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ISOLATION & FORMULATION OF POMEGRANATE JUICE BASED ON PROBIOTIC PROPERTIES

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ABSTRACT:
Probiotics are mainly living microorganisms that used to improve or restore gut flora when ingested. The consumption of probiotics is mainly regarded as safe.A strain of lactobacillus bacteria strain Streptococcus faecalis strains were isolated from bifilac probiotic medicine and identified by colony morphologies cultured on MRS (Man, Rogosa and Sharpe) media and biochemical testing were done in this study. Isolated bacteria were chosen for probiotic screening and further investigation was done based on biochemical properties and further screening. In this study, Gram staining, Catalase test, carbohydrate fermentation, protease test, starch hydrolysis test, motility test, urease, catalase, nitrate test, indole test, growth optimization (pH optimization, and temperature optimization) were all used . Based on the OD (Optical Density), isolated bacteria may thrive at pH 9.0 and at room temperature of 25 °C, making them suitable for use as a probiotic.The shelf life of prepared health drink was also check by checking the growth of bacteria on to the EMB agar media. So, different test revealed that *S. faecalis* can used as a probiotic and also for the development of probiotic health drink. So Probiotics used in gut microbiota by eliciting an immune response that recognises them as beneficial and forms a protective barrier in the intestine against infections.
Keywords: Probiotics,Catalase ,Gram staining, immune response,gut microbiota

1. INTRODUCTION:

Probiotics are live bacteria that, when taken or administered to the body, provide specific health advantages. They're present in yoghurt and other fermented foods, as well as nutritional supplements and cosmetics.. Although bacteria and other microorganisms aren't considered healthy, but there are some microorganisms which can provide many benefits to the body.In recent years, probiotics have been shown to have a wide range of benefits, including strengthening the immune system and promoting a healthy digestive tract. Probiotics can be obtained through food, beverages, and dietary supplements(1).

Many types of bacteria have been classified as probiotics like ***Lactobacillus, Bifidobacterium, Saccharomyces boulardii*** etc. The German scientist Werner Kollath coined the term probiotic in 1953 to describe "active molecules that are required for a healthy growth of life." In 1965, Lilly and Stillwell coined the phrase "substances produced by one organism that promote the growth of another" to describe "substances released by one organism that stimulate the growth of another."(2).

TYPES OF PROBIOTICS

Probiotics" include different types of microorganisms. They are described by genus, species, tribe and their strain designation. ***Lactobacillus, Bifidobacterium,*** and ***Saccharomyces*** are among the most regularly utilised probiotic bacteria. *Bacillus, Propionibacterium, Streptococcus,* and *Escherichia coli* are among the other genera of probiotics. Novel potential probiotic species isolated from various places of healthy human beings are the topic of ongoing investigations(3).

Lactococcus strains with Probiotic Properties:-

Lactococcus is a Gram-positive lactic acid bacterium genus that ismainly used in the dairy sector to produce different type of fermented foods. These help to reduce the formation of different kind of

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spoiling microorganisms in milk products caused by acidification. *Lactococcus lactis subsp*. lactis strains with probiotic features including adherence to vaginal epithelial cells and nisin synthesis ***(Lactococcus lactis subsp. lactis CV56)*** are also used in conjunction with other probiotics to treat antibiotic-associated diarrhea(8).

MECHANISM OF ACTION OF PROBIOTICS:-

Mainly Probiotic microorganisms have a wide range of effects on the host. The intestine luminal environment, mucosal barrier function, epithelial and the mucosal immune system can all be influenced by different type of species. They also affect dendritic cells, monocytes/macrophages, epithelial cells,B cells, T cells, particularly T cells with regulatory features, and NK cells, among other cell types involved in innate and adaptive immune responses(4).Enhancement of the epithelial barrier, associated inhibition of pathogen adhesion, enhanced adherence to intestinal mucosa,competitive exclusion of pathogenic microorganisms, generation of anti-microorganism chemicals, and immune system regulation are all major probiotic modes of action(5)

Characteristics of good probiotics:-

Bacteria should be able to withstand the digestive system's passage.Probiotic bacteria should be able to colonise and adhere to the intestinal epithelia.It must be able to sustain a high level of viability.Probiotic bacteria should be able to use the nutrients and substrates found in a typical diet and in a colonisation form.It should be able to withstand intestinal transit and adhesion.It should be able to keep the microbiota of the host in check.It should not be pathogenic or harmful.It should be able to have a positive impact on the host.Probiotic bacteria should be resistant to organic acids and low pH.It should be sturdy and capable of surviving in storage for long periods of time(6,7).

2. Experimental Methods or Methodology:

SAMPLE COLLECTION:- Cottage cheese sample was collected from supermarket.

ISOLATION OF BACTERIA :- Bacteria can be isolated by growing them over the various types of media. There are several types of media which can be used to isolate the bacteria from various types of samples. Generally, selective and differential medias are used to isolate the particular type of bacteria .For example:- **MRS (DE MAN, ROGOSA and SHARPE)** selective media was used to isolate the probiotic bacteria i.e *lactobacilli, Lactobacillus acidophilus* .

PREPARATION OF MRS MEDIA:-

PRINCIPLE:-

De Man, Rogosa, and Sharpe1 created the MRS formulation in 1960 which promotes the growth of all lactobacilli strains, particularly the problematic and slow-growing *Lactobacillus brevis and Lactobacillus fermenti strains. Lactic acid bacteria,* such as *Lactobacillus, Streptococcus, Pediococcus* and *Leuconostoc* species, thrive in this medium. These organisms are all capable of producing large volumes of lactic acid. They're Gram positive, catalase and oxidase negative, It contains enzymatic digest of animal tissue, beef extract and yeast extract which are the carbon, nitrogen and vitamin sources used to satisfy the general growth requirements in MRS medium. The medium contains dextrose, a fermentable carbohydrate. Sodium acetate and ammonium citrate serve as both energy sources and selective agents to keep contaminating organisms from overgrowing. The buffering agent is potassium phosphate. Magnesium sulphate and manganese sulphate are cations that are necessary for metabolism. Polysorbate 80 is a surfactant that helps *lactobacilli* absorb nutrients. The turbidity on the medium indicates the organism's development, while bubbles on the Durham tube in the broth show the formation of gas during the sugar fermentation.

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PROCEDURE:-
Take 25ml distilled water in culture bottleAdd all the reagents of MRS media (except agar)Measure the pH of the solutionAdd agar and autoclave the media at 121℃ for 15min at 15psi.Pour the media on to the petri plate and leave it for 20 min to solidify it.

SAMPLE PREPARATION:-
Dissolve 4 to 5 gram of sample in broth.Make 0.9% normal saline water (0.9gm NaCl in 10ml dist. Water).Sterilize it for 20 min and cool it.

SPREADING :-
After solidification, spreading of the sample is done with the help of glass spreader rod. (sample should be evenly spread on the media plate).Cover the plate and fix it with parafilm.Place the plate in incubator at 35℃.Observe the growth of bacteria after 48 hrs.

STREAKING:-
Prepare a new MRS agar media plate.Place it under the UV for 20 min.Pick a single isolated colony from the spreading plate and streak it over the new MRS media plate.Keep the plate in the incubator at 35℃ for 48hrs and observe it.

Gram's staining:-
Gram staining, established by Danish Bacteriologist Hans Christian Gram in 1884, is the most frequent, significant, and widely used differential staining method in microbiology. This test separates bacteria into Gram positive and Gram negative bacteria, which aids in microbial categorization and distinction.Reagents used are Primary stain -Crystal violet,Mordant- Gram's IodineDecolorizer- 95% ethanol or 1:1 acetone with ethanol,Counter stain- Safranine(9).

Catalase test:
Principle:- This test used to test the presence of enzyme catalase which is a common enzyme that is found in all living beings that survive in oxygen and catalyzes the decomposition of hydrogen peroxide, releasing water and oxygen. This enzyme protects the organism from oxidative damage from the reactive oxygen species. Under the aerobic condition, 3% H_2O_2 is used, whereas 15% H_2O_2 is used under anaerobic conditions.

H2O2 ——catalase——> H2O + O2

Procedure: Take a slide and made a smear of test bacteria on to it.Place a few drops of 3% H_2O_2 on the smear.

Interpretation:-Catalase +ve :- bubble formation as O_2 gas is liberated from the H_2O_2 .Catalase -ve :- no bubble formation.

3. Result and Discussion:

3.1 Preparation of MRS media:

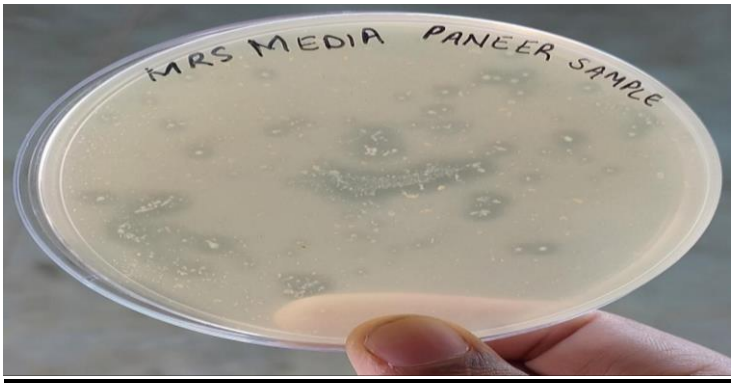

Fig 1 Prepared MRS media for the growth of lactic acid bacteria i.e ***Lactobacillus acidophilus.***

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The nutritional foundation of LMRS agar is made up of proteose peptone no. 3, beef extract, yeast extract, and dextrose. As a source of fatty acids and extra development needs, the medium includes polysorbate 80 (Tween 80) and magnesium. Gram-negative bacteria, oral flora, and fungus may be inhibited by sodium acetate and ammonium citrate. In the presence of these selective agents, *Lactobacilli* recovery is improved. To promote the development of *Lactobacillus spp*., the pH is changed to 6.3–6.7. To prevent the production of oxidised products before use, this medium is produced, distributed, and packed in an oxygen-free environment.

3.2 Spreading &Gram staining of bacteria:

Isolated bacteria was found to gram positive bacteria.When crystal violet which is primary stain is added over the smear of bacteria , it absorbed immediately because gram positive bacteria contains thick peptidoglycan cell wall. It does not decolorize even after the addition of ethanol so that's why gram positive bacteria give positive result and appear as purple colored and also appear as cocci in pairs and short chains or in cluster(10).

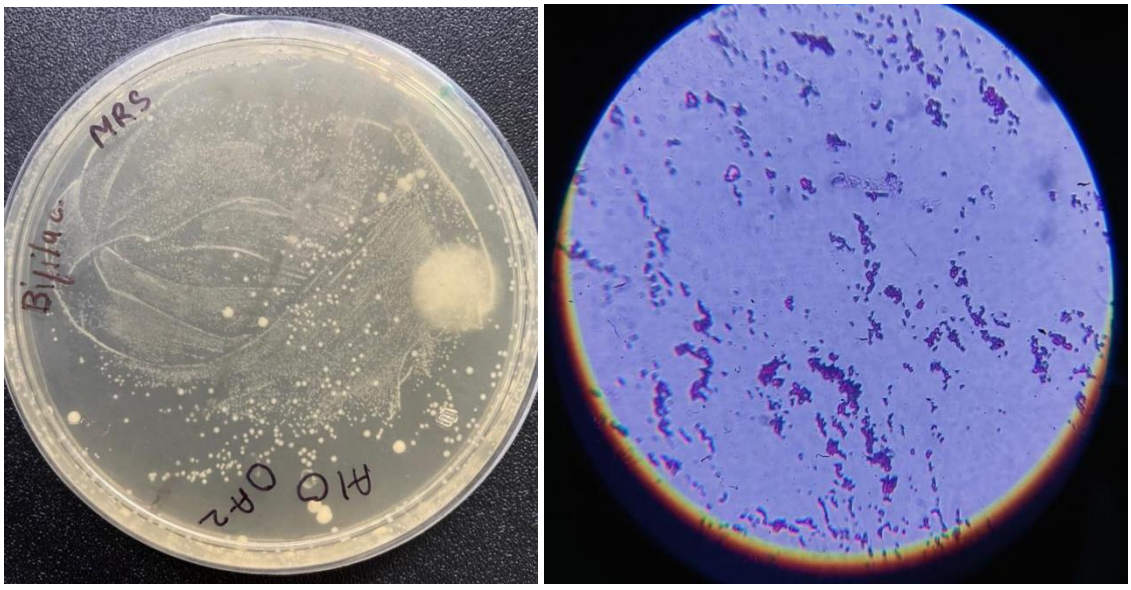

Fig 2 it shows the colonies formed over the MRS media after spreading of bifilac sample with the help of L shape rod spreader.

3.3 Biochemical test:-

Catalase test:-

This test shows that catalase, an enzyme that catalyses the release of oxygen from hydrogen peroxide, is present or not .

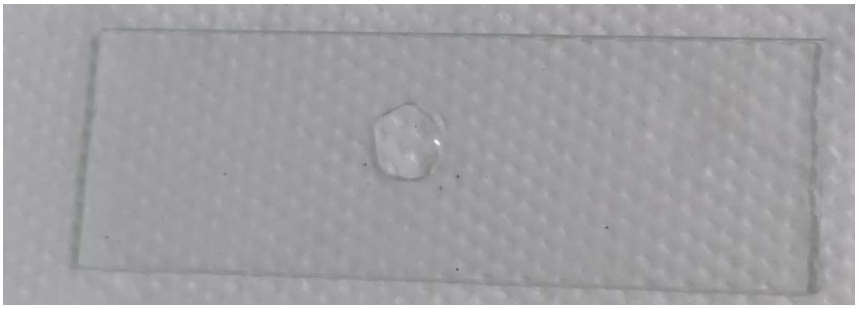

Fig 3. Result of catalase test

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Isolated bacteria was found to be catalase negative bacteria because there were no formation of bubble after addition of H_2O_2. This bacteria do not produces catalase enzyme which catalyse the breakdown of hydrogen peroxide in to oxygen and water. Lactobacillus acidophilus do not produces catalase enzyme.

3.4. Formulation of probiotic health drink by pomogrenate juice:

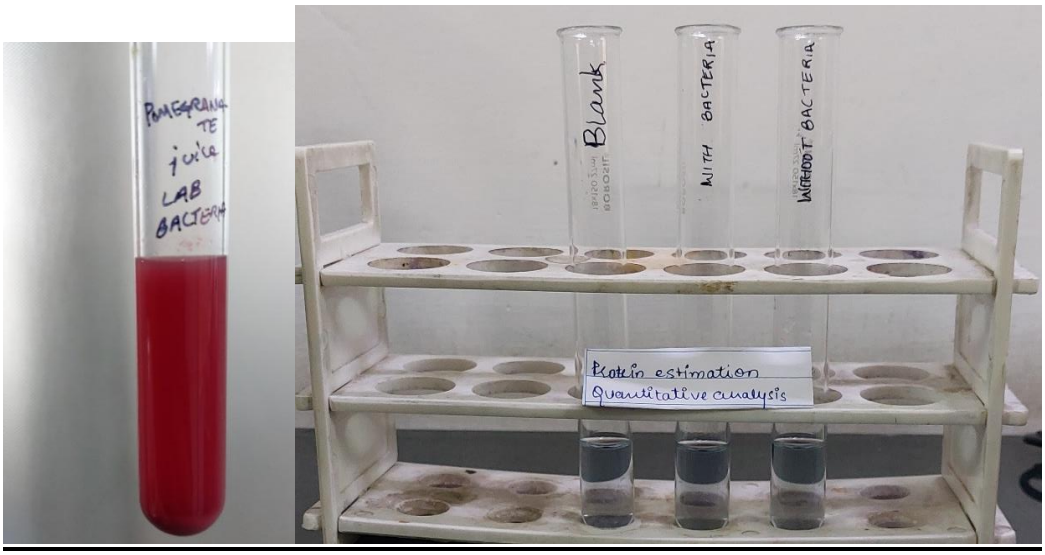

Fig 4. development of probiotic health drink & quantitative protein result of pomogrenate.

Tubes	Concn. (mg/ml)	O.D. at 660nm
Blank	0	0
T1	0.03	0.157
T2	0.06	0.306
T3	0.12	0.623
T4	0.24	1.234
S1	0.1467	0.442
S2	0.2620	0.788

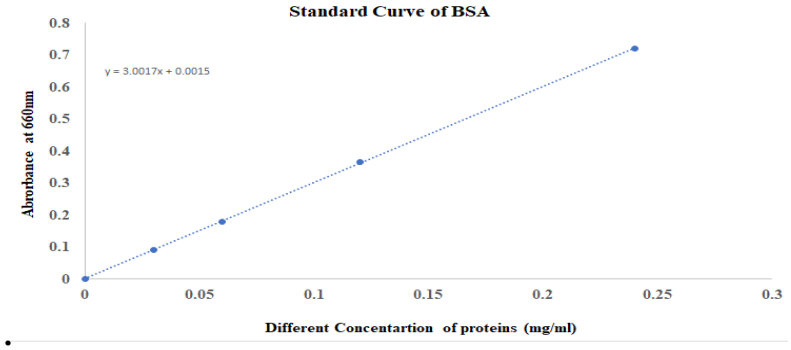

Fig 5 Standard curve of BSA.

For calculations of unknown sample:-

y= mx ±c

x= y-c/m

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x= concn. , y= absorbance , c= intercept , and m=slope.
For S1(control):- X=0.442 - 0.0015/3.0017= 0.1467
For S2(with bacteria):- X= 0.788 - 0.0015/3.0017 = 0.2620
From above result, it is confirmed that isolated bacteria i.e. Lactobacillus acidophilusdoes not reduces the amount of protein from probiotic health drink.

CONCLUSION:

From the above report, it can be concluded that probiotics are useful bacteria for the human health and their well being.It can be utilize to cure several disease like diarrhea , digestive system and also helps in boosting immune system so, can be used it as daily purpose.Isolated bacteria i.e ***Lactobacillus acidophilus*** is also a good probiotics and can be utilized as for the development of probiotic health drink which is more beneficial to human health as it contain carbohydrate in medium amount but proteins and other phytochemicals in good amount. The shelf life of the prepared juice was more than that normal juice as *Lactobacillus acidophilus*reduce the growth of bacteria which deteriorate the properties of juice.So, probiotic health drink are very useful for humans as well as for animals.

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