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COMPREHENSIVE STUDY AND PREPARATION OF CULTURE AGAR MEDIA AND MICROSCOPIC EXAMINATION OF CULTURED LACTOBACILLUS BACTERIA BY USING STAINING TECHNIQUES

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ABSTRACT
Culture media is used in the laboratory for the cultivation of micro-organism supply for nutrients required for growth and maintenance The cultivation of a wide variety of bacteria is commonly done using nutrient agar, or NA, as a general-purpose medium. When studying bacteria, it's important to isolate them from different natural sources and facilitate their growth on artificial media in laboratories. The cultivation of bacteria means promoting the growth of the desired type of bacteria. A culture may be either pure or mixed. Based on this study it is concluded that agar medium is used for isolating (Lactobacillus) microbes and to determine characteristics of colonies. The appearance of colonies and changes that occur in the surrounding media help in the identification of bacterial species. For the identification of bacteria, different staining techniques are used along with microscopes in laboratories. In the differential staining process, There are several chemical stains utilized for better differentiation between different micro-organisms. Structures, cells, and sub-cellular structures. The gram stain is the differential stain for bacteria that is most widely employed (gram-positive and gram-negative). The amount and strain of useful bacteria in curd vary from place to place. The number of different lactobacillus bacteria isolated from curd preparation in India is high as 250 species. But can curd technically called probiotics and also can be consumed by a human with no harmful effects. Nowadays only lactic acid bacteria especially lactobacillus are used as probiotics. The intake of which can be beneficial for the immune system. Probiotics provide good bacteria to the gut, thus improving gut health. Therefore, This laboratory investigation looked at the lactobacillus bacteria's size, shape, and structure and it was compared to what differences were found between the cultured colonies of the bacteria and direct using a sample of fresh curd smear on the slide, where also lactobacillus is present. Gram-positive bacteria are generally considered to belong to the genus Lactobacillus.

Keywords: A Culture media (agar media), lactobacillus bacteria, micro-organism, staining techniques, microscopy, (gram positive- gram negative), probiotic, cultured colonies.

INTRODUCTION

In a culture or growth media, the development of microorganisms is intended to be supported by solid liquid or semisolid. Culture media are used for the general cultivation and maintenance of bacteria stored in the laboratory culture collection because they contain all the components that the majority of bacteria require for growth and are not selective. An indeterminate medium, often referred to as a basal or complicated medium, includes an amino acid source, different salts, and a carbon source, such as glucose and nitrogen like beef and yeast extract. Microbiological studies are dependent on the capacity to cultivate and maintain microorganisms under laboratory conditions by providing suitable culture media that offers favorable conditions. Culture media is a nutritional medium prepared for the development of microorganisms in a lab. Microorganisms can obtain energy directly from sunlight while carbon can be made available in organic form such as carbohydrates or inorganic forms such as carbon-di-oxide and water. Nutrient agar medium is commonly used as a general-purpose medium for the cultivation of a broad range of bacteria. It is a basic medium composed of peptic digest of animal tissue, beef extract, yeast extract, sodium chloride, and agar. Lactic acid bacteria [LAB] were first recognized as a distinct group at the beginning of the 20th century. This category currently demonstrates tremendous technological potential and is found in numerous fermentative food production processes for foods intended for human consumption, including dairy, vegetables, meat, and bakery goods as well as silage for animal feed. These bacteria not only help to shape the nutritional, rheological, and organoleptic qualities of food, but they also create settings that are adverse to the growth of dangerous microbes including Bacillus, Pseudomonas, listeria, and Escherichia coli, among others. Some strains of lactic acid bacteria from the genus Lactobacillus play a significant part in bio-conservation processes and are therefore advantageous to both human and animal health.

MATERIALS AND METHODS

Preparation of Agar Media

The apparatus must be sterilized by moist heat in an autoclave. After then suspend 2.8 gm of [Agar powder] in 100ml of distilled water, in a conical flask. Mix well and other ingredients like (Meat Extract, Peptone, Nacl, Gelatin), etc. one by one (Table-1). Heat gently by using a hot plate and stir until dissolve the medium is complete. The pH must be adjusted, it's checked by using a pH strip. Cool to 45-50c into Petri dishes. then dispense In the Petri dishes, add 15–20 ml of the prepared media. To solidify, let stand for 30 minutes. After that sample streaking out on the solidifying agar media in a Petri plate by using incubating loop. All procedure was done under the laminar airflow. After that, it is kept in an incubator at a fixed temperature for the bacterial colonies' development [1-5].

Table-1: Preparation of Culture media

S.No	Ingredient	Quantity Prescribed for 1000 Ml	Quantity Taken For 100 Ml
1.	Beef extract	3gm	0.3 gm
2.	Peptone	5gm	0.5 gm
3.	Agar- agar	28gm	2.8 gm
4.	Nacl	5gm	0.5gm
5.	Gelatin	5gm	0.5gm
6.	Distilled Water	1000 ml	100 ml

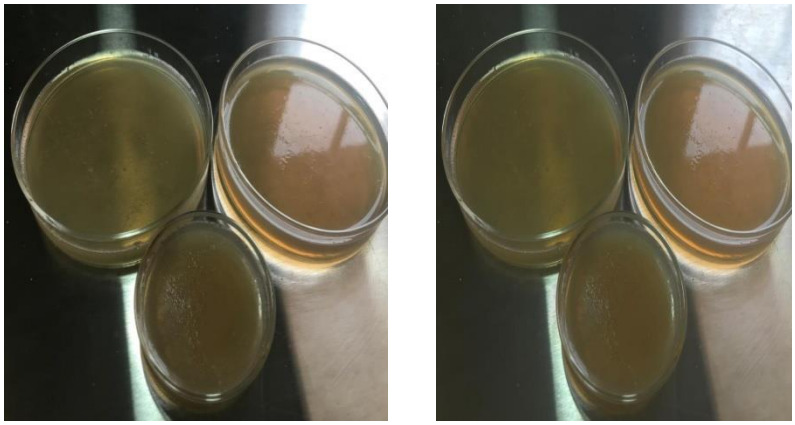

Figure 1: Preparation of Culture media

Isolation Methods for Pure Cultures of Bacteria

Culture is a procedure during which microorganism is cultivated in a medium; if a culture has a population of a single type of microorganism, it is known as a pure culture. A colony is a visible collection of cells that develops when a single bacterial cell is spread out across the surface of a TSA agar plate and given 24 to 48 hours to multiply. The same microbial species will develop colonies on the same medium that are all similar in appearance. This is so that the progeny cells can stack on top of one another in a pattern that creates a distinctive colony form, which is caused by cell morphologies, pigmentation, division plane, rate of cell division, and other distinctive characteristics [6].

(a) Zig Zag Streak

The zigzag streak's steps are as follows: Utilizing an inoculating loop or sterile cotton swab, take a sample aseptically. Right-side up the agar plate. Hold the plate lid in place so that it functions as a barrier against

airborne bacteria, shielding the agar surface. Beginning with the side of the plate furthest from the dominant hand, pass the loop on the agar's surface in a zigzag pattern, covering the whole surface of the plate. Incinerate the loop or discard the cotton swab right away after replacing the lid. For incubation, flip the plate over [7].

(b) Streak Plate Technique

In this technique, a transfer loop is used to streak a portion of the mixed culture across the surface of the agar-containing medium. This operation thins down the germs on the agar's surface, making it easier to separate some specific bacteria from one another. The figure shows various techniques for streaking a culture plate to produce isolated colonies. It is advised to streak the second plate with the same loop and needle without reinoculation it. These plates are then placed in an incubator to promote colony expansion[8].

Figure 2: Streak Plate Technique for pure bacteria culture

Preparation of Smear from (Curd Microorganism) and Preparation of Cultured Bacterial Smear (from Agar Plate for Staining)

Bacteria can be grown on an agar plate and then prepared for staining. Take two clean and grease-free slides for making a smear. In one slide, take one or two loops full of watery curd sample and place them on the slide with a bacteriological loop, and on another slide take cultured bacteria (lactobacillus) from colonies of the prepared agar plate. Then with a circular movement of the loop spread the cell suspension into a thin area. Allow the smear to airdry. Heat fixes the smear while holding the slide at one end and by quickly passing the smear over the flame of the Bunsen burner two to three times [9-13].

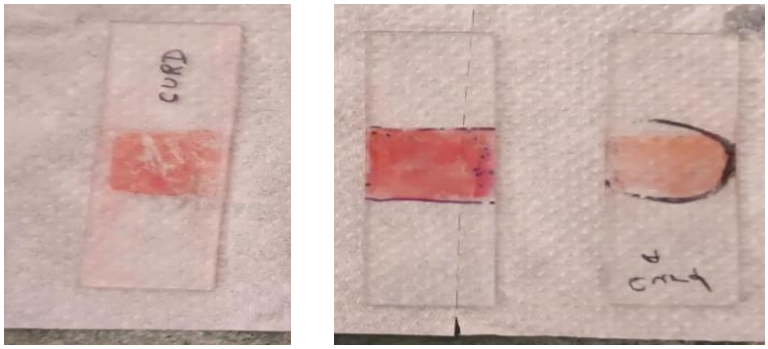

Figure 3: Preparation of culture bacteria Smear from agar plate

Crystal violet should be applied to the smear (both slides of the curd sample and the cultured lactobacillus from the agar plate) and left to stand for a minute. Gently run tap water over the smear to rinse it. Gram's iodine should be applied over the stain, and it should stand for a minute. Gently rinse the smear under running water once more. Pour 95 percent alcohol on the smear to remove colour. Gently rinse the smear under running water once more. Safranine should be applied gently one more over the smear for a minute. Rinse the smear under running water once more, then let it air dry. Utilize a compound microscope to examine the smear [14].

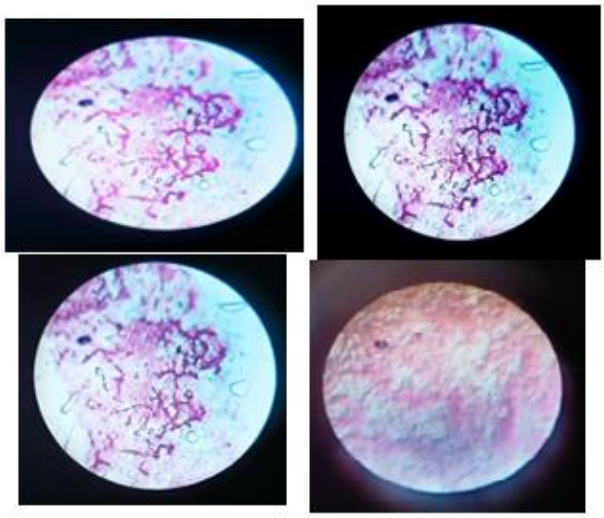

Figure 4: Smear of Cultured (Curd Sample)

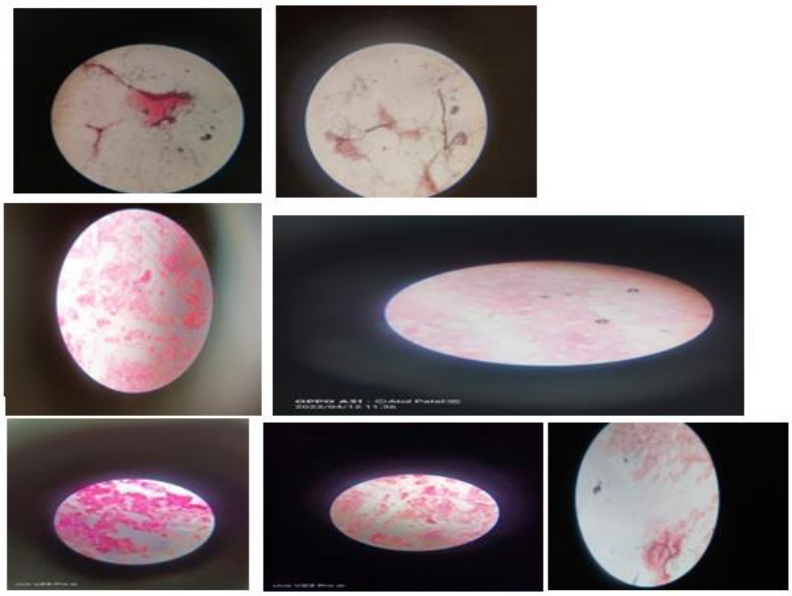

Figure 5: Smear of Cultured (Curd Bacteria) From Agar Plate

Table 2: Results of Smear of Cultured (Curd Sample) and Smear of Cultured (Curd Bacteria)

S.No	Morphology	Arrangement	Colour of cytoplasm	Colour of background	Interference
1.	Rod (Bacilli) Shape	Singles Chain	Violet\Purple	Colourless	Gram Positive
2.	Oval yeast cell (Roundshape)	Singles. Budded	Violet\Purple	Colourless	Gram Positive

RESULT AND DISCUSSION:

Bacteria that appear blue\ violet\ purple are assigned as Gram positive (Figure 4) while as those appearing red\pink (Figure 5) are assigned as Gram negative. Gram-positive bacteria retain primary dye that is violet and do not become discoloured. and purple colour on different prepared slides of smear. Gram-positive bacteria were found to be on the fresh curd sample (Figure 4) and gram-negative bacteria were also found in (Figure 5) that was prepared by the cultured bacteria in the laboratory which appeared red and pink in colour and shape of bacteria was seen like round and rod (cocci and bacilli) on microscopy after staining.

CONCLUSION:

It was concluded that fresh curd samples in the entire study showed 95% positive results (Gram positive bacteria identified) with crystal violet/safranin dye compared to curd bacteria isolated from agar plate. Numerous effects are seen because microbes need a wide range of circumstances to grow and reproduce, including nutrients, oxygen, moisture, and temperature. Despite the fact that there have been relatively few instances of its gram-negative stain appearances. Final conclusion after microscopy and staining techniques found well identified bacterial species, shape and size.

REFERENCES

1. Manivannam R, Singh B, Patel KL and KumarY. Pharmaceutical Microbiology. Thakur publication pvt. Ltd, Lucknow, edition 2018, reprint edition. 2020; 114-119, 69-70, 79.
2. NurHussain et al., Advance in Microbiology, edible growth medium;a new window for probiotic research. Department of Microbiology. Jaganath university, Dhaka, Bangladesh. 2020.
3. Franklund C. Microbiology Laboratory Manual. Observing and Recording the Microbial World Farris Stale University, 2018. https\\github.com websites microbiology laboratory manual.
4. Prathameshs K. Isolation and Identification of Bacteria Fromcurd. European Journal of Experimental Biology. 2014; 4(6): 95-97.
5. Uthayasooriyan M et.al. Formulation of Culture Media for Bacteria Growth. Der PharmaciaLettre, 2016; 8(1): 431-436.
6. Rehman A, Tashiyo MA and Sonomoto K. Advance in Lactic Acid Production by Microbial Fermentation Processes. Biotechnology Advances. 2013; 31(6): 877- 902.
7. Dhir A. Textbook of Applied Microbiology including Infection. Control and Safety. CBS Publishers and Distributors Pvt. Ltd. 2020; 74-84, 110-129, 130-143, 144-158.
8. Kalplan ML and Kaplan L. The Gram Stain and Differential Staining. au of Laboratories. Journal of Bacteriology, 1932.
9. Beveridge TJ and Graham LL. Surface Layer of Bacteria Microbial Rev. 1991; 55: 684-705.
10. Brown E. Alfred Benso's Microbiological Application. Edition 9, MC Graw Hill Publication, New York.
11. Choudhary T et al. Isolation, Identification and Determination of the Probiotic Potential of Lactic Acid, Bacteria from Local Curd, International Journal of Scientific Research. 2016; 7(4): 263-267.
12. Shah G, Shaikh M. Determination of Probiotic Properties of Lactic Acid Bacteria from Curd Global Journal of Biology. Agriculture and Health Science, 2019; 2(2): 119-122.

13. Gomez CEM et. al. Isolation, Characterization and Continuous Culture of Lactobacillus Spp. and its Potential Use for Lactic Acid Production From Whey Biotransformation Group, School of Microbiology University, Medellin, Colombia, Food Science, Campinas. 2020; 40 (11): 1021-1028.
14. Wani I and Rosy W. Experiments in Microbiology. S Vikas and Company (Medical Publishers) PV books. Edition 2. 2017; 32, 96, 97.