used for healthful and religious functions at intervals standard of living. In providing a spotlight for moral, property and ecological farming practices that gives a resource for thousands of farmers, the cultivation of Tulsi goes on the far side providing edges for people and households and begins to handle broader social, economic and environmental problems.

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12. To Evaluate Antibacterial Activity against Pathogens and Phytochemical Screening of Turmeric *(Curcuma Longa)*

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Abstract

The ethanolic extract of Turmeric (Curcuma longa) was subjected to microbial susceptibility test using the agar well diffusion method. The extract was found to be active against the bacteria used as it inhibited all the organisms with the least inhibition zone of 7. 2 mm recorded against Pseudomonas aeruginosa and the highest inhibition of 17.2 mm recorded against Staphylococcus aerues. The ability of rhizome of C. longa extracts to inhibit the growth of test pathogen is an indication of its broad spectrum antimicrobial potential which may be employed in the management of microbial infections

The presence of these phytochemicals like tannins, alkaloids, phenols, steroids, flavonoids, glycosides, saponin, etc; is indicative of the antimicrobial activity of Turmeric. The results so far obtained are indicative of the good medicinal value of Turmeric in both pharmaceutical and pharmacological formulations.

Keywords - Turmeric, Phytochemicals, Antibacterial Sensitivity, Ethanolic Extract, Pathogens.

1. Introduction

Medicinal plants have provided a reliable source for preparation of new drugs as well as combating diseases, from the time of civilization. The extensive survey of the literature revealed that Curcuma longa L. or turmeric (from Zingiberaceae family) is highly regarded as a universal panacea in the herbal medicine with a wide spectrum of pharmacological activities. Turmeric is a plant distributed throughout tropical and subtropical regions of the world. It is widely cultivated

In Asian countries, mainly in China and India. Turmeric is an essential spice all over the world with a distinguished human use (Ammon HP. et al., 1992) . Apart from the uses as spice, it is used as traditional medicine in Asian countries such as India, Bangladesh and Pakistan because of its beneficial properties (Wahl MA. et al., 1991) It is called turmeric (Zarchooveh in Iran) and has been used for its flavoring, and medicinal properties (Bernard GaT, et al., 1982) . Current traditional medicine claims that its powder is used against gastrointestinal diseases, especially for biliary and hepatic disorder, diabetic wounds, rheumatism, inflammation sinusitis, and cough (Chattopadhyay aI. et al., 2004) The coloring agent of turmeric is called curcumin, which has yellow coloration and is the essential component of this plant . Recent studies have proven turmeric as anticancer, anti-diabetic, antioxidant, anti-inflammatory, antimicrobial, anti-fertility, antivenom, hepatoprotective, nephroprotective, anticoagulant, etc. The plant has also shown to possess anti HIV activity to combat AIDS. These medicinal properties of turmeric caused it to be considered as a spice with multifunctional medicinal properties.

India produces nearly all of the world's turmeric crop and consumes 80% of it. With its inherent qualities and high content of the important bioactive compound curcumin, Indian turmeric is considered to be the best in the world The South Indian state of Tamil Nadu, is the world's largest producer of and the most important trading center for turmeric. It is also known as "Yellow City," "Turmeric City," or "Textile City." Sangli, a city of Maharashtra, is second only to Erode in size and importance as a production and trading site for turmeric. Before turmeric can be used, the turmeric rhizomes must be processed. Rhizomes are boiled or steamed to remove the raw odor, gelatinize the starch, and produce a more uniformly colored product.

The objectives of this study was to evaluate the antimicrobial activity of the extracts from turmeric (C. longa L.) against common pathogens.

2. Material and Methods

2.1. Plant Material and Extraction

The rootstalk of C. longa (turmeric) was purchased from market and 25 gm of dry powder was packed in Soxhlet equipment for extraction of individual soluble bioactive molecules from the rootstalk by the employment of various solvent (methanol and water). Fractions containing volatile solvents, were focused with the assistance of rotary evaporator (rota vapor) beneath cut back pressure. The focused extract was blank to sterilized aggregation tube. Fig.1 has shown the focused extract of turmeric.

Fig. 1: Concentrated extract of Turmeric

2.2. Test Microorganisms

Prior to sensitivity testing, Escherichia coli, Pseudomonas aeruginosa, staphylococci aerues and Salmonella typhi strains were civilised onto culture medium and incubated for twenty-four h at 37°C. one colony was then civilised in five metric capacity unit Nutrient Broth for four h at thirty seven °C. The density of bacterium culture needed for the take a look at was adjusted to 0.5 McFarland customary, (1.0 x 108 CFU/ml) measured mistreatment the Turbidometer.

2.3. Phytochemical analysis of the plant extract

Preliminary phytochemical screening of plant was done following the quality procedures custom-made by the assorted staff (J.B. Harborne, 1998., C.K. Kokate., et al., 2004). The extracts were subjected to phytochemical tests for determination of plant secondary metabolites like tannins, saponins, steroid, alkaloids and glycosides in accordance with C.K. Kokate., et al., 2004.

2.4. Medicinal drug activity

Antibacterial activity was tested by agar well diffusion methodology wherever totally different concentrations of designated plant extract were used. The organisms were planted in sterile Petri plates mistreatment medium by softly commixture of 0.1 ml of the 24h recent cultures alone with thirty five metric capacity unit sterile liquid agar. seven millimetre diameter wells were created mistreatment sterile borer once Harding the agar of Petri plates. All wells were crammed with 0.1 ml of extract then incubated at 37 °C for 24 h. The diameter of the inhibition zone around of every well was measured to observe the medicinal drug activity. The experiment was wiped out triplicate and mean diameter of inhibition zones were recorded.

2.5 ANOVA analysis by MINITAB 14.

3. Results and Discussion

3.1. Phytochemical Analysis of Curcuma Longa Extract

Phytochemical analysis of C. longa extract showing antimicrobial activity unconcealed the presence of various active constituents in numerous extracts (Table 1). Turmeric extract contained alkaloids, tannin, flavonoid, organic compound and saccharide. There are reports showing that alkaloids and flavonoids are the accountable compounds for the medicinal drug activities in higher plants (G.A. Cordell, 2001).

Table No. 1 : Phytochemical Analysis of Curcuma Longa Extract

Sr. no	Phytochemical tests	Name of test	Various extracts of Neem	
			Methanol	Water
1	Alkaloides	Dragner's Test	+	-
		Wagoner's Test	+	-
2	Glycolides	Benedict's test	+	+
		Fehling's test	+	+
3	Reducing sugar test	Benedict's test	+	+
		Fehling's test	+	+
4	Tannin	Lead acetate Test	+	+
		Ferric Chloride test	+	+
5	Phenolic Compounds	Phenolic Comound Test	+	+
6	Phytosterol	Salkowksi Test	+	++
7	Saponine	Foam Test	+	+
8	Protein	Ninhydrin test	-	--
		Biuret test	-	-

3.2. Antibacterial Activity of Curcuma Longa Extracts

Antimicrobial status tests of various fractions of C. longa rootstalk extract against the pathogens. The extract was found to move against the bacterium used because it pent-up all the organisms with the smallest amount inhibition zone of 7 mm recorded against Pseudomonas aeruginosa and also the highest inhibition of 17.2 mm recorded against Staphylococci aerues (Table no. 2). S. aureus ATCC 6571 and clinical isolates show that each one fractions of C. longa rootstalk are extremely active against customary and clinical isolates of S. aureus showing zone of inhibition ranges between 9 mm and 21 mm that was kind of like the study of Negi et al. (1999). World Health Organization according the repressing effects of alcohol and methane series extract of turmeric against S. aureus. any observations unconcealed that water (aquous) extract was least active showing zone of inhibition of concerning 11.5 mm at the concentration mg/ml whereas methanolic extract was most active showing zone of inhibition of concerning 13 at the concentration of 50 mg/ml. whereas repressing activity of all different fractions ranges

between the two. Similar observations has been according for species like C. longa, genus Curcuma zedoaria, aromatic and Curcuma amada from the study of (T. Yoshioka et al., 1998, A.M. Majumdar, et al., 2000). All information analysed as a statistically important (p value < 0.05) by ANOVA.

Table No. 2: Antibacterial Activity of different Extract of Turmeric Against Pathogens

	Methanol			Aqueous		
	50 mg/ml	100 mg/ml	200 mg/ml	50 mg/ml	100 mg/ml	200 mg/ml
S. aureus	13	15.2	17.5	11.5	-	-
E. coli	9.7	11	13.5	12	-	-
S. typhi	12.5	14	15.5	11	12.5	-
P. aeruginosa	-	-	7.2	-	-	-

4. Conclusion

Turmeric is that the vital supply of assorted varieties of chemical compounds, that ar answerable for several plant dependent activities. Although, tons of experiments are done on turmeric, however, additional investigations are required to take advantage of different therapeutic utility to combat diseases. A drug development programme ought to be conducted to develop fashionable medicine though crude extracts from leaves or rhizomes of the plant have medicative applications, fashionable medicine may be developed once intensive investigation of its pharmaco medicine, bioactivity, mechanism of action, and toxicities, once correct standardization and clinical trials, because the world state of affairs is currently ever-changing towards the employment of non-toxic plant product having ancient medicative use, development of recent medicine from C. longa ought to be emphasised for the management of assorted diseases. any analysis has to be dole out on C. longa so as to explore the hid areas and their sensible clinical applications, which might be used for the welfare of human beings.

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13. To Determine Antibacterial Activity of Neem *(Azadirachta Indica)* Against Pathogens

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Abstract

Neem (Azadirachta indica) is wide employed in the Ayurvedic drugs system for treating protozoal infection and fever. the current study was undertaken to organize crude extracts of Melia Azadirachta leaves with completely different polarity organic solvents by employing a maceration methodology and assessing phytochemical screening, the whole phenol content by the qualitative analysis methodology. The leaf samples collected from the near garden region. Organic chemistry screening was resolute by established strategies. The whole phenol content and antioxidant activity were assessed by standard Folin–Ciocalteu chemical agent (FCR) strategies. Phytochemical screening of the crude extracts of Melia Azadirachta leaves unconcealed positive results of flavonoids, saponins, steroids, alkaloids and tannins. However, reducing sugars, glycosides and proteins weren't detected within the crude extracts of Azadirachta leaves. Azadirachta extract has shown the medicinal drug activity against pathogens.

Keywords - Neem, phytochemical screening, Total phenol, UV–visible qualitative analysis.

1. Introduction

Neem (Azadirachta indica) is associate evergreen tree that belongs to the family Meliaceae family and is found throughout the globe (P. Sithisarn and W. Gritsanapan, 2011). Its English name is Melia Azadirachta, and its Arabic name is Al Shurisha. Melia Azadirachta could be a massive tree that's about twenty five meters tall with a semi straight trunk. It's a spermatophyte and commonly starts mature when 3–5 years. The tree becomes productive among ten years .(B. Annadurai, et al., 2012). The bark of this tree is gray and rough. The leaves

area unit up to thirty centimeters long. Every leaf has 10–12 rough leaf lets that area unit seven centimeters long by 2.5 centimeters wide. All elements of the chosen plant area unit used as drugs for the treatment of the many diseases and diseases. Historically, the leaves and their paste area unit used for hardening allergic skin reactions and antiviral treating pox and varicella (K.H. Khine., et al., 2013). Most urban Nepalese, Indian and Bangladeshi use Melia Azadirachta twigs to scrub their teeth. The juice from the leaves is employed as a tonic to extend appetence and to get rid of enteric worms (A.G, Kumar, et al., 2009). It's additionally used for its hypoglycemic, hypolipidemic, hepatoprotective and hypotensive activities and to manage fever (A. Bag, et al., 2011). Therapeutically, the leaf extract is employed for its antimicrobial activity against dental pathogens (I Khan, et al.,2010).

A literature survey unconcealed only a few publications on medicinal drug activity and phytochemical screening Melia Azadirachta species. Therefore, there the current study was conducted on antibacterial activity against pathogens and phytochemical screening of various crude extracts of the chosen plant.

2. Material and Methodology

2.1 Preparation of plant extract

The plant extract was ready in line with Odey et al., (2012). Briefly, the healthful plants when assortment were completely washed to get rid of scrap and therefore the earth remains. From these usable elements of the plant were separated and cut into bits and allowed to dry underneath shade. The dried sample then mingling for creating them into powder from by electrical liquidizer and preserved in airtight instrumentation. 100g of every powder was weighed victimization associate electronic measurement balance. Every powder was otherwise soaked in 400ml of ethyl group alcohol at a quantitative relation of 1:4 powder /solvent and was agitated victimization an electrical liquidizer at the moment each mingling mixture was poured into associate airtight plastic instrumentation and unbroken within the icebox at four degree Centigrade for forty eight hour. The mixture then initial filtered by material so filter by whatman no. one paper. The filter solutions were hold on in instrumentation and preserved in icebox for detection of antimicrobial activity.

2.2 Screening for Antimicrobial Activity

Antimicrobial activity was tested by agar well diffusion methodology wherever completely different concentrations by elite plant extract were to used . The organisms were

planted during a sterile petri plate victimization medium by softly combination 0.1 ml of the 24h recent cultures alone with 20ml sterile liquid agar wells were stuffed with 0.1 ml of extract so incubated at 37 degree Centigrade for twenty-four hours. The diameter of the inhibition zone around every well was measured to find the antibacterial activity. The experiment was carried triplicate and mean diameter of inhibition zones were recorded .

2.3 Baacterium and Bacterium Culture

Prior to sensitivity testing, E.coli and S.aureus strains were civilized into EMB and Mannitol Salt Agar severally and incubated for 24 h at 37 degree Celsius. One colony was then civilized in 5ml Nutrient Broth for 4 h at 37degree Celsius. The density of microorganism culture needed for the check was adjusted to 0.5 McFarland customary, (1.0 X108 CFU/ml) measured victimization the Turbidometer.

3.4 Confirm Phytochemical Activity

Qualitative Phytochemical Analysis

All extracts of Neem tree leaves were subjected to qualitative phytochemical analysis to spot presence of varied phytochemicals viz. alkaloids, glycosides, proteins, reducing sugar, tannins, resins, sterols, synthetic resin compounds and saponins as per the tactic delineate by Rosenthaler (1930).

3.4.a Check for Detection of Alkaloids: A tiny low quantity of extract was taken in tube and further with 5ml of 0.5 N HCl (v/v) so filtered Some drops of every of the subsequent reagents were further to the filtrate and mixed well, look of turbidness or any changes in color to the check indicates the presence of alkaloids.

Wagner's chemical agent test: The chemical agent was ready by dissolving iodine 1.27 gm and 2 gm of iodide in 5ml of H2O and diluted to a 100ml. The tiny quantity of the higher than extract (filtrate) was further to the present chemical agent, look of brown to woolly precipitation unconcealed the presence of organic compound.

3.4.b.Test for Detection of Glycoside

a. Benedict's chemical agent test: Equal amount of each the extract and benedicts chemical agent was further and heated to boil for 2 minutes, look of dark-brown to red color indicate presence of organic compound.

b. Folin Chinese copper chemical agent test: a bit quantity of extract was further to few drops of folin Chinese copper chemical agent, the event of red color provides positive reaction for organic compound.

3.4.b.Test for Detection of Glycoside

a. Benedict's reagent test: Equal quantity of both the extract and benedicts reagent was added and heated to boil for two minutes, appearance of brownish to red colour indicate presence of glycoside.

b. Folin Wu copper reagent test: A little amount of extract was added to few drops of folin Wu copper reagent, the development of red colour gives positive reaction for glycoside. 3. Test for

3.4.c Detection of Proteins

a. Biuret test: Few amounts of the extracts were added to 4% sodium hydroxide solution followed by a drop of 1% copper sulphate solution, the development of violet to pink colour indicates presence of proteins.

3.4.d Test for Detection of Reducing Sugar

a. Benedict's reagent test: The extract was added with benedicts reagent in equal amount and mixture was heated for 2 minutes, appearance of brown to red colour indicates presence of reducing sugar.

b. Foiln copper reagent test: Few quantities of the extract were added with few drops of folin Wu copper reagent, the development of red colour indicates presence of reducing sugar. 5. Test for

3.4.e Detection of Tannins

A little quantity of alcohol extract taken in a test tube was warmed and filtered. The filtrate was used to carry out the tests.

a. Lead acetate test: Few drops of 5% lead acetate solution were added to the filtrate, formation of precipitation indicates the presence of tannins.

b. Ferric chloride test: Few drops of ferric chloride were added to the little amount of the filtrate, development of green color revealed presence of tannins.

3.4.f. Test for Detection of Phytosterols

a. Salkowski reaction: A small amount of extract was added with 2 ml of concentrated H2SO4 and was shaken for few minutes and mixed well, the development of red or brown colour indicates the presence of sterols.

3.4.g.Test for Detection of Phenolic Compounds

A small amount of extract was treated with 2 ml of ferric chloride solution and shaken for few minutes. The appearance of pale brown colour to the test indicates presence of phenolic compounds.

3.4.h. Test for Saponins

a. Foam test: A small amount of extract was treated with 2ml of sodium bi-carbonate and added with distilled water, the mixture shaken vigorously. The development of froth to the test indicates presence of saponins.

3. Result and Discussion

3.1 Evolution of the Antibacterial of Neem (Azadirachta indica).

The medicine result of Neem tree on E.coli, S. aureus, P. aeruginosa and B. subtilis has been represented in table 1. We tend to used four completely different concentration of plant extract for bacterium to live the inhibition zone within the media. From the table we tend to found that the various concentration of Azadirachta indica extract has shown the medicine activity against all pathogens. The antimicrobial activity of Azadirachta indica has been rumored in previous studies. It absolutely was found that the Azadirachta indica has restrictive result against P. aeruginosa , Aeromonas hydrophilateria, Monocytogenes and Salmonella typhimurium DT104 and penicillin - resistant S.aureus. During this study, the results showed antibacterial activity of Azadirachta indica fermentation alcohol extract against E.coli and S. aureus . The result from this study could support the antimicrobial activity and somehow the confirmation of antimicrobial activity of Azadirachta indica. Moreover, it could support the used of Azadirachta indica for antimicrobial treatment sickness or hindrance of bacterium growth .

Table No. 1: Evaluation of Antibacterial Activity of Neem

Conc.of Plant Extract	E.coli	Staphylococcus aureus	Pseudomonas.aregunosa	S.typhi
10ml	10 mm	12.5 mm	7.5 mm	7.8mm

20ml	12.5 mm	14 mm	8.6mm	9.5 mm
30ml	14 mm	16 mm	9 mm	8.3 mm
40ml	17 mm	18.5 mm	12.5 mm	11 mm

3.2 Phytochemical Analysis - The Alkoloides, Phenolic Copmpouds, Saponin, Tannin and Phytosterol Were Present in Plant Extract.

Table No. 2: Phytochemical Analysis of Neem (Azadirachita Indica)

Sr. no	**Phytochemical tests**	**Name of test**	**Various extracts of Neem**	
			Methanol	Water
1	Alkaloides	Dragner's Test	+	+
		Wagoner's Test	+	+
2	Glycolides	Benedict's test	-	-
		Fehling's test	-	-
3	Reducing sugar test	Benedict's test	-	-
		Fehling's test	-	-
4	Tannin	Lead acetate Test	+	+
		Ferric Chloride test	+	+
5	Phenolic Compounds	Phenolic Comound Test	+	+
6	Phytosterol	Salkowksi Test	+	++
7	Saponine	Foam Test	+	+
8	Protein	Ninhydrin test	-	--
		Biuret test	-	-

The results showed that alkaloids, tannins, phytosterols and saponins were detected all extracts of A. indica leaves. Phytochemical compounds were detected in Azadirachta indica leaves (methanol) and extract of Azadirachta indica leaves (Distilled water), whereas not detected in Azadirachta (Distilled water) and liquid extract of A. indica. Glycosides, proteins and reducing sugars weren't detected all told extracts of A. indica. The healthful values of the secondary metabolites thanks to the presence of chemical substances that made by the plant

extracts against explicit organism manufacture a certain physiological action on the body. (Susmitha et al., 2013). The phytochemicals exhibit varied pharmacologic and organic chemistry action once eaten by animals. The plant bioactivity depends on chemical compounds which can inhibit insect feeding. Agro ecosystems square measure a crucial system for secondary plant metabolites and their degradation product. hepatotoxic effects to insects/pests square measure made by the compounds viz. terpenoids and steroids, phenols, coumarins, flavonoids, tannins, alkaloids, and cyanogenetic glycosides (Ogbonna et al.,) Alkaloids organic element substances (alkaline in nature) having outstanding physical and medicine properties like stimulant, medicine, vasodilative, antiasthmatic, anti-arrhythmic etc. (Kumar R, et al., 2014). The presence of alkaloids represents the chance of some biological activity of the extracts of A. indica like anti-cholinergic, anti-tumour, anti-hypertensive, cough medicinal drug, anaesthetic, analgesic, relaxant, anti-pyretic,anti-malarial (Emran TB, et al., 2015). Aqueous leaf extract of A. indica showed the presence of alkaloids, saponins that have nematocidal properties (Muhammad , et al., 2017).

4. Conclusion

In the study, analysis of the phytochemical activity and total phenol content showed that principally the wood alcohol crude extracts of the chosen plant square measure potent sources of natural antioxidants. Therefore, the chosen crude extracts are often used as a natural inhibitor rather than an artificial inhibitor. Any studies square measure designed for the isolation and identification of individual phytochemicals compounds; conjointly, in vivo studies required to raised perceive their mechanism of action as associate inhibitor.

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14. Scale UP Studies on Production of Bacteriocin from Marine Mutant Strain of *Lactobacillus Pentosus B25*

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Abstract

The bacteriocin concentration was inflated and viability of the cells of mutant strain of Lactobacillus pentosus B25 was maintained until the tip of the fermentation. However, the commercial application of the batch production of the bacteriocin is treatment batch fermentation has worked in an exceedingly commercial level. In batch fermentation, Sugar consumption was equally initiated once 9 h and led to the biogenesis of lactic acid, that obtained up to 1.14 % at the tip of fermentation. The pH scale was ablated because the lactic acid concentration inflated. The utmost biomass and bacteriocin production reached 4.65 gm/500 ml (9.3 g/L) and 3440 AU/ml severally once 18 h, corresponding the cell viability obtained 2.128 OD at 620 nm. Considering the high yield and stability of the bacteriocins even within the batch fermentations, it's essential to rescale the method as endless system which may offer far better yield of bacteriocin in future.

Keywords - Lactobacillus pentosus B25, Batch fermentation, scale up studies, Klebsiella pneumoniae 535.

1. Introduction

The demand for foods with minimum process or foods while not chemical preservatives is quick gaining momentum visible of accelerating issues on health. It's documented that varied unhealthful and spoilage microorganisms, already gift in foods might survive and multiply once there's negligible process and/or within the absence of preservatives. During this respect natural

bio preservatives like bacteriocins that are non-detrimental to human health, have surfaced as an alternate to process and therefore the use of chemical preservatives in foods.

The use of bacteriocin and therefore the producer strain are of explicit interest to the food business since they'll facilitate to confirm the microbic safety of the food merchandise. The limiting consider mistreatment bacteriocin as food preservatives is their low yield throughout production (Bertrand et al., 2001). Since high biomass density well-tried to be helpful for bacteriocin production (Parente and Ricciardi, 1988), it's seemingly that bacteriocin production can be increased in an exceedingly system with high cell density. Acceptable exploitation of each cell biomass and bacteriocin may result in more improvement of the profit of the fermentation method.

According to Meng and Pinglan, (2013) Pentocin 31-1 was made by eubacteria pentosus 31-1, isolated from the standard Chinese soured Xuan-Wei Ham. The study of pentocin 31-1 production, that they had allotted in perennial batch cell recycle fermentations in five L bioreactor and up to fifty L pilot bioreactor for bacteriocin production. Batch fermentation of L. pentosus 31-1 gave bacteriocin production and productivity of 1395.3 IU/ml and 66.4 IU/(mL/h), severally. The bacteriocin production (2186.2 and 2004.5 IU/ml, respectively) and productivity (260.7 and 237.4 IU/(ml/ h), respectively) was measured in perennial batch cell recycle fermentations in five and fifty L bioreactors, that were corresponding to 1.48-and 1.8-fold will increase compared with the biomass (3.2 g/l) obtained from batch fermentation. His results prompt that an inexpensive resolution to develop large-scale production of each pentocin 31-1 and L. pentosus 31-1 in food business.

In this studies, centered on up the assembly of bacteriocin by scale up studies. Batch fermentation that was developed to enhance each biomass and bacteriocin production in changed MRS medium (mMRS) by mistreatment eubacteria pentosus B25. In rescale studies production changes as per rising size from (50 ml to 2000 ml).

2. Materials and Methods

2.a. Batch Fermentation

2.A . I Bacterial Strains, Maintenance, Inoculum Preparation, and Media

Strain of L. pentosus B25 was used because the producer strain of the bacteriocin. K.pneumoniae 535 was used as a sensitive indicator organism for detection of bacteriocin activity. The strains were hold on at −80°C in MRS medium (HiMedia, Mumbai, India)

containing 2 hundredth (vol/vol) alcohol. Cultures were propagated double in MRS medium (HiMedia; initial pH scale half dozen.5, 24h, 37°C) before use because the matter two (V/V) for the fermentation experiments. Fermentations were allotted in changed MRS medium adjusted to pH scale 7.0 (i.e., MRS medium containing completely different concentrations of carbon and complicated chemical element supply. Medium was sterilized by being heated at 110°C for twenty min (Meng and Pinglan, 2013).

2.a. ii. Fermentation Experiments

A 1000 ml flask was used for this study. The flask was full of 500ml of mMRS (Modified MRS) medium, and inoculated with two (v/v) L. pentosus B25. Fermentation was allotted at a temperature of 300C and constant pH scale of 7.0, about 48 h. The 10 ml sample was withdrawn aseptically from fermentation medium at a daily measure of 2 h throughout the cultivation; bacteriocin activity, Cell viability, bio-mass, macromolecule concentration, sugar consumption, lactic acid production and pH scale were analysed. Fermentations were allotted in duplicate.

2.a. iii. Analysis of Sample

10 ml of aliquot of the culture broth was aseptically withdrawn from the flask at intervals throughout fermentation and used for assessment of following analysis.

- **A.** Optical Density Determination - The optical density was measured by mistreatment photometer (Single Beam UV-Visible photometer, BioEra) at 620 nm.
- **B.** Biomass Determination - Once sampling, 10 ml of sample was centrifuged at 12,000 revolutions per minute for 10 min. at 40C. The pellet was washed and freeze dried. Tubes were weighed once freeze drying.
- **C.** pH Scale Determination - The pH scale of medium was firm mistreatment pH scale probe (Lab Serv, AcuStar+ V, pH/ physical phenomenon Meter).
- **D.** Antibacterial activity was done by agar well technique. -
- **E.** Estimation of protein concentration by Folin's Lowry technique.
- **F.** Estimation of reducing sugar concentration by DNSA technique (Sengupta et al., 2006).
- **G.** Estimation of Lactic acid concentration by acidimetrically volumetric analysis with 0.1 N NaOH to the purpose.

2.a(iv) Statistics Analysis

Each experiment was carried double and every determination was worn out duplicate. The information were examined by analysis of variance (ANOVA) using Minitab 14 at grade of significance of $p < 0.05$. Graphs of the fermentations were given.

2.B. Scale Up Studies on the Assembly

Scale-up of the procedure for the assembly of bacteriocin B25 was done on laboratory level. For the optimized medium as higher than, was ready in (50ml , 100 ml, 300 ml, 500 ml, 700 ml, 1000 ml, 1500 ml and 2000 ml) inoculated with improved culture (2% matter volume) and incubated at 300C for 18h. After incubation check the bacteriocin production. The data were examined by paired t-test using MINITAB 14.

3. Result and Discussion

Scale-up studies on Bacteriocin production by Batch Fermentation

3.a Batch Fermentation

Figure 1 and 2, shows the fermentation profile of improved (mutant) strain of true L. pentosus B25 with batch fermentation in 500 ml fermentation medium viz., changed MRS (mMRS). Throughout the primary 6 h of cultivation, the biomass will increase up to 1.7 gm/500ml (3.4 g/L) and so the culture passed to the exponential section. Most of the bacteriocin was accumulated within the exponential section and stable up to the tip of stationary section. Sugar consumption was equally initiated when 9 h and production of Lactic acid, that obtained up to 1.14 % at the tip of fermentation. The hydrogen ion concentration was weakened because the carboxylic acid concentration augmented. The utmost biomass and bacteriocin production reached 4.65 gm/500ml (9.3 g/L) and 3440 AU/ml severally when 18 h, corresponding the cell viability obtained 0.128 OD at 620 nm.

All comparison were supported the quality deviation of the mean (SD). Information were analysed in a technique analysis of Variance (ANOVA), result were thought of important once p values were < 0.05.

Similar work performed by user interface and Li, (2013) pentocin production by L.pentosus, in line with his result the assembly augmented apace when exponential biomass growth started, bacteriocin activity reached their most level when 21 h of fermentation and failed to decline phase. The decrease of bacteriocin could also be attribute to associate in nursing interesting producers cells or degradation by specific proteolytic enzyme and also the former bacteriocin has been established by most bacteriocins (Maghrous et al., 1992; Parente et al.,

1994). In our result, sugar consumption happens sharply once bacteriocin and lactic acid created within the medium in batch fermentation that indicates largely sugar was accustomed support for production. equally Zannini according the strains L. plantarum DiSA 33 and bacteria L. casei DiSA 27 completed the use of protein supply, whereas the very best values of residual (>50% of the original) at the tip of fermentation. Fed-batch fermentation was dispensed as a result of it absolutely was believe that it might take away lactic acid and sustain the productivity of batch operation.

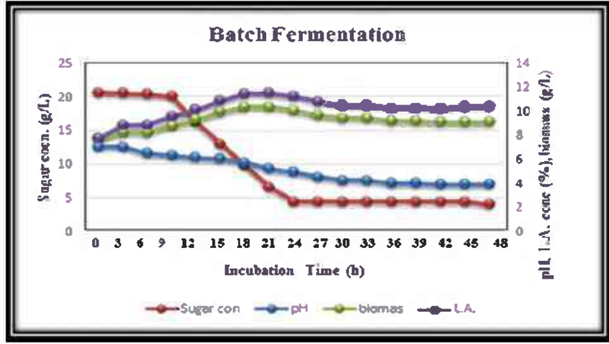

Fig. 1: Batch Fermentation Profile Contain Reducing Sugar Concentration, Ph, Biomass and Lactic Acid Concentration

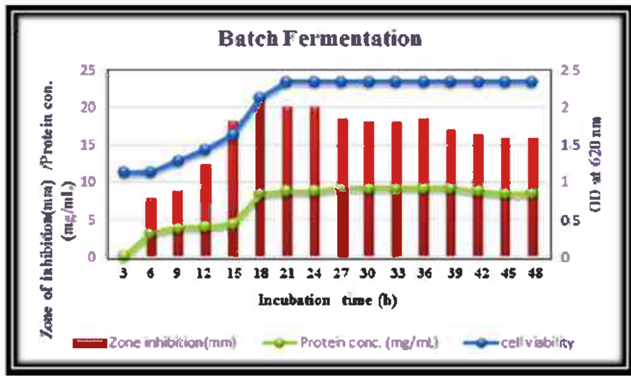

Fig. 2: Batch Fermentation Profile Contain Antibacterial Activity, Protein Concentration and Cell Viability

3.c Scale-UPStudies

Table No. 1: Scale UP Study of Bacteriocin Production

Sr. No	Production medium volume (ml)	AU/mL
1	50	4640
2	100	4650

3	300	4640
4	500	4640
5	700	4640
6	1000	4642
7	1500	4643
8	2000	4640

According to Lancini, (1993), the difference of method studied in flasks or tiny fermenters up to industrial level may be a branch of the comparatively recent discipline of technology. Also, Essam et al., (2013) explicit that enlarging the assembly scale is sometimes related to reduction thanks to complexness of fermentation method. Therefore, it's an excellent Therefore, it's an

importance to review the scaling-up of the fermentation method and adopt appropriate strategy so as to extend the productivity of desired product on the commercial level. Antibacterial activity and macromolecule concentration it absolutely was noted that the activity was virtually same within the flasks has shown within the table one (Wadekar and Dharmadhikari, 2021). All the info valid by paired t-test as important. Photoplate one has shown the medicinal drug activity of various volume created bacteriocin against microorganism.

Photoplate 1: Antibacterial Activity against Pathogen

3. Conclusion

From the results of the batch fermentation on bacteriocin production, it's over that the bacteriocin concentration was augmented and viability of the cells was maintained until the tip of the fermentation when put next with the free cell fermentations. Considering the high yield and stability of the bacteriocins even within the batch fermentations, it's essential to proportion the method as a nonstop system which may offer far better yield of bacteriocin in future.

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15. Isolation of Enterococcus Sp. from Different Milk Samples

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Abstract

The goal of this research was to isolate Enterococcus spp. from milk samples of cow and vaginal. Enterococcus spp was identified by morphological, biochemical tests. This was accomplished by the collection of 5 samples of milk growth in de Man rogosha Sharp, Bile esculine Agar and incubated aerobically for 24 hours at 370C. In the present study LAB, was isolated from different Milk samples. Antibiotic sensitivity testing was performed on some isolates that showed high resistance to (vancomycin, pencillin, Ofloxacin, ciprofloxacin, nitrofurantoin, tetracycline, and amikacin). The percentage of antibiotic resistant was 100% in amikacin, nitrofurantoin, and tetracycline for all isolates isolated from dairy milk samples.

1. Introduction

Enterococci are common bacteria found in the gastrointestinal tract, and were found to be non motile and catalase negative. Because of their high flexibility and resilience to unfavourable environmental circumstances, Enterococcus spp. are one of the most frequent bacteria in a variety of environments. They've been isolated from a variety of natural sources, including soil, water, plants, and mammals' gastrointestinal (GI) tracts (Franz et al., 1999). They are also an important component for many naturally fermented, traditional kinds of cheese, where they aid in the fermentation and ripening process (Franz, Holzapfel, and Stiles 1999; Giraffa 2002). They are responsible for the particular characteristics of certain dairy products. And since they are naturally present in the gut of humans, Enterococci tends to have a beneficial effect on the health of consumers and sometimes, they act as probiotic bacteria. (Franz et al., 1999)

The growth of bacterial cells within dairy products is highly influenced by parameters such as water activity, pH and salt concentration levels as well as temperature. Microorganisms are present in milk and dairy products, which vary depending on the variety of products obtainable in the dairy market. Endogenous contamination of milk and dairy products by pathogenic bacteria can occur after discharge from an affected animal's udder. Exogenous contamination can occur through direct contact with sick cattle or via the surrounding (e.g., water, handling personnel). Milk is an ideal source for the growth of organisms. In addition, organisms can grow on equipment because of their ability to withstand adverse conditions. It may, therefore, account for their increased incidence in dairy products. Enterococci can multiply during milk chilling and survive after pasteurisation due to their cryophile nature, heat resilience, and flexibility to various substrates and growth circumstances. As a result, Enterococci can be found in both raw and pasteurised milk microbiota. Different enterococci species can be detected in dairy products, however E. faecalis and E. faecium are the most important (Gelsomino et al., 2001).

The goal of this research was to isolate Enterococcus spp. from different milk samples and identification using the conventional method and determine the antibiotic sensitivity for Enterococcus spp.

2. Material and methods

2.1 Collection of Samples

5 milk samples of different types were collected from different local markets in the city and cultured in the frame of this study.

- Sample 1 was collected from local dairy,
- Sample 2 was collected from a local tea shop ,
- Sample 3 was collected from an Amul packet ,
- Sample 4 was collected from Mother dairy and
- Sample 5 was collected from Gokul milk .

2.2 Isolation and Enrichment of Samples

All samples was inoculated into de Man Rogosha Sharp Agar, Bile Esculin Agar and incubated aerobically at 37 °C for 24 hours , the growing colonies examined physically by naked eye according to colour, size and shape etc.

2.3 Identification of Microorganism by Gram Nature and Biochemical Technique

Biochemical tests were conducted according to (Quinn PJ., et al., 2001) such as catalase, oxidase and sugar fermentation.

3. Result and Discussion

3.1 Isolation and Enrichment of Milk Samples

Isolation of Enterococcus spp. from dairy milk samples. Out of 5 milk samples, isolation of Enterococcus spp was 41 isolates from local dairy sample and 27 isolates from mother dairy as shown in Table 1 and Fig 1.

Table 1: Growth of Isolates on Agar

Sr. No.	Milk Sample	De Man Rogosha Sharp		Bile Esculin Agar	
		No. of Colonies	Growth on agar	No. of colonies	Growth on Agar
1	Local dairy	39	Circular, white milky,	41	Blackening of the medium around growth.
2	Local tea shop	51	Circular, white milky,	12	Blackening of the medium around growth.
3	Amul packet	31	Circular,white milky,	2	Blackening of the medium around growth
4	Mother dairy	27	Circular, white milky,	27	blackening of the medium around growth.
5	Gokul milk	36	Circular, white milky,	8	Blackening of the medium around growth

Fig 1: Growth of isolates on MRS and Bile Esculin Agar

3.2 Identification of Microorganism by Gram Nature and Biochemical Technique

Table 2. Cultural, Morphological and Biochemical Characteristics of Bacterial Isolates.

Sr. No.	**Samples**	**Characteristics of organisms**			**Biochemical Tests**		
1	Local dairy	Colony morphology on Bile Esculin Agar	Microscopic observation	Catalase test	Tellurite Tolerance	Tetrazolium Reduction	Mannitol Fermentation
2	Local tea shop	Blackening of the medium around growth.	Cocci, single, in pair or short chain	-	+	+	+
3	Amul packet	Blackening of the medium around growth.	Cocci, single, in pair or short chain	-	+	+	+
4	Mother dairy	Blackening of the medium around growth	Cocci, single, in pair or short chain	-	+	+	+
5	Gokul milk	Blackening of the medium around growth	Cocci, single, in pair or short chain	-	+	+	+

All the isolate were cultured on selective media such as Bile Esculin agar, which is selective for Enterococcus spp. Because of the presence of sodium azide, which inhibits the growth of gramme negative bacteria, isolation is required. While Black zone around colonies are formed on bile Esculin agar (Mossel DAA, et al., 1973). Based on the result of biochemical tests, Tellurite tolerance, mannitol fermentation, and tetrazolium reduction and inability to ferment arabinose, 18 E. faecalis strain were identified from local dairy milk and 14 E. faecalis

strain identified Mother dairy milk sample (Facklam RR, Collins MD, 1989, 2000., Manero A, Blanch AR, 1999., Day AM, et al., 2002). In another study

(Citaki S, et al., 2005), E. faecalis (54.2%), other enterococcal isolates E. durans (6.2%), E. gallinarum (3.0%).

4. Conclusion

These study conclude that Five milk samples, Enterococcus spp. were isolated from all milk samples. Highest percentage of Enterococcus spp. were isolated in Local dairy and mother dairy milk samples.

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16. Isolation of Phenol Degrading Microorganism from Waste Water Samples

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Abstract

Phenol and its derivatives are pollutants present in the soil, water samples effluents of major industries such as paper mill, oil refineries and petrochemical plants. Removal of phenol from waste water is extremely important because of its toxicity to the aquatic life and environment. In the present study, an attempt has been made to eradicate the phenol from wastewater using isolated bacteria. The pH value of the waste water has been observed to be 8.2 to 9.5. The presence of high concentration of phenol has been observed in the effluent samples. The total nine bacterial isolates as obtained were checked for growth on minimal salt medium amended with different concentrations of phenol by flask culture technique. In the present study, the three isolate species of N3, N5, and N8 were found to be very tolerant to degrade a phenol concentration up to 1250 mg/L.

Keywords: Bacteria, Waste Water Sample, Phenol, Flask Culture Technique.

1. Introduction

Phenol is a naturally occurring and man-made aromatic molecule that plays an essential role in the biodegradation of natural and industrial aromatic compounds. Only a few phenol-degrading anaerobic organisms have been described so far, despite the fact that many microbes capable of aerobic phenol degradation have been discovered. Enrichment of culture was used to obtain phenol-degrading denitrifying pure cultures from different industries waste water samples collected. The phenol-degrading isolates were also closely related to members of the genus Pseudomonas sp., according to morphology nature.

Phenol is both a natural and a synthetic aromatic chemical. Natural phenolic compounds and their derivatives can be found all over the place. 4-hydroxybenzoate and phenolic acids such as ferulic, p-coumaric, vanillic, cinnamic, and syringic acids (Sparling G P, et al., 1981) (are exuded by plant roots . Phenolic compounds are also released into the environment as byproducts of the biodegradation of natural polymers with aromatic rings, such as lignin's and tannins, as well as aromatic amino acid precursors. Furthermore, they may enter the environment as intermediates during xenobiotic chemical biodegradation. Pulp mills, coal mines, refineries, wood preservation factories, and numerous chemical businesses, as well as their wastewaters, are all known sources of phenol contamination. The toxicity of phenol to sediment bacteria in phenol-contaminated sites has been studied, and it has been discovered that bacteria can adapt to ambient phenol concentrations, but that increasing phenol concentrations appears to reduce overall phenol biodegradation (Dean-Ross D., 1989, Dean-Ross D and Rahimi M. 1995)

Despite the presence of phenols in most soils and sediments, only a few anaerobic phenol-degrading bacteria have been isolated and studied. While aerobic phenol-degrading bacteria were originally described in 1908 (Störmer K. and Ueber die, 1908), Desulfobacterium phenolicum (Bak F and Widdel F. 1986) was the first obligately anaerobic phenol-degrading bacterium to be discovered in 1986. Two more sulfate-reducing phenol-degrading microbes have recently been discovered (Boopathy R., 1995, Kuever J., et. al., 1993). Phenol degradation has also been described under methanogenic circumstances, and various researchers have been able to extract or identify organisms from methanogenic cocultures that can conduct the initial step(s) of phenol degradation in methanogenic conditions (Knoll G. and Winter J., 1989). Denitrifying phenol-degrading isolates first described in 1977 could grow in phenol-containing nutrient broth, but only a small amount of growth occurred when phenol was the sole carbon source (Bakker G. 1977). Only two pure cultures of denitrifying bacteria, strains K172 and S100, have been documented earlier that can use phenol as their sole source of carbon and energy (Tschech A and Fuchs G. 1987). The strain K172 has been employed in research on the metabolic mechanisms of anaerobic phenol and anaerobic toluene degradation (Lack A., and Fuchs G. 1992) .The isolation and characterization of phenol-degrading denitrifying bacteria isolated from waste water samples from nearby industries in Mumbai.

2. Materials and Method

2.1 Sample Collection

The waste water samples were collected from close industries that area unit located in city, India

The samples were collected in screw cap bottles antecedently cleansed by washing in anionic rinsed with H2O and soaked in 10% HNO3 for 24h before final remotion completely with deionized water. Thereafter, the screw cap bottles were sterilized in autoclave and therefore the collected samples were unbroken at four °C.

2.2 Media and Screening of Phenol Tolerant Bacteria

The strains were screened for phenol tolerance in phenol amended MSM agar and broth within the presence of 1 Chronicles aldohexose (w/v). The medium constituents (g/L) are: Na2HPO4:1.6; KH2PO4:0.4; NH4NO3:0.5; MgSO4.7H2O:0.2; CaCl2:0.025; FeCl3:0.0025; and Agar: two.0, that was supplemented with variable concentration of phenol (500–2000 mg/L) along side management and with/or while not aldohexose supplementation and pH scale of the medium was seven.2. Phenol tolerant microorganism were screened from enrichment flask containing MSM with phenol as a sole carbon supply. The broth was soft on totally different concentrations of phenol go fifty mg/L to 2000 mg/L. The isolated strains were inoculated into medium with the assistance of immunization loop and incubated on a shaking brooder (Bio-Era) at one hundred thirty revolutions per minute at temperature for forty eight h in 500-mL round shape flasks. Growth of microorganism cells at totally different phenol concentrations make up my mind by microorganism cloudiness measuring at 610 nm at each 2-h interval up to a 48-h incubation along side management by victimization UV–Vis photometer. The isolates were screened for more studies on the premise of their tolerance to phenol. the chosen isolates were refined by recurrent streaking on mineral salt medium containing one hundred mg/L of phenol, and therefore the operating culture was maintained by culturing in mineral salt medium containing one thousand mg/L of phenol at 2-weeks intervals (Ali et al., 1998).

3. Result and Discussion

3.1 Isolation and Culture of Bacteria from Waste Water Samples

The effluent samples within the gift study were measured in terms of viable counts per cubic centimeter of sample. It was found to be 27×107 cells per cubic centimeter of WWS (Table No. 1). Haritash and Kaushik (2009) discovered that the phenol-degrading enzymes area unit broadly speaking distributed in different microorganisms that play a very important role within the degradation of phenol. Marihal and Jagadeesh (2013) rumored the loss of vital

microbes thanks to addition of untreated chemicals and alternative contaminants. The enrichment technique for isolation has been utilized in the current study so as to get specific microorganism amongst various natural population. However, a complete of 09 differing kinds of microorganism isolates were isolated from the collected sample. For enumeration of microorganism, agar medium was used.

Table no. 1: Isolation of bacterial isolates from WWS

Sr. No	Name of Industry	Isolates	Positive isolates
1		37 x 106	2
2		27 x 107	5
3		11x 105	2

To obtain pure culture, the cultures were repeatedly streaky on agar medium and incubated at 37 °C for 24 h. Pure culture of all total 09 microorganism isolates was developed and categorized serially as N1 to N9 (Fig. 1). These isolates were more checked for growth on MSM broth medium amended with totally different concentrations of phenol. Growth has been found to be negligible on applying higher concentration of phenol (1250 mg/L). this might flow from to the sensitivity of those microorganism towards higher concentration of phenol, or they will need acclimatization on phenol before its degradation (Abd-El-Haleem et al. 2002). it's additionally been discovered that with relevance time and acclimatization, the expansion of microorganism seems within the phenol medium, indicating that the microorganism step by step adapt themselves to the compound. An oversized variety of phenol tolerant microorganism and plant species are isolated from phenol contaminated sites (Bhushan et al., 2000). Arutchelvan et al., (2005) rumored isolation of competent microorganism cells from the waste of trade producing phenol–formaldehyde resins.

Fig. 1: Growth of Bacterial Isolates on Medium

3.2 Screening by Growth Studies Phenol and its Derivatives have shown shocking capability in phenol elimination with microorganism having quick replica when acclimatization.

Therefore with isolation, purification and growth of species, that has high capability of phenol removal, will be utilized in areas with wastewaters containing high phenol concentrations. However, logically it will be inferred that the rise within the phenolic resin concentration causes the choice and utilization of the phenol degrading microorganisms with less diversity. On the opposite hand, choosing teams will be simpler which might stand up to additional tolerance of phenol concentration in comparison with the non-degrading microorganisms. Hussain et al., (2008, 2009, 2010) conducted a study victimization membrane bioreactor in treatment of phenolic resin waste. The study unconcealed the assorted vital options concerning diversity, physiology and performance of genus Pseudomonas population that's found in industrial phenol-degrading bioreactors. However, a major physiological nonuniformity within the tolerance limit of microorganism isolates has been discovered in treatment of phenolic resin waste (Whiteley et al., 2001).

In the study, 3 microorganism isolates N3, N5 and N8 showed luxuriant growth on phenol-amended borderline salt medium (MSM) within the presence of 1 Chronicles aldohexose (w/v), whereas no growth has been discovered within the absence of aldohexose. These strains showed quick and luxuriant growth at phenol concentration of 0–1000 mg/L.

The results obtained from present study concerning growth studies indicate that N3, N5 and N8 area unit tolerate the phenol up to a phenol concentration of 900 mg/L, 1250mg/L and 1800 mg/L. Similar study being conducted by principle and Lee (2007) rumored that phenol encompasses a doubtless restrictive impact on cell growth supported the very fact that the high phenol concentration of 2000 mg/L causes substrate inhibition. it's additionally rumored that the following exposure to the increasing phenol concentration from zero to 2000 mg/L on isolated microorganisms will degrade it effectively and with efficiency. it's a standard technique utilized in the enrichment method additionally

4. Conclusion

The removal of phenol in industrial effluents is extremely crucial thanks to its persistent and ototoxic effects. The obtained strains N1, N3 and N9 were found to be economical amongst the sixteen strains established by checking their capability of phenol tolerance with relevance the incubation time. The current study will be utilized in real-scale systems as identification of

phyletic ally closely connected species for phenol degradation is a very important side. This may facilitate in treatment of commercial wastes, and therefore the utilization of such isolated microorganisms proves to be additional economical and possible. This may cut back the environmental burden with the event of technology as an efficient and economical method.

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17. Production of Biofertilizer from *Rhizobium Sp.* Isolated from Garden Soil

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Abstract

Bio fertilizers area unit outlined as preparations containing living cells or latent cells of economical strains of microorganisms that facilitate crop plants' uptake of nutrients by their interactions within the rhizosphere once applied through seed or to soil. They accelerate the extent of availableness of nutrients in a very kind simply assimilated by plants. Fairly often microorganisms aren't as economical in natural surroundings collectively would expect them to be and thus area unit fast the microorganism processes in soil. Use of biofertilizers is one among the necessary part of integrated nutrient management, as they're price effective and

Supplement the chemical fertilizers for property agriculture. within the present study biofertilizer with carrier materials was more practical than the while not carrier materials.

Keywords - Rhizobium, Biofertilizer, chemical element Fixing bacterium, Carrier Material, cereal, crop attention, yield.

1. Introduction

Biofertilizer area unit outlined as preparations containing living cells or latent cells of economical strains of microorganisms that facilitate crop plants' uptake of nutrients by their interactions within the rhizosphere once applied through seed or to soil. They accelerate bound microorganism processes within the soil that augment the extent of availableness of nutrients in a very kind simply assimilated by plants. Mostly often microorganisms aren't as economical in natural surroundings collectively would expect them to be and thus by artificial means increased cultures of with efficiency elect microorganisms play a significant role in fast the microorganism processes in soil. Use of biofertilizer is one among the necessary parts of integrated nutrient

management, as they're a value effective and renewable supply of plant nutrients to supplement the chemical fertilizers for property agriculture. In this study biofertilizer with carrier materials were more practical than those while not carrier materials.

Biofertilizer could be a substance that contains living microorganisms that, once applied to the vascular plant surfaces or soil colonizes the rhizosphere or the inside of the plant and promotes growth by increasing or availability of primary nutrients to the host plant. Bio-fertilizers add nutrients through the natural processes of organic process, (G.S Naganandha, Arijitdas, Sourav Bhattacharta and T. Kalpana) solubilizing phosphorus, and stimulating plant growth through the synthesis of growth-promoting substances. Bio-fertilizers is expected to scale back the utilization of chemical fertilizers and pesticides. Bio-fertilizers offer eco-friendly organic agro-input and area unit cheaper than chemical fertilizers. Since a bio-fertilizer is technically living, it will symbiotically keep company with plant roots. Concerned microorganisms may pronto and safely convert complicated organic material in straightforward compounds, so plants area unit simply concerned. It maintains the natural environment of the soil. It will increase crop yield by 20-30%, replaces chemical element and phosphorus by twenty fifth, and stimulates plant growth. It also can offer protection against drought and a few soil-borne diseases. (Nilabja Ghosh, 2007)

Very often microorganisms aren't as economical in natural surroundings collectively would expect them to be and thus by artificial means increased cultures of with efficiency elect microorganisms play a significant role in fast the microorganism processes in soil. Use of biofertilizers is one among the necessary parts of integrated nutrient management, as they're a value effective and renewable supply of plant nutrients to supplement the chemical fertilizers for property agriculture. In study biofertilizer with carrier materials were more practical than those while not carrier materials.

Several crop plants area unit being exploited within the production of biofertilizers. They will be classified in numerous ways in which supported their nature and performance.

In present study, isolated the biofertilizer manufacturing bacterium and prepare biofertilizer by mistreatment carrier

2. Materials and Methods

Sampling

Method of biofertilizer production

The production of carrier based biofertilizer involves five

Stages

1. Isolation and characterization of Biofertilizer producing microorganisms
2. Culturing of microorganisms- starter culture and inoculum preparation.
3. Processing of carrier material.
4. Mixing the carrier and broth culture.
5. Packing.
6. Proper storage.

2.1 Isolation of Biofertilizer Producing Microorganism

The soil ought to be collected from the garden space and also the isolation of being is distributed. Waste soil sample of 4-5 days recent is taken directly from that portion of crammed from wherever chance of finding Rhizobium sp. is most one metric weight unit of soil sample is taken and serially diluted mistreatment saline to arrange dilution of 10-1 to 10-9 severally in numerous sterile check tubes. 1ml of sample from every tube is enamoured the assistance of micropipette and adjoin the freshly ready agar plates within stratified air flow. Then petri plates square measure unbroken in associate apparatus at 370 C for twenty-four hours.

2.2 Isolation of Rhizobium sp.

The strains of various microorganisms square measure isolated by serial dilution methodology on Rhizobium isolation media, N-free diuretic drug agar medium, grape sugar Agar medium, Triple Sugar Iron Agar Media, germicide agar media. All the samples were pure cultivated by agar plating methodology and characterised by colony morphology, Gram's staining and organic chemistry tests.

2.3 Isolation and Identification Rhizobium sp.

The Rhizobium isolates were obtained from root nodules of Bean, Gram. Nodules placed on the roots were spherical (2-4 metric linear unit in diameter) and pink. Root nodules were sterilized in ninety fifth (v/v) alcohol for ten s and so washed seven times with sterile H2O. Individual nodules were crushed with sterile glass rods and streaky onto Rhizobium isolation agar. once incubation for 2-3 days at 30°C, single colonies were elect and restreaked on this agar for purity.

2.4 Important Steps of Biofertilizer Production

Samples of 1g of rhizosphere (roots and soil) were added 10 ml of culture broth Rhizobium isolation agar. Associate aliquot of 1ml from every broth was additional to a selective medium to purify the microorganism. Identification of full-grown isolated colonies was supported morphologic and culturing characteristics.

2.5. Culturing of Microorganism

Biofertilizers carrier based mostly preparations containing economical strains of nitrogen fixing or phosphate solubilizing microorganisms. Biofertilizers developed typically as carrier based mostly inoculants. The organic carrier materials square measure more practical for the preparation of microorganism inoculants. The solid inoculants carry additional microorganism cells and support the survival of cells for extended periods of your time.

2.6 Methodology of Biofertilizer Production

Although several microorganism are often used beneficially as a biofertilizer the technique for production of Rhizobium is mentioned here. The expansion mediums used for mass culturing of various microorganism biofertilizers as follows:

Rhizobium sp. Growth on Yeast Extract broth, add 10 ml of azo dye stock resolution (dissolve 250 mg of azo dye in 100mL water) to 1liter once adjusting the hydrogen ion concentration to 6.8 and before adding agar. Rhizobium forms white, semi-transparent, glistening, elevated and relatively tiny colonies on this medium. Moreover, Rhizobium colonies don't take up the colour of azo dye additional within the medium. Those colonies that promptly take up the azo dye stain aren't rhizobia however presumptively genus Agrobacterium, a soil microorganism closely associated with Rhizobium, Sodium molybdate 200 mg; Manangeous sulfate 235 mg; chemical element acid 280 mg; sulfate 8 mg; white vitriol 24 mg; H_2O 200ml; $FeSO_4$.Trace; Yeast Extract-0.5 g; Distilled Water-1000 ml

2.7 Mixing

The on top of broths square measure ready in separate flasks and matter from individual mother cultures is transferred to flasks. The culture is full-grown below shaking conditions at 30±2°C as submerged culture. The culture is incubated till a most cell population of one 105 to 1010 cfu/ml is created. Below optimum conditions this population level can be earned among four to five days for Rhizobium. The culture obtained within the flask is termed starter culture.

for big scale production of inoculum, matter from starter culture is transferred to giant flasks and full-grown till needed level of cell count is reached.

Table 1. Production of Different (High & Low) Ratio of Biofertilizer with Carrier Materials

Sr. No.	Coal	Soil	Sand
1	50%	50%	50%
2	50%	50%	-
3	70%	30%	30%

2.8 Preparation of Inoculants Packet

The carrier with totally quantitative relations and also the broth culture and packing Inoculants packets square measure ready with different ratios with carrier materials by intermixture the broth culture obtained from fermenter with sterile carrier material as delineated below with high and low ratio of biofertilizer production.

2.9 Storage of Biofertilizer Packet

The neutralised and sterilized carrier material is unfold during a clean, dry, sterile gilded or plastic receptacle. The microorganism culture drawn from the fermenter is additional to the sterilized carrier and mixed well by manual (after sporting sterile gloves) or by mechanical mixer. The culture suspension is to be additional to level of forty - five hundredth water holding capability relying upon the population. The inoculants packet of two hundred g quantities in synthetic resin baggage, sealed with electrical sealer and allowed for action for two -3 days at temperature (curing are often done by spreading the inoculants on a clean floor/polythene sheet or by keeping in open shallow tubs/ trays with synthetic resin covering for two -3 days at temperature before packaging).

Specification of the Synthetic Resin Baggage

The synthetic resin baggage ought to be of denseness grade. The thickness of the bag ought to be around fifty - seventy five metric linear unit. Every packet ought to be marked with the name of the manufacturer, name of the merchandise, strain variety, the crop(s) to that recommended, methodology of vaccination, date of manufacture, batch variety, date of expiration, price, full address of the manufacturer and storage directions etc.

The packet ought to be hold on during a cool place off from the warmth or direct daylight. The packets could also be hold on at temperature or in cold storage conditions in heaps

in plastic crates or polythene/gunny baggage. The population of inoculums within the carrier inoculant packet could also be determined at a fifteen days interval. There ought to be quite 109 cells per gram of inoculum at the time of preparation and 107 cells per gram on a dry weight basis before expiration date.

3. Result and Discussion

3.1: Preparation of Inoculants Packet

Table 1. Production of Different (High & Low) Ratio of Biofertilizer with Carrier Materials

Ratio	Coal	Soil	Sand
1	50%	50%	50%
2	50%	50%	-
3	70%	30%	30%

The neutral and sterilized carrier material is unfold in a very clean, dry, sterile aluminiferous or plastic receptacle. The microorganism culture drawn from the fermenter is value-added to the sterilized carrier and mixed well by manual (after carrying sterile gloves) or by mechanical mixer. The culture suspension is to be value-added to grade of forty - five hundredth water holding capability relying upon the population. The inoculants packet of two hundred g quantities in polyethylene baggage, sealed with electrical sealer and allowed for solidifying for 2 -3 days at temperature (curing may be done by spreading the inoculants on a clean floor/polythene sheet or by keeping in open shallow tubs/ trays with polyethylene covering for 2 -3 days at temperature before packaging).

3.2: Specification of the Polythene Bags

The polythene bags should be of low density grade. The thickness of the bag should be around 50 - 75 micron. Each packet should be marked with the name of the manufacturer, name of the product, strain number, the crop(s) to which recommended, method of inoculation, date of manufacture, batch number, date of expiry, price, full address of the manufacturer and storage instructions etc.

3.3: Storage of Bio Fertilizer Packet

The packet ought to be hold on in a very cool place faraway from the warmth or direct daylight. The packets could also be hold on at temperature or in cold storage conditions in tons in plastic crates or polythene/gunny baggage. The population of inoculums within the carrier inoculant packet could also be determined at a fifteen days interval. There ought to be over 109

cells per gram of inoculum at the time of preparation and 107 cells per gram on a dry weight basis before expiration date (Fig. 1).

3.4: Identification of Isolated Microorganism

The potent biofertilizers production bacteria is identified by morphological and biochemical tests. According to table no. 2, the most potent biofertilizer producing bacteria is Rhizobium sp.

It is a natural methodology with none issues like salinity, alkalinity, eating away etc., In huge areas of low input agriculture as in crops like sugarcane these product are going to be of a lot of use to provide property to production. Biofertilizer soaks up and hold these dissolved nutrients substances in order that the roots have longer to soak up them. Biofertilizer conjointly adds copper, chemical element and different very important nutrients to the soil and protects water quality (Biofertilizer a completely unique tool for agriculture Boraste.A, Vamsi.K. K, Jhadav. A, Gupta.M, Joshi. B, Gupta. N, Gupta. G). A bio-fertilizer is technically living, it will symbiotically escort plant roots. Concerned microorganisms might promptly and safely convert complicated organic material in easy compounds, in order that plants are simply obsessed. being perform is in long length, inflicting improvement of the soil fertility. It maintains the natural environment of the soil. Biofertilizer immunization was found to enhance plant growth and biomass considerably. The organism has high ability to extend N,P and K further as different nutrition acquisition for plants.(Seemaparoha, K.K Chandra and Rakhiyadav).

Table 2. Morphological Identification and Biochemical Test of the Isolated Microorganism

Serial No.	Test	Rhizobium
A	Growth on Rhizobium Isolation Agar	White, Translucent Colonies
B	Gram's Nature	Fine and rod shape , pink coloured, shaded fine structure
C.	Biochemical Tests	
1	Urease	Positive
2	Nitrate reduction	Positive
3	Starch hydrolysis	Positive
4	Gelatin hydrolysis	Negative
5	Casein hydrolysis	Negative
6	Methyl Red	Positive

7	Vogues Proskauer	Negative
8	Indole negative	Negative
9	Citrate utilization	Positive
10	Hydrogen sulphide	Negative

Fig. 1: Biofertifizers Packet by Rhizobium sp.

4. Conclusion

Information from the assorted literatures on the market presently depicts that association either rhizospheric or endophytic between Rhizobium and cereal could be a phenomenon. Progressive information of this space could bring advantages for exploitation this technology. For exploitation, in depth optimisation and comparative study of the once effects of the applying is needed. Hence, additional analysis is required on the interaction between cereal grains and rhizobia or rhizobia-like microorganism. there's conjointly ought to totally assess whether or not the rhizobia fix N2 in association with cereals beneath conditions within which this microorganism promote plant growth and if therefore what portion of the plant-N may be derived from BNF method. Our understanding of the aptitude of rhizobia to inhibit cereals and enhance their growth is barely commencing to be explored. notwithstanding, the novel findings delineated here represent major advancement in achieving the technically difficult goal of accelerating cereal productivity by reducing its dependence on the chemical fertilizer-N through sweetening of its natural association with rhizobia.

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18. Impact of Encapsulated Marine Strain *Lactobacillus Rhamnosus* L43 in Alginate Matrices for Bacteriocin Production

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Abstract

Strains of Lactobacillus rhamnosus L43 turn out the bacteriocin were immobilized in sodium alginate beads. The free cells of them were optimized viz., temperature and pH for optimum bacteriocin production. The bacteriocin production was maximized at 18 h at 30°C and in pH 7.0. Free cells refined at the optimum culture conditions were accustomed compare the impact of immobilization on bacteriocin production. Bacteriocin production peaked at 21 mm. A decline in bacteriocin production occurred at the centre of the fermentation with the free cells in distinction to future stability detected with the immobilized cells. The immobilized cells were subjected to end increased yield with increase in biomass

Keyword: Lactobacillus rhamnosus L43, Sodium Alginate, Beads, Antibacterial activity

1. Introduction

Cell immobilization is outlined because the fix or work cells to a limit space of house with the preserve of chemical change activity (Karel et al., 1985). Immobilized molecule is one whose movement in house has been restricted either utterly or to a tiny low restricted region by attachment to a solid structure (Yu-Qung et al., 2004). Immobilization of cell is helpful to stabilize the activity of bioreactors in ordered operations, inclusion stability, increasing phage resistance and lowering the inhibition by antibiotics or salts (Champagne et al., 1994). especially, cell immobilization has shown to supply several blessings for biomass and substance productions compared with the free-cell (FC) systems like high cell density and extremely high volumetrically productivity, apply of biocatalysts, high method stability (physical and biological) over long fermentation periods, retention of plasmid-bearing cells, improved resistance to

contamination, uncoupling of biomass and substance productions, stimulation of production and secretion of secondary metabolites and physical and chemical protection of the cells (Scannell et al., 2000; Grattepanche et al., 2007).

In this study, the practicability of bacteriocin L43 will increase victimisation alginate immobilized cells of L. rhamnosus L43, was investigated and therefore the production was compared with free-cells.

2. Materials and Methods

2.1.a Preparation of Beads

Immobilization was administered victimisation the wild and mutant strain of L. rhamnosus L43. Cells were immobilized victimisation sodium alginate. Changed MRS (mMRS) broth was used for learning bacteriocin production with encapsulated cells to extend the soundness of sodium alginate matrix, MRS broth was changed. (Scannell et al., 2000; Ivanova et al., 2002; Rao et al., 2008, 2009). Before immobilization, one OD of L. rhamnosus L43 (107 cfu/ml) were inoculated into mMRS broth and incubated at 300C for 48h. Cells were recovered by action, washed in 0.1% buffered organic compound water (Hi Media) and re suspended in 10 ML mMRS. Sodium alginate was ready in water, autoclaved (1100C, 15 min), and cooled to 450C before use.

Cell suspension of Bacteriocin manufacturing strain was inoculated to the alginate resolution (10% v/v) and aseptically transferred to a sterile syringe. The mixture was forced through a sterile syringe needle (0.8 millimeter diameter) by air-pressure drop-wise into a stirred resolution of twenty-two (w/v) zero.5 M amalgamate salt. The ensuing beads were hardened in salt for one h, wet sieved and surface dried during a streamline flow hood. when preparation of beads of compound matrices, additional in fifty cubic centimetre of mMRS broth and incubated their several temperature and time and in conclusion assayed for bacteriocin activity by agar well diffusion methodology.

2.1.B Determination of the Concentration of free Cells within the Media

The viability of cells was counted by dilution plating on MRS agar and when incubation for 40 h at 300C. The info were expressed as cfu/ml (colony forming unites per ml) (Ivanova et al., 2002; Sarika et al., 2012).

2.1.c Determination of Cell Concentration within the Beads

The viable cell concentration within the beads make up volume by suspending 1ml of alginate resolution in 0.1 M phosphate resolution followed by light shaking for 30 min for destruction of the beads (Suthasinee, 2010). The suspension was submitted to serial dilutions victimisation 0.1% sterile water. Every dilution was plated in duplicate on MRS agar and incubated at 300C for 48h. the quantity of viable cells were expressed as cfu/ml by dilution plating on MRS agar when incubation for 48 h at 300C.

2.2.a Comparative Study of Bacteriocin Production by Free and Immobilized Cells

The fermentations with the free and immobilized cells were performed in 2 completely different Erlenmeyer flasks containing 50 ml mMRS broth with the optimized pH and temperature conditions and while not shaking for 90 h.

2.2.b Determination of Bacteriocin Activity

The fermentation broth was centrifuged (10,000 rev for ten min), knowledgeable Millipore filter (pore size zero.22 µm, Hi Media) and therefore the ensuing supernatant fluid was evaluated for bacteriocin activity by agar well methodology.

2.3.c Statistical Analysis

Each experiment and determination was perennial in duplicate. The info were examined by unidirectional multivariate analysis victimisation MINITAB 14 at level of significance of $p < 0.05$.

3. Results and Discussions

3.1.a: Stability of Beads

The highest stability and antibacterial activity was found in sodium alginate beads. Sodium alginate has eco-friendly nature because it is nontoxic and safe for nature, cheap, merely used and gentle conditions needed for immobilization (Rao et al., 2009). Porousness of the beads limits the nutrient offer and chemical element diffusion to the immobilized cell (Dey et al., 2003 and Adinarayana et al., 2004). Photoplate A, 1 and 2 shows the immobilization of beads and stability

3.1.b: Cell Concentration within the Bead

The immobilized beads with the scale of 2mm diameter were subjected to determination of the cell concentration. The utmost cell concentrations of 39 X 107 cfu/mL. These observations were akin to the studies conducted earlier in Lactococcus sp. (Prevot and Divis, 1992),

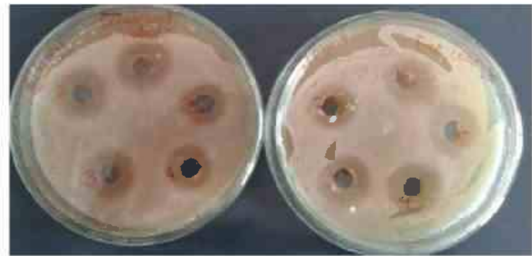
L.pentosus B25 (Wadekar and Dharmadhikari, 2019), Bifidobacterium longum ATCC 15707 (Doleyres et al., 2002; Sarika et al., 2012).

3.2.a: Bacteriocin Production by free and Immobilized Bacteria

The batch fermentation was dole out with the free and immobilized cells of each the strains in controlled temperature and pH conditions. The bacteriocin production profiles were similar in each the strains within the immobilized cells. Bacteriocin production peaked at 21 mm and 23.5 mm in free and immobilized cells

Photoplate B has represents the antibacterial activity of free and immobilized cells against microorganism (Escherichia coli). However, in comparison with the free cell fermentations, the fermentation with the immobilized cells raised the soundness of yield of bacteriocin and tried long run suggests that of manufacturing bacteriocin in optimized media. Once reaching a peak, the bacteriocin activity attenuate considerably within the matter. Such results were according for alternative bacteriocins (De Vuyst 1994; Diamond State Vuyst et al., 1996). It may be supposed that the alginate beads had a protecting role separating the bacteriocins within the medium from the peptidase within the bead.

Photoplate A: 1 And 2: Immobilized Beads of Cell and their Stability



Photoplate B: Antibacterial Activity of Free and Immobilized Beads of Lactobacillus Rhamnosus L43

4. Conclusion

The immobilization of the mutant strain of L. rhamnosus L43 in Ca-alginate beads created potential the increasing of bacteriocin production as a results of the increasing of the

biomass. The calcium-alginate beads was found to boost the survival of cells throughout exposure to variety of product tested presumably as a results of factors related to the altered cell microenvironment. So Immobilised Cell Technology has potential increasing the effectiveness of Lactic acid bacterium probiotic preparations by enhancing cell resistance throughout bacteriocin treatment. Considering the high yield and stability of the bacteriocins even within the batch fermentations, it's essential to rescale the method as a nonstop system which might provide far better yield of bacteriocin in future.

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19. Application of Bacteriocin B25 by Marine Lactobacillus Pentosus B25 as a Food Biopreservative in Milk

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Abstract

Bacteriocin is employed as a food preservative thanks to its medicine activity. It principally used for increase the period of time of food. It works as natural preservative similarly as mix with chemical preservatives. In study, we tend to use the 5% bacteriocin in raw milk and pasteurized milk. The raw milk extend their life up to eight days and pasteurized milk extending up to forty two days. The preservative activities of the bacteriocins tested on milk showed that the bacteriocin B25 had most reduction of microorganism population and thereby extending the period of time and enhancing the protection of food merchandise.

Keywords: Bacteriocin, Milk Sample, Preservatives, MBRT Test.

1. Introduction

Lactobacillus species are primarily employed in the food trade in probiotics like Yakult and Vitagen, however also can be found in yoghurts and alternative hard dairy farm merchandise like cheese, wherever they're used as starter cultures. Daeschel, (1989) declared that in some food system carboxylic acid microorganism represent the dominant micro flora. The organisms are ready to manufacture antimicrobial compounds against competitive flora, as well as food borne spoilage and morbific microorganism. The antimicrobial impact exerted by carboxylic acid microorganism is that the production of carboxylic acid and reduction of hydrogen ion concentration, and lactic acid, diacetyl, oxide, fatty acids, aldehydes and alternative compounds. Fuller, (1989) according that lactic acid microorganism are used as adjuncts in food to supply a good selection a health advantages. The establishment of the gut by probiotic microorganism prevents the expansion of harmful microorganism by competitive exclusion and by the assembly of matter viz., organic acids and antimicrobial compounds. Piard and Desmazeaud, (1991) had

made antimicrobial compounds from the lactic acid microorganism, which may be classified as low mass compounds like oxide, greenhouse emission, diacetyl and high mass compounds like bacteriocins. Agar well diffusion methodology and neutral living thing culture filtrate obtained from isolates of L. acidophilus, L. delbrueckii spp. bulgaricus, L. salivarius and Lactococcus lactis spp. lactis from Dahi showed weak to moderate inhibition of Staphylpcoccus aureus, Bacillus caryophylloid dicot genus, E. coli, Bacillus brevis, Bacillus circulans, Bacillus coagulans, Bacillus laterosporus, Bacillus, and P. aeruginosa and according the potential of exploitation neutral living thing cellular filtrate of lactic acid microorganism within the bio preservation of foods. Yoneyama et al., (2004) showed that nisin and a few of the recently isolated novel bacteriocins were effective bio-preservatives in sure food systems during which focused bacteriocin preparations or the bacteriocin manufacturing strains had been used. The Lactic acid microorganism strains owe their antagonistic activity to either decrease within the hydrogen ion concentration or to bacteriocin production creating them ideal for cheese creating and preventing the expansion of S. aureus. In 2007, Vuyst and Leroy, according that bacteriocins may be used as food additives and as an alternate to the addition of bacteriocins to foods, bacteriocins could also be made directly within the food as a results of starter culture or co-culture activity.

In present study, we tend to confirm the period of time of milk by exploitation bacteriocin that is made by true bacteria pentosus (B25) as a food preservative.

2. Materials and Methods

The bio preservative potency of the bacteriocins B25 obtained from L.pentosus B25 made up our minds as represented by Joshi et al., protocol with slight modification. Every flask contained 50 ml raw milk and pasteurized milk, out of that one is management i.e. while not bacteriocin and one is sample i.e. with bacteriocin. for every sort 50 ml sample used and bacteriocin was side at 5ml and analysed for 2, 4, 6, eight and ten days (Raw milk) and most up to two months (Pasteurized milk). For raw milk, after every 1 day and for pasteurized milk, after every 6 days, the quality and stability of milk was studied with respect to (1) colony count (2) pH (3) Clotting test (4) Alcohol test (5) Acidity test (6) Resazurin test and (7) Methylene blue reductase test.

2.1 a Determination of Colony Count

The residual activities were taken every 2 days interval and serially diluted at 10-6 were made and the plated on nutrient agar and was incubated at 370C for 24 hours. The colony count was recorded and compared with the control (without bacteriocin).

2.2 b Quality control test of Milk

2.2 b(i) pH Determination

The pH of medium was determined using pH probe (Lab Serv, AcuStar+ V, pH/ Conductivity Meter).

2.2. b (ii) Clot on Boiling (C.O.B) Test

The test is quick and simple. It is one of the old tests for too acid milk (pH<5.8) or abnormal milk (e.g. colostral or mastitis milk). If a milk sample fails in the test, the milk must contain many acid or rennet producing microorganisms or the milk has an abnormal high percentage of proteins like colostral milk. Such milk cannot stand the heat treatment in milk processing and must therefore be rejected. Boil a small amount of milk in a spoon, test tube or other suitable container. If there is clotting, coagulation or precipitation, the milk has failed the test.

2.3 The Alcohol Test

The test is quick and simple. It is based on instability of the proteins when the levels of acid and/or rennet are increased and acted upon by the alcohol. Also increased levels of albumen (colostrum milk) and salt concentrates (mastitis) results in a positive test.

The test is carried out by mixing equal amounts of milk and 68% of ethanol solution in a small bottle or test tube. (68 % Ethanol solution is prepared from 68 mL 96% (absolute) alcohol and 28 ml distilled water). If the tested milk is of good quality, there will be no coagulation, clotting or precipitation, but it is necessary to look for small lumps. The first clotting due to acid development can first be seen at 0.21-0.23% Lactic acid. For routine testing 2 ml milk is mixed with 2 ml 68% alcohol.

2.4 Acidity Test

Bacteria that normally develop in raw milk produce more or less of lactic acid. In the acidity test the acid is neutralised with 0.1 N Sodium hydroxide and the amount of alkaline is measured. From this, the percentage of lactic acid can be calculated. Fresh milk contains in this test also "natural acidity" which is due to the natural ability to resist pH changes .The natural

acidity of milk is 0.16 - 0.18%. Figures higher than this signifies developed acidity due to the action of bacteria on milk sugar.

To 9 ml of the milk measured into the porcelain dish/conical flask, 1 ml Phenolphthalein is added and then slowly titrated again 0.1 N Sodium hydroxide present in the burette under continuous mixing, until a faint pink colour appears. The amount of ml of Sodium hydroxide solution required was noted and % lactic acid was estimated.

2.5 Resazurin Test

Resazurin test is the most widely used test for hygiene and the potential keeping quality of raw milk. Resazurin is a dye indicator. Under specified conditions Resazurin is dissolved in distilled boiled water. The Resazurin solution can later be used to test the microbial activity in a given milk sample. Resazurin can be carried out as 10 min test, 1 h test, 3 h test. The 10 min Resazurin test is useful and rapid, screening test used at the milk platform. The 1 h test and 3 h tests provide more accurate information about the milk quality, but after a fairy long time. They are usually carried out in the laboratory.

10 ml of milk and 1 ml of Resazurin was added in test tube, mixed the dye thoroughly and incubated in water bath and response was observed as change in colour and quality of milk was decided. Following table 1 has shown the reading and result of this test.

Table 1: Readings and Results (10 Minute Resazurin Test)

Resazurin Test	Colour	Grade of milk	Action
1	Blue	Excellent	Accept
2	Light blue	v. good	Accept
3	Purple	Good	Accept
4	Purple pink	Fair	Separate
5	Light pink	Poor	Separate
6	Pink	Bad	Reject
7	White	Very bad	Reject

2.6 Methylene Blue Reductase Test

Transfer 10 ml of each milk sample into appropriately labelled test tube. Add 1 ml of redox indicator, methylene blue to each test tube containing milk sample. Tighten the test tube mouth with stoppers. Gently invert the tubes at about four or five times to ensure proper mixing of the methylene blue solution. Keep the tubes in the water bath at 370C. Note the incubation

time. That is, the time elapsed for the colour to turn whitish appearance. Stabilize the tubes for 5 minutes.

Table 2: Result of Methylene Blue Reductase Test

Sr. No.	Time in reduction	Quality of milk
1	Within 30 min	Very poor quality
2	Between 30 min and 2 h	Poor
3	Between 2 and 6 h	Fair
4	Between 6 and 8 h	Good
5	Not reduced in 8 h	Excellent

3. Result and Discussion

Application of bacteriocin as a Food bio Preservative

3.a Application of Bacteriocin as Food bio Preservative in Raw Milk

Bacteriocin is employed as a food preservative thanks to its bactericide activity. It principally used for increase the period of time of food. It works as natural preservative likewise as mix with chemical preservatives. The milk extend their life up to eight days and milk extending up to forty two days (photoplate 1). The preservative activities of the bacteriocins tested on milk showed that the bacteriocin B25 had most reduction of microorganism population and thereby extending the period of time and enhancing the protection of food merchandise.

Table 3a, b represents the clotting formation, odour, clotting, colour modification in Resazurin take a look at, you look after carboxylic acid and thiazine enzyme take a look at of milk (photoplate two and 3). Figure one represents the colony count, hydrogen ion concentration and carboxylic acid concentration of milk.

Table 3a: Quality Control studies of Raw Milk

Sr. No.	Coagulation Formation		Odour		Clot on Boiling test		Alcohol Test	
Day	C	S	C	S	C	S	C	S
1	No	No	N	N	-	-	-	-
2	No	No	N	N	-	-	-	-
3	No	No	Ab	N	+	-	+	-
4	Y	No	Ab	N	+	-	+	-
5	Y	No	Ab	N	+	-	+	-
6	Y	No	Aci	N	++	-	++	-

7	Y	No	H. Aci	N	++	+	++	+
8	Y	Yes	H. Aca	Ab	++	+	++	+

Legends: N-Normal, Ab.-Abnormal

Table 3b: Quality Control Studies of Raw Milk

Sr. No.	Resazurin Test		Lactic Acid Test (%)		MBRT Test (h) Reduction	
Day	C	S	C	S	C	S
1	Blue	Blue	0.58	0.58	8	8
2	Blue	Blue	0.58	0.58	8	8
3	Purple Pink	Blue	0.67	0.58	5	8
4	Light pink	Blue	0.95	0.67	1	8
5	Pink	Purple	1.165	0.67	1	5
6	Pink	Purple	1.506	0.81	½	5
7	White	Purple pink	1.963	0.83	½	5
8	White	Light pink	2.358	1.43	½	3

3.b Application of Bacteriocin as a Food Bio-Preservatives in Pasteurized Milk

Table 4a, b represents the clotting formation, odour, clotting, colour modification in Resazurin take a look at, you look after carboxylic acid and thiazine enzyme take a look at of milk (Photoplate 1 and 2). Figure two represents the colony count, hydrogen ion concentration and carboxylic acid concentration of milk. Similarly, the bacteriocin of L. fermentum had most reduction on microorganism population. These results additional disclosed that microorganism count drastically reduced in each the treated and untreated sample. (Joshi et al., 2006). Combined impact of warmth and bacteriocin additionally extended storage lifetime of milk by suppressing growth of various microorganisms (Sharma et al., 2008)). Similar work done by Cao-Hoang et al., (2010); Mills et al.,(2011).

Table 4a: Quality Control Studies of Pasteurized Milk

Sr. No.	Coagulation Formation		Odour		Clot on Boiling test		Alcohol Test	
Day	C	S	C	S	C	S	C	S
1(1)	No	No	Normal	Normal	-	-	-	-
2(6)	No	No	Normal	Normal	-	-	-	-
3(12)	No	No	Abnormal	Normal	+	-	+	-
4(18)	Yes	No	Abnormal	Normal	+	-	+	-
5(24)	Yes	No	Abnormal	Normal	+	-	+	-

6(30	Yes	No	Acidic	Normal	++	-	++	-
7(36)	Yes	No	Highly acidic	Normal	++	+	++	+
8(42)	Yes	Yes	Highly acidic	Abnormal	++	+	++	+

Table 4b: Quality Control Studies of Pasteurized Milk

Sr. No.	Resazurin Test		Lactic Acid Test (%)		MBRT Test (h) Reduction	
Day	C	S	C	S	C	S
1(1)	Blue	Blue	0.58	0.58	8	8
2(6)	Blue	Blue	0.58	0.58	8	8
3(12)	Purple Pink	Blue	0.67	0.58	5	8
4(18)	Light pink	Blue	0.95	0.67	1	8
5(24)	Pink	Purple	1.165	0.67	1	5
6(30	Pink	Purple	1.506	0.81	½	5
7(36)	White	Purple pink	1.963	0.83	½	5
8(42)	White	Light pink	2.358	1.43	½	3

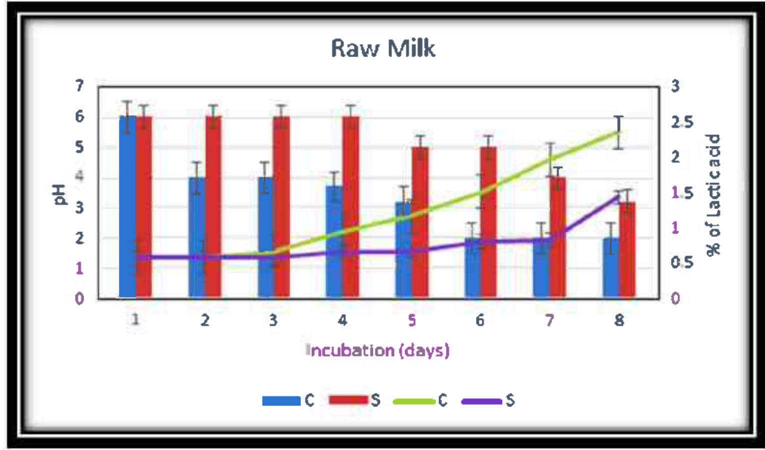


Fig. 1: Ph and Lactic Acid Concentration

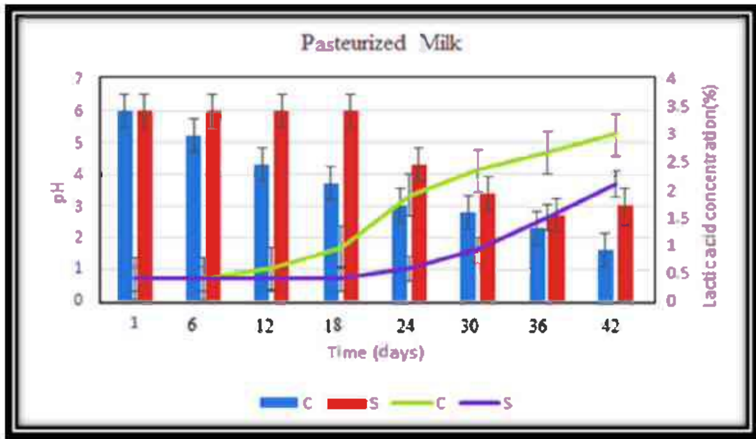
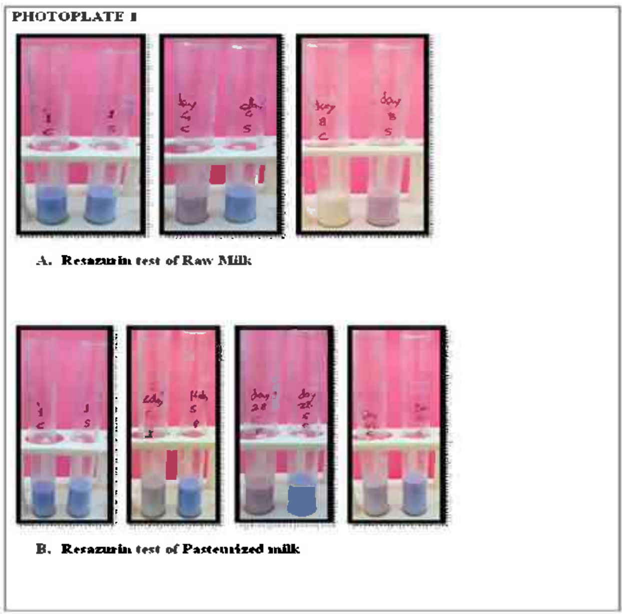


Fig. 2: Ph and Lactic Acid Concentration



4. Conclusion

These bacteriocins have tolerance to wide environmental factors, it can be used as a bio preservative in food industries. Bacteriocin is employed as a food preservative thanks to its bactericide activity. It principally used for increase the period of time of food. It works as natural preservative likewise as mix with chemical preservatives. In this study, we tend to use the 5 % bacteriocin in raw milk and pasteurized milk. The milk extend their life up to eight days and milk extending up to forty two days. The preservative activities of the bacteriocins tested on milk showed that the bacteriocin B25 had most reduction of microorganism population and thereby extending the period of time and enhancing the protection of food merchandise.

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20. Isolation and Identification of PHB Microorgnisms from Different Samples

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Abstract

Many bacteria produce polyhydroxybutyrate, which are biodegradable polyesters. Because of their hydrolysable ester linkages, biodegradable polymers are the most common biodegradable material. PHB is made by a variety of microorganisms when they are given the right conditions, such as nitrogen, calcium, magnesium, iron, or necessary vitamins. Because of their biological origin, PHBs breakdown spontaneously and entirely to carbon dioxide and water in the natural environment, thanks to microbial enzymatic activity. The current study describes the isolation and screening of soil bacteria, as well as the subsequent generation of PHB under normal circumstances. It was discovered that red soil was capable of producing the highest yield of PHB.

Keywords:- Polyhydroxy Butyrates (PHB), Biodegradable polymers

Introduction

Polymers are a wide class of materials that are made up of tiny molecules called monomers that are bonded together to form lengthy chains and are utilised in a variety of everyday products and goods. Polymers are these new materials, and their effects on our current lifestyle is almost impossible to comprehend.(Namazi, 2017) Clothing composed of synthetic fibres, polyethylene cups, Kevlar, nylon engine parts, plastic bags, polymer-based paints, epoxy glue, polyurethane foam pillow, silicone stents, and Teflon-coated cookware are all examples of polymer-based products. The list goes on and on.(Belgacem & Gandini, 2008)

A huge industry for environmentally compatible products has been developed in the recent decades. Biologically degradable polymers are currently underdeveloped in this context. Although these materials are well-known in principle, they have only been used in medicine for a

limited time. However, biodegradable plastics have undeniable commercial promise as a replacement for polyolefin commodities such as polypropylene (PP) and polyethylene (PE), but high costs and restricted availability of these materials currently prevent a widespread introduction to a ready market.(Mecking, n.d.; Zhang, 2015).

Due to their inherent biodegradability, sustainability, and environment-friendly features, biodegradable polymers such as poly- hydroxyalkanoates (PHAs) are more promising. (Salehizadeh & van Loosdrecht, 2004). PHAs are biodegradable polymers that are completely biodegradable. Many microorganisms (Gram-negative and Gram-positive bacteria) produce thermoplastic polyesters of several R-hydroxyalkanoic acids as carbon and energy reserves or reducing power storage materials in the presence of excess carbon, particularly when another essential factor such as oxygen, nitrogen, or phosphorus is limiting or after pH shifts.(Shah et al., 2008)

Polyhydroxybutyrate (PHB) was initially found in the bacterium *Bacillus megaterium*, which has intracellular granules, by Maurice Lemoige in 1926. It is a biodegradable and biocompatible thermoplastic. Polyhydroxyalkanoates (PHAs) are a type of bacterial polyesters that many bacteria amass intracellularly as reserve granules in adverse environmental circumstances.(Salehizadeh & van Loosdrecht, 2004)

PHB is largely a carbon assimilation product that microorganisms employ as an energy storage molecule. PHB is largely a carbon assimilation product that microorganisms employ as an energy storage molecule. It can be moulded into a variety of shapes and forms. PHB (polyhydroxy valeric acid) and PHV (polyhydroxy valeric acid) are all being investigated for a range of uses.(Gerngross et al., 1993)

Other biodegradable polymers are moisture sensitive and water soluble, however PHB has unique qualities such as insoluble in water, highly resistant to hydrolytic destruction, oxygen permeability, and UV resistance. PHB has low acid and basic resistance, is soluble in chloroform and other chlorinated hydrocarbons, and is biocompatible, making it ideal for medical applications.

PHB is generated as an internal storage substance and accumulates as discrete black inclusion granules in the cytoplasm of the cell during imbalanced growth. PHB is used by the cell as an internal reserve of carbon and energy during unfavourable situations. Many bacteria, especially those found in the soil, can produce and degrade PHB.(Anderson & Dawes, 1990)

PHB and other comparable bacterial alkanoate polyesters may be moulded into precise shapes since they are water insoluble, and several have been utilised commercially to make disposable thermoplastics for packaging material, disposable utensils, medical equipment, and other items.(Ym & Savitha R, 2011)

PHB-producing organisms can gain access to these storage granules by developing internal depolymerases, which hydrolyze PHB into the 3HB monomer, which can be used as a carbon or energy source. Other bacteria have evolved extracellular (exoenzyme) PHB depolymerases to scavenge PHB released by deceased PHB-producing bacteria, which is not surprising.(Ym & Savitha R, 2011)

For several years, our undergraduate research group has focused on isolating and characterising microorganisms that can degrade PHB.

Materials and Methods

2.1 Isolation of PHB from different cultures:

The bacteria utilised in this study were gathered from soil samples such as vegetable soil, market soil, playground soil, college garden soil, and potted plant soil for the purpose of screening the best PHB generating bacteria, with the medium listed below serving as the nutrition source. The 10-5 dilution was plated on nutritive medium using Peptone-2gm, Yeast extract-2gm, NaCl-1gm, Agar-4gm, Distilled water-1lt, and the media was then autoclaved at 121°C for 20mins at 15lbs pressure to prevent contamination. Overnight, these plates containing soil samples were incubated.

2.2 Screening for PHB producing bacteria:

All of the bacterial isolates were qualitatively evaluated for PHB synthesis using the Sudan Black Dye viable colony technique of screening (Jeran et al. 1998). For this PHB producer screening, nutritional agar media containing 1% glucose were autoclaved at 121°C for 20 minutes at 15 pounds of pressure. This mixture was placed into sterile Petri plates and allowed to set. Bacterial isolates were identified on plates that had been separated into five equal portions. For 24 hours, these plates were incubated. Now, a 0.02 percent Sudan Black B ethanolic solution was distributed over the colonies on Petri plates and left undisturbed for 30 minutes. To remove extra discoloration from colonies, they were rinsed with 96 percent ethanol.

2.3- Quantification of PHB production and selection of isolates

All Sudan Black B positive isolates were submitted to PHB production quantification using Jhon and Ralph's technique (1961). To remove undesirable components, the bacterial cells containing the polymer were centrifuged at 10,000 rpm for 10 minutes and the pellet was washed with an equal volume of acetone and ethanol. The pellet was resuspended in 4% sodium hypochlorite and incubated for 30 minutes at room temperature. The supernatant was removed after centrifuging the entire mixture once more. The PHB-containing cell pellet was washed again with an equal volume of acetone and ethanol. Finally, the polymer granules in the pellet were dissolved in hot chloroform

The chloroform was filtered, and 10 mL of concentrated hot sulphuric acid was added to the filtrate. Sulphuric acid transforms the pellet into crotonic acid, which has a brown tint. After cooling the solution, the absorbance was measured at 235nm against a sulfuric acid blank.

2.4 Screening Techniques

The culture was gram stained after 24 hours and the slide was examined under a microscope for gram response. In addition, IMViC, oxidase, and peroxidase assays were performed.

3. Result

Among the five soil samples which were used Playground soil gave a greater number of isolated single colonies. Ten colonies from Playground soil were again grown in nutrient broth and then again incubated overnight and then preserved for rest of the experiment.

3.1 Screening for PHB producing bacteria:

Colonies of black colour were viewed as a positive result.

3.2 Screening for PHB producing bacteria

Table No: 1

Sr No.	Sample Description	No. of Bacteria Isolated	No. of Sudan black colonies obtained.
1.	Vegetable soil,	10	6
2.	Market soil,	15	8
3.	Playground soil,	21	4
4.	College garden soil,	13	7
5.	Potted plant soil	19	2

3.3 Quantification of PHB production and selection of isolates: Selected isolates for PHB production

Table No: 2

Sr no.	Source	Isolates	Yield(g/100ml)
1.	Playground soil	I	0.170
2.	Playground soil	II	0.150

Screening Test

Table No: 3

Sr No.	TEST	Isolates	
		I	II
1.	Gram Staining	Gram negative rods	Gram negative rods
2.	Citrate Test	Positive	Positive
3.	Indole Test	Positive	Positive
4.	Methyl Red	Positive	Positive
5.	Voges-Proskauer	Negative	Negative
6.	Oxidase	Positive	Positive
7.	Peroxidase	Positive	Positive

Conclusion

Among the five soil samples taken, Playground soil yielded the best results when evaluated using a quantitative test. Ten best colonies were extracted, six of which tested positive for PHB-producing bacteria, and I and II yielded the best results. I and II's physical and chemical properties were changed and verified for maximum yield.

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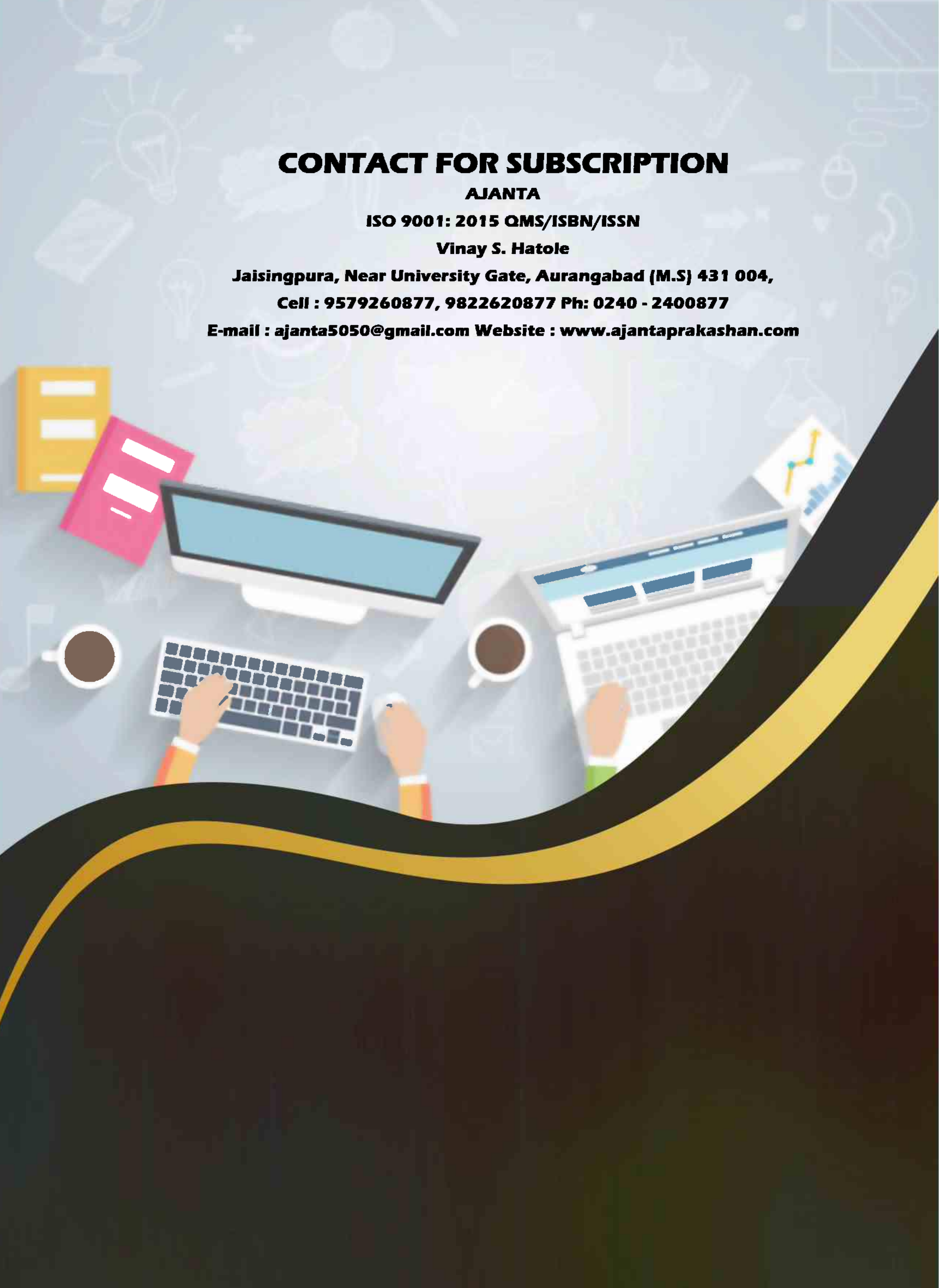

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