PROFILING OF MICROBES FROM CURD

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ABSTRACT

When looking upon the people's consciousness over health after (covid-19 pandemic) has made them to take nutrients and supplements in various forms like natural vegetables, fruits juices, chewable etc. to enhance and prevent the serious level of any circumstances. The hygiene among the people has become a good thing which is to be appreciated, which serves to be the first weapon to break the transmission of pathogenic diseases from an individual to other .During this phase of lockdown people came to know about the ancient techniques and medicines to overcome flu, cold, aches. Apart from getting an updated version of medications and facts, people all over the world got acknowledged that microbes do exist in our environment, their pros and cons were clearly assessed by the people. In order to lead a healthy life, maintenance of the microbiota became a primary goal to most of the individuals who were infected; those who just recovered had a task of replenishing their lost microbiota. As lactobacillus is important for maintenance of the gut microbiota, Lactobacillus is one of the microorganisms essential for proper functioning of the system. It plays a fundamental role in various microbiota like gut, oral and vagina. It is a gram positive organism, its size ranges from 1-1.5 micrometers in length and diameter of 0.7-1 micrometer. The work intended here is to undergo an intensive study of lactobacillus microorganism and look up for the chances of applying it into a commercial product like either into a juice or squash, etc.

KEYWORDS: Lactobacillus, microbiota, health, diseases.

INTRODUCTION

Curd is formed from milk. Milk contains the sugar lactose and protein casein. Bacteria like lactobacillus grow on lactose and produce lactic acid as a byproduct (process of fermentation). Production of lactic acid decrease the pH of the milk which further denatures or curdles the milk protein casein. Homemade curd contains different species of the genus lactobacillus like *L.fermentum, L.acidophilus, L.confusus, L.delbruckii, L.lindneri and L.helveticus. Leuconostoc lactis* is also reported to occur in homemade curd. Lactobacillus converts lactose, the sugar in milk, to lactic acid during fermentation. Lactic acid has a pleasant, sour taste. It is completely miscible with water, alcohol and ether although it is insoluble in chloroform thus; it does not crystalline from solution as do other acids. Also, its low melting point means that its liquid at most commonly encountered temperatures. It is a weak acid with good solvent properties, and it polymerizes readily for the production of polymers. In addition many of it salts are quite soluble in water. Thus, these various properties have allowed lactic acid to found wide commercial usage. It provides acidity in food and beverage applications and serves as a preservative in foodstuffs. Crude grades are used for the declaiming of hides in the leather industry, and it is utilized for fabric treatment in the textile and laundry industries. Its ability to form polymeric polylactic acids finds application in production of various resins. Calcium lactate is employed in baking powders, as an animal- and poultry-feed supplement, and as a means for providing a calcium source in pharmaceutical preparations. Copper lactate is used in electroplating. Finally, various chemical derivatives of lactic acid are used in the production of plastics.

Lactobacillus species can be divided into three groups: Obligate homofermentative (Group l) including: *L.acidophilus, L.delbrueckii, L.helveticus, L.salivarius.* Facultatively heterofermentative(Group II)including*: L. casei, L. curvatus, L. plantarum, L.sakei and* Obligately heterofermentative(Group III)including*: L. brevis, L.buchneri, L.fermentum, L.reuteri.* Lactobacilli have an important role in controlling undesirable microflora in the gut and are able to prevent the rise of pathogenic bacteria by producing antimicrobial metabolites. They can be used as biological preservatives and are raised naturally in foods (LE Casisa JR,2016).

MATERIALS AND METHODS

SAMPLING COLLECTION AND PROCESSING:

Curd is best source of Lactobacillus species. Among the other dairy products such as milk, butter milk, etc. sample chosen for the study was curd. Curd was serially diluted from 10^{1} to 10^{14}. In this 10^{-5},10^{-7},10^{-9} are selected. These 3 tubes are performed with spread plate technique and streak plate techniques are performed on MRS medium. They are incubated in incubator at 37°C. After incubation, 3 isolated colonies were grown. Colony characterization is done for these 3 colonies and found to be lactobacillus. A colony shows 100% resemblance with lactobacillus acidophilus. Further tests are to be performed (M.H Helland *et al.,* 2003).

MORPHOLOGICAL CHARACTREIZATION:

STAINING:

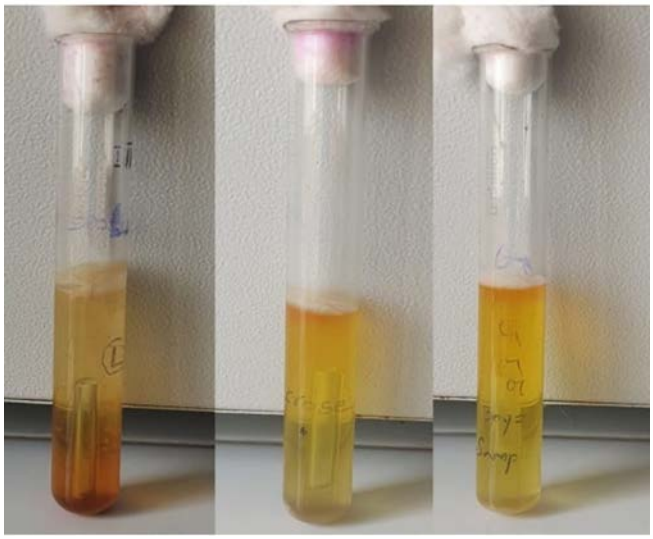
Sterile glass slides were taken and disinfected with 70% ethyl alcohol. A loopful of culture was taken and a smear was made on the glass slide. The smear was heat fixed. The smear was flooded with 2-3 drops of crystal violet and washed after 1min.2-3 drops of gram's iodine was added and washed after 1 min and the smear was decolorized with decolourizer. The smear was counter stained with safranin and washed after 30 seconds. The smear was air dried and viewed under 40x and 100x objectives of microscopic to study the morphology (R.Temmerman *et al.,* 2003).

BIOCHEMICAL TESTS:

CARBOHYDRATE FERMENTATION TEST:

It detects the ability of microorganisms to ferment a specific carbohydrate.3 carbohydrates were taken: Lactose, Glucose, Fructose. Organisms of the sugar were weighed on the weighing balance and added to the test tubes containing peptone water. Methyl red indicator was also added. Durham's tube is placed in inverted position. It was Autoclaved for 15 min at 115 degree Celsius. Bacterial culture (lactobacillus) was inoculated with the help of inoculation loop and 37 degree Celsius in the incubator.

Fig 1: Carbohydrate fermentation



MOTILITY TEST:

Used to determine whether an organisms is motile or non motile. With the sterile inoculation needle, 24 hours old culture of lactobacilli was touched from agar medium. Inoculated tubes were then incubated at 37 degree

Fig 2: Motility test

CASEIN HYDROLYSIS TEST:

To determine the ability of organisms to degrade protein casein. Skim milk agar plate was prepared and inoculated with the simple streak. Inoculated plates were incubated at 25 to 37 degree Celsius for 18-4 hours.

Fig 3: Casein hydrolysis

CATALASE TEST

This test demonstrate the presence of catalase, an enzyme that catalyses the release of oxygen from hydrogen peroxide. Slide method was done to check for the presence of catalase enzyme. Inoculation loop was used to transfer small amount of colony growth in surface of clean, dry glass slide. A drop of 3% H_2O_2 was placed in glass slide. No air bubbles was observed

Fig 4: Catalase test

RESULT ANS DISCUSSION:

In Carbohydrate fermentation, yellow color colonies were observed indicating that acid is produced by fermenting the carbohydrates. No bubbles were observed in Durham's tube, indicating that it does not produce gas. In Catalase test, the test demonstrate the presence of catalase an enzyme that catalyses the release of oxygen from hydrogen peroxide. It interprets that lactobacillus were found to be negative. In Motility, the test indicates that lactobacillus was found to be motile in nature. In Casein Hydrolysis, the clear zone around the simple streak was not observed. In Oxidase test, it indicates that Lactobacilli are oxidase negative.

Table 1. Result

TEST	RESULTS
H2S	Negative
Motility	Positive
Catalase	Negative
Voges Proskauer	Negative
Oxidase	Negative
Carbohydrate fermentation	Positive
Casein hydrolysis	Clear zone was not observed

In our Present study, we isolated lactic acid forming bacteria from two curd Samples. Both the strains were gram positive, non-spore-forming and showing catalase Negative. Identification of the Lactic acid bacteria was performed through morphological characteristics. Besides, other biochemical tests and all of the LAB were identified to its generic level in that *Lactobacillus spp.*were found as whitish, small to large size, the circular

margin on MRS media and *Lactococcus spp*. were found to be creamy white to yellowish color and small to large in size. Gram stain characteristics of the bacterium revealed that all of the bacteria were Grampositive, cocci Shaped, and have different arrangements. *Streptococcus spp*.was found in chain arrangement*; Leuconostoc spp*. were Found more in paired arrangement (Yeshambel Tay *et al.,* 2021).

DISCUSSION

We reported that lactic acid bacteria are facultative anaerobes with a Preference of anaerobic conditions. They cannot synthesize porphyrins and consequently they do not synthesize cytochromes or catalase. Oxygen is sometimes used for formation of hydrogen peroxide, which is toxic for lactic acid bacteria and do not contain catalase to break it down. Lactobacillus species can survive in a highly acidic environment with a pH of 4 to 5 or even lower, and it is Lactobacillus that is responsible for the final stages of fermentation in the various products, as a result of these traits it demonstrates that Lactobacillus spp. survives in low pH environments (Tadesse Degu *et al.*, 2021).

CONCLUSION

Lactic acid bacteria are a group of ubiquitous, heterogeneous, and ecologically diverse bacteria with significance in food fermentation processes. Lactic acid bacteria are also therapeutically useful as an antidote for many food borne related diseases. The impact of lactic acid bacteria is therefore critical in promoting a healthy microbiota and increased immunity against diseases and infections. In addition, probiotics supplementation in human diets cannot be overemphasized based on the countless derived therapeutic health benefits. Probiotics and the advent of lactic acid bacteria are underpinned in the One Health Concept because stable-to-optimum health status requires a well-balanced micro biota composition and a strong immune system. The enlightened culture of food safety now advocates natural remedies that are environmentally friendly while inhibiting pathogens and food spoilage organisms. Thus, the concept of bio-preservation through lactic acid fermentation is a highly recommended alternative for product shelf life extension. The COVID-19 pandemic has resulted in a greater focus on preventive health and innate immunity as pro-active approaches to dealing with this novel corona virus. As a result, it has been suggested that the augmented use of probiotics and greater consumption of lactic acid fermented foods could be among the best ways to boost the immune system and ward off viral infection. As it is widely accepted

that probiotics and lactic acid fermented foods are capable of boosting the body's immune system, the augmented use of these natural food products could be among the best ways to boost immunity and build the first line of defense as the virus. Thus, another potential application of this immune system enhancement would be to explore the use of lactic acid bacteria as a live vaccine prophylaxis against COVID-19. Because lactic acid bacteria are capable of delivering antigens to the mucosal and systemic immune systems and generating specific antibody responses in serum and secretions, lactic acid bacteria as a live vaccine could build an effective immune response. It is also possible to construct biologically contained food grade strains for such a vaccine. This could be a promising vehicle not only for antigens but also for other biologically active compounds such as immunomodulators, antibodies, enzymes, or peptides (Ibrahim, 2020).

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