Gopal S, 2021, 9:6
ISSN (Online): 2348-4098
ISSN (Print): 2395-4752

Formulation of Media for Lactic Acid Bacteria

Gopal S, Shivalkar Yadav, Rajsekhar P. And Prabha R.
Dept. of Dairy Microbiology,
Dairy Science College,
KVAFSU, Hebbal, Bengaluru
*corresponding author - raoprabharao@gmail.com

Abstract- Bacteria are single celled prokaryotes present ubiquitously in nature. Good bacteria are also known as beneficial bacteria, involved in natural or controlled fermentations and some are probiotic in nature enhancing the human gut health. Lactic Acid Bacteria (LAB) that include Lactococcus, Streptococcus thermophilus, Leuconostoc, Lactobacillus bulgaricus, Lactobacillus acidophilus and Bifidobacterium are among the most important groups of microorganisms used in food fermentation. They contribute to the taste and texture of fermented products and inhibit food spoilage bacteria by producing growth inhibiting substances and large amounts of lactic acid. LAB is a group of Gram positive, non-spore forming cocci or rods, catalase negative, produce lactic acid only or other acids or ethanol and gases. . In a mixed lactic culture, enumeration of each group of LABS is difficult as one may predominate on others. These lactic cultures used in fermented milk products require enumeration and sometimes isolation using selective media. M17 agar, ST agar, LUMS, MRS agar and bifidobacteria agar are used for selective enumeration of lactococci, Streptococcus thermophilus, leuconostoc, lactobacilli and bifidobacteria respectively, by serial dilution and anaerobic incubation at optimum growth temperature of the genus 30°C or 37°C. If fermented milk has yeast and aerobic spore formers as contaminants then any of them predominate and isolation of the LAB become difficult. Addition of antimycotic (Nystatin, sodium benzoate) and antisporulating (calcium propionate) agents into the selective lactic acid media may help in controlling the contaminants from LAB.

Keywords: Lactic acid bacteria; Contaminants; Antimycotic agent; Fermentation.

I. INTRODUCTION

Bacteria are ubiquitous in nature. They are single celled prokaryotes. There are good and bad bacteria on earth. Good bacteria are also known as beneficial bacteria, involved in natural or controlled fermentations and some are probiotic in nature enhancing the human gut health.

Spoilage and pathogenic bacteria spoil foods and cause disease respectively. Lactic Acid Bacteria (LAB) are among the most important groups of microorganisms used in food fermentation. They contribute to the taste and texture of fermented products and inhibit food spoilage bacteria by producing growth inhibiting substances and large amounts of lactic acid. LAB is a group of Gram positive, non-spore forming cocci or rods, catalase negative, produce lactic acid only or other acids or ethanol and gases. Lactic acid bacteria most commonly used in fermented milk industry include species of Lactococcus, Streptococcus thermophilus, Leuconostoc, Lactobacillus bulgaricus, Lactobacillus acidophilus and Bifidobacterium [1].

Fermented milk products prepared using the combined or mixed lactic cultures the difficulty encountered is separation of genus during enumeration. Microbial contaminants like Bacillus spp. or yeast enter the product during processing or

International Journal of Science, Engineering and Technology

An Open Access Journal

packaging may predominate over lactic cultures when plated even on selective media mentioned above.

Antimycotic agents such as nystatin, calcium propionate and sodium benzoate may help in inhibition of the yeast as well bacterial spores. Emphasis has to be given for avoiding microbial contaminants and selective enumeration in a mixed lactic flora from fermented milk products by use of proper inhibitors and selective agents in a medium [2].

II. HISTORY OF BACTERIOLOGICAL MEDIA

Arguably the first to cultivate microorganisms on a growth medium, with a degree of reproducibility, was the French chemist and microbiologist Louis Pasteur (1922-1985). Robert Koch (1843-1910) discovered that broths based on fresh meat extracts produced optimal growth that used gelatin a solidifying agent and his contribution towards culture medium formulation, named him as 'The Father of Culture Media'.

In 1882 that Fannie Eilshemius (1850-1934)3, later the wife of Dr. Walther Hesse (1846-1911) (who was Koch's research assistant), suggested replacing gelatine with agar. Fannie Hesse had been inspired by the use of agar to prepare fruit jams and jellies (agar had been used as a gelling agent in parts of Asia for centuries.

Agar (or 'agar-agar') is a phycocolloid water soluble polysaccharide derived from red-purple seaweeds (the various species of Rhodophyceae belonging to the genus Gelidium and Gracilaria). A further important development for the manufacture of solid media occurred in 1887 when Julius Richard Petri (1852-1921), worker in Koch's laboratory, was involved in modifying the flat glass plate into a shallow, circular glass dish with a loose-fitting cover to culture bacteria later called Petri dish.

In 1960, de Man, Rogosa and Sharpe, designed MRS, as selective culture medium to favour the luxuriant growth of Lactobacilli and named as de Man Rogosa Sharpe agar (MRS). Later in 1975, Terzaghi and Sandine composed M17 agar for Lactococcus species isolated from milk products.

Tanaka and Mutai in 1980 introduced bifidobacterial selective agar for selective enumeration of bifidobacteria from probiotic foods [3].

III. LACTIC ACID BACTERIA (LAB)

Lactic acid bacteria are among the most important groups of microorganisms used in food fermentation. They contribute to the taste and texture of fermented products and inhibit food spoilage bacteria by producing growth inhibiting substances and large amounts of lactic acid. Lactic acid bacteria (LAB) are a group of Gram positive, non-spore forming cocci or rods, catalase negative and fastidious organism with high tolerance of low pH (5.5 to 5.8), produce lactic acid through homo or hetero fermentation.

Some of the lactic acid bacteria produce bacteriocins and vitamins. LAB are involved in the commercial preparations of natural or controlled fermented products like batters, pickles and fermented milks like curd, yogurt, probiotic milks and so on [4]. Lactococci is used in preparation of curd, CBM, Cheddar cheese ex: L.lactis ssp.lactis L.lactis ssp.cremoris L.lactis ssp.lactis bv. diacetylactis Streptococcus thermophilus used in the preparation of yogurt, Emmental cheese.

Leuconostoc is used in the preparation of curd, cultured butter milk, Gouda cheese. ex: Leu.mesenteroides ssp. mesenteroides Leu .lactis. Lactobacilli is used in the preparation of acidophilus milk, yogurt and bioyogurt. ex Lb.acidophilus Lb. bulgaricus. Bifidobacteria, is used in the preparation of bifidus milk ex: B.bifidum [5].

IV. ENUMERATION OF LAB

LAB used for preparation of FMP can be enumerated by serially diluting the sample and pour plating using selective media. If single LAB is used then problem of enumeration is not difficult. In a mixed culture, enumeration of each group of LABS is difficult as one may predominate on others [6].

These lactic cultures used in fermented milk products require enumeration and sometimes isolation using selective media. M17 agar (Disodium β-glycero phosphate as buffering agent maintaining pH of 5.7 to avoid acid injury to cells), ST agar, LUMS, MRS

International Journal of Science, Engineering and Technology

An Open Access Journal

agar and bifidobacteria agar are used for selective enumeration of Lactococci, Streptococcus thermophilus, Leuconostoc, Lactobacilli and Bifidobacteria respectively, by serial dilution and anaerobic incubation at optimum growth temperature of the genus 30°Cor 37°C [7].

Harrigan & McCance (1966) [8], used NRCLA with pH of 6.8 for the detection of lactic Streptococci in milk and milk products and yellow colonies were considered as lactococcal colonies.

Terzaghi and Sandine (1975) [9], used M17 agar for isolation of lactococci from fermented milk products which formed white coloured subsurface colonies.

Pradeep (2007) plated curd for Lactococci on NRCLA and M17 agar with Counts of (log10cfu/g) 7.87 on NRCLA agar and 8.20 on M17 agar and declared that M17 as better medium for lactococcal and streptococcal enumeration. Streptococcus thermophilus from yoghurt formed yellow colonies on ST agar as it has pH indicator bromocresol purple.

Dave and Shah (1998) [10], obtained viable count of 8.50 log10cfu/g on ST agar while count on M17 was 8.00 log. They found ST agar is better than M17 agar.

LUSM (Leuconostoc Selective Medium) has sorbic acid, sodium azide to inhibit yeast, and lactobacilli respectively, while Vancomycin controlled lactococci growth and tetracycline inhibited lactobacilli, Cysteine and tomatojuice (manganese sulphate) stimulated leuconostoc forming slimy, smooth and round grayish colonies. MRS media for the enumeration and isolation of Leuconostoc spp. in dairy products that formed Viable counts obtained for Leuconostoc on MRS agar was 7.10 while on LUSM medium counts were 8.00. LUSM was considered as best medium for Leuconostoc as per Benkerroum et.al. (1993)[11].

MRS agar with pH .5 was designed as selective culture medium to maintain the pH with acetatto favour the luxuriant growth of lactobacilli by de Man, Rogosa, & Sharpe (1960)[12], that formed white coloured submerged colonies. Trabulsi and Ewing (1962)[13] used Acetate agar (pH 5.4) for the isolation and cultivation of Lactobacillus species having triammonium citrate (buffering agent).

Nwadiuto et al., (2018) [14], plated yoghurt sample and found viable count (log10cfu/g) of lactobacilli as 7.41 on MRS agar and 4.59 on Acetate agar. They declared MRS agar as best medium for lactobacilli.

Tanaka and Mutai (1980) [15], recommended Bifidobacterium agar with supplement of propionic acid and lithium chloride as selective agents for the enumeration & isolation of Bifidobacteria.

Teraguchi et.al (1978)[16], isolated bifidobacteria using MG agar (pH 6.4 with cysteine and lithium chloride as selective agents) infant faeces forming subsurface cream coloured colonies.

Samona and Robinson (2007) [17], found Bifidobacterium agar with supplement of propionic acid and lithium chloride as best with 8.5 log viable count in Korean probiotic milk.

V. PROBLEMS ENCOUNTERED IN MEDIA USED FOR ENUMERATION OR ISOLATION OF LAB

If fermented milk has yeast as contaminant then yeast predominates over LAB. Once yeast sometimes even Bacillus spp. make colonies then isolation of the LAB become difficult. Media may require addition of antimycotic (Nystatin, sodium benzoate) and antisporulating (calcium propionate) agents.

Nystatin (C47H75NO17) has MW of 926. Nystatin is produced form bacterium Streptomyces nouresi. It is soluble at 28°C in methanol, poorly soluble in water but better solubility is observed in phosphate-citrate buffer at 5.7. The concentration of 50 mg/Lt of the media after sterilization just before pouring inhibits yeast & molds. Alonso-Vargas et.al. (2000)[18], found reduction in 5 log10Cfu of candida when 8mg/lt was used in PDA. Nystatin binds to ergosterol of yeast cell membrane. Forms pores in the membrane that lead to K+ leakage, acidification and lysis of yeast cell.

Calcium propionate (C6H10CaO4) is having MW of 186, soluble in water. The compound is effective at pH 5.5. It prevents microbes from producing the energy as they are protonophores at 0.4 -1 % that inhibited Bacillus spores as well yeast and molds. Sodium benzoate (C6H5COONa) is having MW of 144.

International Journal of Science, Engineering and Technology

An Open Access Journal

Optimum activity of this compound is observed at pH 5-6 as it is soluble in water. The concentration of 0.1-0.3 % inhibited yeast and molds by leakage of PMF and thus ATP synthesis of fungi is affected [19; 8].

VI. CONCLUSION

Lactic acid bacteria play important role in the production of fermented milk products. The viability of the lactic acid bacteria used during storage especially probiotics as well as isolation of these bacteria require optimized media.

The media used should inhibit contaminants like yeast and aerobic spore forming bacteria (Bacillus spp.) with the addition of antimycotic and antisporulating agents.

VII. ACKNOWLEDGEMENT

Authors are grateful to Dairy Science College, Karnataka Veterinary, Animal and Fisheries Sciences University, Hebbal, Bengaluru-24 for providing this opportunity to publish this review article.

REFERENCES

[1] Mokoena, 2017. Lactic Acid Bacteria and Their Bacteriocins: Classification, Biosynthesis and Applications against Uropathogens: A Mini-Review. Molecules. 22(8):1255 doi: 10.3390/ molecules22081255

[2] Mann D.A and Beuchat L.R. 2008. Combinations of antimycotics to inhibit the growth of molds capable of producing 1, 3-pentadiene. Food microbiol. 25(1):144-153.

[3] Sandle, t. (2011): 'history and development of microbiological culture media', the journal (Institute of Science and Technology), winter 2010-2011, pp10-14.

[4] Tamang, J. P., Watanabe, K., and Holzapfel, W. H., 2016. Diversity of microorganisms in global fermented foods and beverages. Front. Microbiol. 7:377. doi: 10.3389/fmicb.2016.00377

[5] Panesar. P.s., 2011. Fermented dairy products: starter cultures and potential nutritional benefits. Food and nutri. Sci. 2:47-51.

[6] Briggs,M.J., 1953. An improved medium for lactobacilli. J.Dairy Res. 20:36-40

[7] Lanxin, m., hao, j., lin, p., qiangchuan, h., chuanjuan. L.ptueva, D. Heping, z., and jie yu., 2019. Biodiversity of lactic acid bacteria isolated from fermented milk products in xinjiang, china food biotechnol. 33(2).

[8] Harrigan, W.F and McCance, E.M., 1966. Laboratory Methods in Microbiology. Vol. 54, Academic Press, Cambridge.

[9] Terzaghi, B.E. and Sandine, W.E., 1975. Improved medium for lactic streptococci and their Bacteriophages. Appl. Microbiol. 29(6): 807-813

[10] Dave, R.I. and N.P. Shah, 1998. Ingredient supplementation effects on viability of probiotic bacteria in yogurt. J. Dairy Sci. 81: 2804-2816.

[11] Benkerroum N., Ghouati, Y., Sandine W.E. and Tantaoui-Elaraki A., 1993. Methods to demonstrate the bactericidal activity of bacteriocins. Lett. Appl. Microbiol. 17(2):78-81.

[12] De Man, J.C., Rogosa, M. and Sharpe M.E., 1960. A medium for the cultivation of lactobacilli. J.Appl. Bacteriol., 23(1): 130-135

[13] Trabulsi, L.R. and Ewing W.H., 1962 Sodium acetate medium for the differentiation of Shigella and Escherichia cultures. Publ. Hlth Lab. 20:137

[14] Nwadiuto O., Nwamaioha, Salam A and Ibrahim, 2018. A selective medium for the enumeration and differentiation of Lactobacillus delbrueckii ssp. bulgaricus. J. Dairy Sci. 101(6). DOI:10.3168/ jds.2017-14155

[15] Tanaka R. and Mutai M., 1980. Improved medium for selective isolation and enumeration of Bifidobacterium. Appl Environ Microbiol. 40(5): 866–869.

[16] Teraguchi S, Uehara M, Ogasa K and Mitsuoka T., 1978. Enumeration of bifidobacteria in dairy products. Jpn J Bacteriol. 33:753–761

[17] Samona A. and Robinson R.K.. 2007. Enumeration of Bifidobacteria in dairy products. Int. J. Dairy Technol., 44(3):64-66

[18] Alonso-Vargas, R., González-Alvarez, L., Ruesga, M.T. 2000. In vitro activity of a liposomal nystatin formulation (Nyotran) against Cryptococcus neoformans. Rev Iberoam Micol. 17: 90-92.

[19] Schillinger U and Holzapfel W., 2003. Culture media for lactic acid bacteria. Progress in Industrial Microbiology 37:127-140 In book: Progress in Industrial Microbiology (pp.127-140), Handong Global University.