JOURNAL OF CRITICAL REVIEWS

ISSN- 2394-5125 VOL 5, ISSUE 06, 2018

ISOLATION AND CHARACTERIZATION OF LACTIC ACID BACTERIA FROM MILK PRODUCT

P.D.Khode[1], B.R.Dhakate[2], P.A.Shankhawar[3]

1,2,3 Assistant Professor, Maharashtra Institute of Pharmacy, Betala Bramhpuri (M.H)441206

*Correspondence for Author

***Dr. Sachin B.Dudhe** Department of Pharmaceutics, Maharshtra Institute of Pharmacy ,Betala, Bramhapuri.441206

ABSTRACT

Lactic acid bacteria are the most important and commonly used bacteria in food industrials. Specially selected starter cultures are required for the industrial production of cheese. These starter cultures are mainly composed of lactic acid bacteria (LAB). Starters LAB have many functions in cheese production. They produce lactic acid during the fermentation process and provide formation of the curd.

Furthermore, they show proteolytic activity and also they play a role inthe production of aroma compounds and antimicrobial substances. In order to prevent loss of LAB biodiversity and loss of traditional cheese diversity, it is important to identify novel LAB from traditional cheese.

KEY WORDS: Lactic acid bacteria, starter culture, fermentation and isolation of LBA.

1. INTRODUCTION

Production of cheese is essentially achieved by bringing four ingredients together: milk, rennet, microorganisms, and salt. The process includes the following steps: gel formation, acid production, whey expulsion, salt addition, and finally ripening period. The main biochemical changes that occur in cheese manufacture is the production of lactic acid from lactose. This is achieved by different species of lactic acid bacteria (LAB). The responsible flora that form acid development during cheese production are starter cultures that cause decrease in the pH, formation of curd, expulsion of whey[2].

For the identification of novel starter strains, working with fresh cheese is very important because fermentation occurs at the beginning. Strains participate in fermentation process diminish immediately after fermentation. It is reported that at the fermentation step, starter strain amount may reach up approximately 10^9 colony forming units (cfu) per g of cheese. During ripening, however, the number of starter cells decreases about two orders of magnitude [2]. There have been many reports about the isolation of starter LAB from traditional

ISSN- 2394-5125 VOL 5, ISSUE 06, 2018

cheese [3,7,10,11,14,15,16,17,18,19,20,22].

2. Lactic Starters in Cheese Industry

According to some research conducted, in many dairy industries, starter cultures can bdivided into three groups as; (1) Mesophilic starter cultures, (2) Thermophilic starter cultures,
(3) Artisanal starter cultures. Each of them can be divided further: undefined cultures, in which number of strains is unknown and defined cultures which are composed of a known number of strains [1].

Mesophilic Starter Cultures

Mesophilic group of LAB have an optimum growth temperature at 30 °C. They are composed of species which belong to the two genera, Lactococcus and Leuconostoc. Lactococcus lactis ssp lactis, Lactococcus lactis ssp. cremoris which are acid producers and Lactococcus lactis ssp. lactis var. diacetylactis, Leuconostoc lactis, Leuconostoc cremoris which are flavor producers. According to the nature of flavor produced (citrate positive strains), mesophilic cultures can be divided into 4 group; 1. O type, containing only L. lactis ssp. lactisand L. lactis ssp. cremoris. 2. D type, containing citrate fermenting species as flavor producers only L. lactis ssp. lactis var. diacetylactis. 3. B (or L) type, containing citrate fermenting species as flavor producers only Leuconostoc. 4. BD (or LD) type, containingboth flavor producers as L. lactis ssp. lactis var. diacetylactis and Leuconostoc.

Mesophilic starter cultures are used in the manufacture of broad range of cheese type. It was estimated that two thirds of the milk fermentation is mesophilic type. The dairy industry is concerned with strains which ferment milk as rapidly as possible. This appears to be a property of Lactococci. Strains of other species will lower the pH of milk at a much slower rate [8]. After fermentation of milk, the autolysis of the lactococci is occured during following ripening times. Autolysis of starter cells is due to a muraminidase [2]. This could be originated by NaCl concentration and associated salt in moisture values of cheese [2].

Thermophilic Starter Cultures

They are used in making cheese types where high cooking temperatures are required (Emmental, Gryere, Grana, Comte). The thermophilic LAB belong to two genera which are Lactobacillus and Streptococcus. Although Lactobacillus is a large group that consists of 64 species with both homo- and heterofermentative characteristic. Only a few of them areinvolved in milk fermentation. The commercial lactobacilli starters mainly consist of Lactobacillus delbrueckii ssp bulgaricus, Lactobacillus delbrueckii ssp lactis and Lactobacillus helveticus

which are obligate fermenters. On the other hand, Streptococcus thermophilus is only one dairy and food associated species among 27 Streptococcus species. Until recent times, it was described as Streptococcus salivarus ssp. thermophilus because it was observed to have a very close relationship with Streptococcus salivarus. After a more detailed DNA hybridization analysis, raised it to species level again [24]. Therefore, it is suggested that only the galactose fermenting lactobacilli should be used as starter together with Str. thermophilus [25].

Artisanal or "Natural" Starter Cultures

Artisanal cultures are derived from using part of a previous batch of fermented product to inoculate a new batch. For instance, Kopanisti which is a Greek cheese variety, is produced by mixing cheese from a previous batch with drained curd of new batch. It is obvious that their composition is very complex, relatively variable and often undefined. Several types of species may be present. Although their variable performance contrasts with current trends in starter technology where consistent performance is required, their replacement by defined starter systems has sometimes results in less flavor [1].

Starter Functions 2.4.1.Acid Production

LAB use carbohydrate fermentatively and produce lactic acid. Lactic acid production leads decrease in pH. Fermentation of sugars that cause leading to pH decrease is important for clotting of milk. Beside, increasing acidity initiates following desirable reactions and changes such as whey expulsion. Because there is a correlation between pH and whey expulsion from curd. Additionally, acid production has beneficial effect on formation of texture, aroma and flavor [23].

Proteolytic Activity

Proteolysis is an important event that occurs during cheese ripening. The lactic acid bacteria use the polypeptides. These polypeptides are generated by milk clotting enzymes and by bacterial cell-wall proteins. Rennet which is the milk clotting enzyme, is responsible for casein degradation. Because of the casein degradation peptides are produced which aretransported into the cell. In the cell, peptidases continue degradation to produce smaller peptides and amino acids. It has been known that aminoacid composition plays an essential role in the aroma of cheese [12].

Flavor Formation

The quality of cheese and other fermented food products is dependent on the ability of flavor and aroma production of microorganisms which include starter culture. Flavor compounds

produced by LAB can be divided into two groups; the compounds in fermented milk, the compounds present mostly in maturated cheese. First group consists of organic acids such as lactic acid and acetic acid, which are produced by L. lactis ssp lactis and L. lactis ssp cremoris. Second group consists of acetaldehyde, diacetyl, acetoin, and 2-3 butylene-glycol which are produced by L. lactis ssp. lactis biovar diacetylactis and Leuconostoc species from citrate present in milk. It has been reported that these aroma compouns might be produced to avoid to pyruvate accumulation in the cell. Moreover, improved knowledge of proteolysis and peptidolysis in cheese, analysis on enzymatic systems of LAB and evaluation of different strains, will provide better understanding between flavor development and starter activity. A number of different LAB have been evaluated for their ability to degrade amino acids to aroma compounds. L. lactis subsp. lactis and L. lactis supsp. cremoris , Lactobacillius lactis, Lactobacillius helveticus, Lb. bulgaricus, Lb. casei are capable of degrading methionine to methonethiol, dimethyledisulphide (DMDS) and dimethyltrisulphide (DMTS) [13].

Antimicrobial Property

LAB have been used as natural preservatives because of their antimicrobial capacity a.Through fermentation products: Antimicrobial activity can be exerted through the reduction of pH or production of organic acids (lactic acid, acetic acid), CO2, reuterin, diacetyl, 2- pyrorelidone, 5-carboxylic acid (PCA) (Mayra, Makinen and Bigret, 1998). Effective starter culture activity can prevent the pathogen and contaminant growth that may occur during cheese making process. b. Through bacteriocins: Bacteriocins can be defined as protein antibiotics of relatively high molecular weight and mainly affecting the same or closely related species. It is known that LAB are generally regarded as safe microorganisms and so are their bacteriocins. Thus, these bacteriocins can potentially be used to control the growth of spoilage and pathogenic organisms in food [4]. Bacteriocin producing lactococcal strains have been used successfully as starter cultures for cheesemaking in order to improve the safety and quality of the cheese. In recent work, 79 wild lactococci have been studied and 32 of theese have been found to be antimicrobially active [12]. In 17 of these strains, the well- known antimicrobial peptide nisin has been found, whereas the others produced diplococcin, lactococcin or a unidentified bacteriocin-like compound. Moreover, the use of nisin as an effective preservative in processed cheese has been widely accepted.

Commercial Production of Dairy Starter Cultures

Starter cultures are essential for industrial production of all kinds of cheese. Before adding to

ISSN- 2394-5125 VOL 5, ISSUE 06, 2018

milk, cultures have been pre-grown in milk or milk-based media. Depending on the cheese type, the inoculation volume varies from 0.2% to 2% of volume of milk [8]. Each year ~12.5 x 10^{10} tons of milk are used in order to produce ~12.5 x 10^{6} tons of cheese worldwide. If it is assumed that 0.5% (v/v) in ratio inoculums used for each type of cheese, it indicates that ~6.3x 10^{8} L starter is required. Commercial production of dairy starter cultures refers this mother culture.

a) Liquid Starter Culture

This is a traditional method which is based on the following procedure. First the starter is cultivated as a liquid stock culture, and then sufficient volume is obtained by subculturing. This method has advantages, if the production area and laboratory that provide culture is close. On the other hand, contamination risk due to the number of inoculations, phageinfection could be faced. Strains which are kept in liquid media can easily loose its starter properties also.

b) Air Dried Culture

This method is based on the principle of adsorption of liquid cultures on a special material and drying by pulverization under the vacuum. However, during the vacuum pulvarization most of the cells can die.

c) Freeze Dried Starter Cultures (Lyophilization)

Lyophilization is a process in which the product is first frozen so that a matrix is formed in which the solvent is crystallized and separated from its solute. Solvent is then removed by sublimation and desorption. Despite the advantages as easy to use, significant amounts of cell injury and death occur.

d) Freeze-Dried Concentrated Starter Culture

This method aims of direct inoculation of the milk. Freeze-dried concentrated cultures are being used extensively in Europe while in US has limited usage. This is because of that this typ of starters require more time to reach log phase of growth.

e) Frozen-Concentrated Starter Cultures

Frozen concentrated starters usually contain $10^{1\ 0}$ to 10^{11} cfu/g. In this method, the most critical point is rate of thawing in order to minimize cell injury. It is important to thaw the samples rapidly as possible.

Genetically Modified Lactic Acid Bacteria and Culture Improvement

ISSN- 2394-5125 VOL 5, ISSUE 06, 2018

Due to the considerable economical importance of LAB, culture improvement studies have been accelerated in recent years. Progress in gene technology has allowed this development. Modification has been achieved by introducing new genes to improve bacteria that better fitted to technological processes or enhanced organoleptic properties. It is expected that better understanding of the genetics and physiology of LAB will give rise to better strain use, selection and improvement [23]. Construction of bacteriophage resistant strains is very important. The resistance mechanisms are often carried out by plasmids and transposons. Some high level resistance plasmids were shown to carry more than one resistance mechanisms [6].

In some cases, the starter strains have been engineered for autolysis. These cells will lyse at an appropriate moment during cheese making. Lysis allows the release of many enzymes into cheese matrix that leads to degradation of peptide to amino acids. These free amino acids are the precursors of aromatic substances (Renault, 2003).

In the future, it will be beneficial to determine the following characteristics of the isolated strains.1. Bacteriophage resistance. 2. Proteolytic activity. 3. Lipolytic activity. 4. Production of aroma and flavor compounds. 5. Antimicrobial properties. 6. Dietetic properties (L- Lactic acid produced, probiotic properties). Finally, the isolated strains might also be tried for new fermented food formulations.

REFERENCES

1. Axelsson L. ì.1998. Lactic acid Bacteria: Classification and Physiologyî in Lactic acid Bacteria, Microbiology and Functional Aspects, edited by S. Salminen and A. Von Wright (Marcel Dekker Inc, New York: 1998, pp.1-73.
2. Beresford TP, Fitzsimons NA, Brennan NL, and Cogan TM. ìRecent advances in cheese microbiologyî, International Dairy Journal,2001; 11: 259-274.
3. Bouton Y, Guyot P, Beuvier E, Taillliez P, and Grappin R. ìUse of PCR- based methods and PFGE for typing and monitoring homofermentative lactobacilli during ComtÈ cheese Ripening î, International Journal of Food Microbiology,2002; 76: 27-38.
4. Cardinal MJ, Meghrous J, Lacroix C and Simard RE ìIsolation of Lactococcis lactis strain producing inhibitory activity against Listeria", Food Biotechnology, 1997; 11(2): 129-146.
5. Centeno JA, Menendez S, Hermida M and Rodriguez-Otero JL. îEffects of the addition of Enteococcus Faecalis in Cebreiro cheese manufactureî, International Journal of Food Microbiology,1999; 48: 97-111.
6. Coffey AG, Daly C, andFitzgrald G. ìThe impact of biotechnology on dairy industryî, Biotechnology Advances,1994;12: 625-633.

7. Cogan TM, Barbosa M, Beuvier E, Branchi-Salvodari B, Cocconcelli PS, Fernandes I, Gomez J, Gomez R, Kalantzopoulos G, Ledda A, Medina M, Rea M:C: and Rodriguez E. ìCharacterization of the lactic acid bacteria in artisanal dairy productsî, International of Dairy Reseach, 1997; 64:409-421.
8. Cogan T M, ìHistory and Taxanomy of Starter Culturesî, in Dairy Starter Cultures, edited by T. M. Cogan and J.-P. Accolas (Wiley ñVCH Inc., New York): 1996, pp. 1-25.
9. Coppala R, Nanni M, Iorizzo M, Sorrentino A, Sorrentino E, Chiavari C, and Grazia L. ìMicrobiolgical characteristics of Parmigiano Reggiano cheese during the cheesemaking and the first months of ripeningî, Lait,2000; 80:479-490.
10. Giraffa G and Neviani E. ìDifferent Lactobacillus helveticus strain populations dominateduring Grana Padano cheese makingî, Food Microbiology,1999; 16: 205-210.
11. Lopez-Diaz TM, Alonso C, Roman C, Garcia-Lopez ML and Moreno B. Lactic acid bacteria isolated from a hand-made blue cheeseî, Food Microbiology, 2000;17: 23-32.
12. Wouters JTM, Ayad EHE, Hugenholtz J, and Smit G. Microbes from raw milk for fermented dairyproductsî, International Dairy Journal, 2002; 12: 91-109.
13. Yvon M, and Rijnen L. ìCheese flavor formation by amino acid catabolism International Dairy Journal, 2001; 11:185-201.
14. Ventura M and Zink R. ìSpecific identification and moleculer typing analysis of Lactobacillus johnsonii by using PCR- based methods and pulsed- field gel electrophoresis. î FEMS Microbiology Letters,2002; 217:141-154.
15. Alonso-Calleja C, Carballo J, Capita R, Bernardo A and Garcia-LopÈz ML. Changes in the Mirroflora of Valdeteja Raw Goatís Milk Cheese throughout Manufacturing and Ripeningî, Lebensm-Wiss-Technol, 2002; 35: 222-232.
16. Bouton Y, Guyot P, Beuvier E, Taillliez P and Grappin R. ìUse of PCR- based methods and PFGE for typing and monitoring homofermentative lactobacilli during ComtÈ cheese Ripening î, International Journal of Food Microbiology,2002; 76 : 27-38.
17. Coppala R, Nanni M, Iorizzo M, Sorrentino A, Sorrentino E, Chiavari C, and Grazia L. ìMicrobiolgical characteristics of Parmigiano Reggiano cheese during the cheese making and the first months of ipeningî, Lait, 2000; 80: 479-490.
18. Mannu L, Comunian R, and Scintu MF. ìMesophilic lactobacilli in Fiore Sardo cheese: PCR-identification and evolution during cheese ripeningî, International Dairy Journal, 2000; 10: 383-389.
19. Mannu L, Riu G, Communian R, Fozzi CM and Scintttu FM. ì A preliminary study of lactic acid bacteria in whey starter culture and industrial Pecorino Sardo ewesí milk cheese : PCR

identification and evolution during ripening î, International Dairy Journal, (2002); 17-26.

20. Parente E, Rota MA, Riccciardi A, and clementi F. ìCharacterisation of Natural Starter Cultures Used in the Mnufacture of Pasta Filata Cheese in Basilicata (Southern Italy)î, International Dairy Journal,1997; 7:775-783.
21. PÈrez G, Cardell E and Z·rate V. ìProtein fingerprinting as a complelementary analysis to classical phenotyping for the identification of lactic acid bacteria from Tenefire cheeseî, Lait, 2000; 80: 589-600.
22. Requena T, Peloez C, and Desmazeaud MJ. ìCharacterization of lactococci and lactobacilli isolated from semi-hard goatís cheeseî, Journal of Dairy Reseach, 1991; 58: 137-145.
23. Ross RP, Stanton C, Hill C, Fitzgerald GF, and Coffey A. ìNovel Cultures for cheese improvementî, Trends in Foood Science and Technology, 2000; 11: 96-104.
24. Stiles M, and Holzapfel W. ìLactic acid bacteria of foods and their current taxonomy International Journal of Food Microbiology, 1997; 36: 1-29.
25. Rodriguez E, Arques J, Gaya P, Nunez M and Medina M. ì Control of Listeria monocytogenes and monitoring of bacteriocin producing lactic acid bacteria by colony hybridization in semi-hard raw milk cheese.î Journal of Dairy Reseach,2001; 68: 131- 137.