www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(11): 12-16

www.thepharmajournal.com
Received: 15-08-2023
Accepted: 19-09-2023

Jitesh Tarak
Department of Dairy Microbiology, College of Dairy Science and Food Technology, C.G.K.V., Raipur, Chhattisgarh, India

B Shekhar
Department of Dairy Microbiology, College of Dairy Science and Food Technology, C.G.K.V., Raipur, Chhattisgarh, India

B Narssimlu
Department of Dairy Microbiology, College of Dairy Science and Food Technology, C.G.K.V., Raipur, Chhattisgarh, India

Manorama
Department of Dairy Microbiology, College of Dairy Science and Food Technology, C.G.K.V., Raipur, Chhattisgarh, India

Sarang Dilip Pophaly
Department of Dairy Microbiology, College of Dairy Science and Food Technology, C.G.K.V., Raipur, Chhattisgarh, India

Corresponding Author:
Jitesh Tarak
Department of Dairy Microbiology, College of Dairy Science and Food Technology, C.G.K.V., Raipur, Chhattisgarh, India

Improvement of starter culture using induced aerobic respiration in lactobacillus

Jitesh Tarak, B Shekhar, B Narssimlu, Manorama and Sarang Dilip Pophaly

DOI: https://doi.org/10.22271/tpi.2023.v12.i11a.23950

Abstract
The Lactic Acid Bacteria (LAB) are used for starter preparation in manufacture of various dairy products such as *Dahi*, Acidophilus milk, Yogurt, Cheese etc. Certain LAB species can exhibit a dual metabolic life in which they can switch from fermentative mode to aerobic respiration mode when heme or heme and menaquinone is present in the medium, which results not only in high cell number but also resistance against many stress conditions. Improved starter cultures was prepared by inducing aerobic respiration in indigenous Lactobacillus culture isolated from dairy, vegetable samples and compares their rate of acid production with fermentative starter culture as well as evaluate different heme and menaquinone concentration effect on the growth of Lactobacillus culture by spectrophotometric ally @ 600 nm. The respiratory starters showed a marked increase in rate of acidification as compared to fermentative starters. Excess heme more than 4 µg/ml concentration was toxic to *Lactobacillus* culture but increasing menaquinone concentration had no such effect.

Keywords: Culture, aerobic respiration, lactobacillus

Introduction
Starter culture plays a very crucial role for the production of desirable quality fermented milk products. A starter culture is any active microbial preparation intentionally added during product manufacture to initiate desirable fermentation. The bacteria used in the manufacturing of fermented dairy products are generally LAB (Marth & Steele, 2001) [8]. In special case *Propionibacterium shermanii* and *Bifidobacterium spp*. are also used as starter cultures. LAB are Gram positive, non-sporeforming rod or coccus shaped, catalase negative, obligate fermentative bacteria. It produces lactic acid as a major end product.
LAB have GRAS (Generally Recognized as Safe) status due to their ubiquitous nature in food, Some LAB, notably lactobacilli, occupy important niches in the gastrointestinal tracts of humans and animals and are considered to offer a number of probiotic benefits to general health and well-being (Klaenhammer *et al*., 2002) [6]. The LAB have a long and safe history of application and consumption in the production of fermented foods and beverages (Caplice & Fitzgerald, 1999) [2]. The back-slopping was a standard practice before the advent of preserved starter culture in different forms. However, lack of batch-to-batch consistency of the product quality and chances of contamination are inherited problems associated with back-slopping. To overcome these problems commercial starter culture have been developed for the production of freeze-dried liquid cultures and concentrated frozen starter cultures for the direct inoculation, these are known as direct vat set (DVS) or direct-to-vat inoculation (DVI) cultures. The production of starter cultures inflicts diverse stress conditions on the microorganisms like cooling, freezing, freeze-drying, osmotic and oxidative stress. The microorganisms have to negotiate these stress conditions in order to maintain high viability till their use in the vat. Thus high viable and stress-resistance biomass production is an important requisite in starter manufacture. Fermentation is not an efficient energy production process as only two molecules of ATP are produced. Some lactic acid bacteria can switch to aerobic respiration for cellular ATP production under modified conditions. This switch to respiration from fermentation has distinct changes on the physiology and characteristics of respiring LAB. Respiration results in high biomass production as compared to fermentative conditions and also results in more robust stress tolerance in these bacteria. LAB are different from other respiratory cultures as they don't have Kreb's cycle for the production of NADH so they use sugar source for NADH production and catabolize the sugar through glycolysis and produce

NADH. These NADH donate its electron to electron transport chain for ATP production but lactic acid bacteria don't have fully equipped electron transport chain due to lack of heme & menaquinone (lactobacilli) synthesis gene and it require exogenous heme and menaquinone for respiration. Recently, a change from the traditional anaerobic fermentation to an aerobic respiration process has been reported for some LAB species (Pedersen *et al*., 2005) [9]. This is based on the observation that *Lactococcus lactis* spp. is capable of respiration in the presence of oxygen and heme, resulting in the production of a higher biomass and a greatly reduced amount of lactic acid (Gaudu *et al*., 2002) [3]. The presence of heme allows the formation of a membrane potential via an aerobic electron transfer chain (Brooijmanns *et al.*, 2009) [1], giving a more efficient use of energy sources and resulting in a higher biomass production (Pedersen *et al*., 2005) [9]. This technology is used for the production of lactococcal starter culture. (Yamamoto *et al*., 2005) [11] reported that most of the lactobacilli strains lack heme and menaquinone synthesis genes so they require exogenous heme and menaquinone for respiration. The genus *Lactobacillus* currently contains over 180 species and encompasses a wide variety of organisms *Lactobacillus casei* shirota, *Lactobacillus rhamnosus* GG are some patented probiotic strains from the *Lactobacillus* genus. From starter culture point of view there are many advantages to grow lactobacilli under aerobic respiratory conditions. Respiratory cultures are more robust in comparison to fermentative cells so it provides good viability. Respiratory culture also exhibits enhanced long-term survival for longer time in comparison to fermentative culture so it provides good stability. In this work we improved *Lactobacillus* starter culture using induced aerobic respiration by addition of exogeneous heme and menaquinone in growth medium as well as optimized heme and menaquinone concentration for respiratory culture. The respiratory starter culture was prepared using optimized heme and menaquinone concentration and rate of acid production was compared between respiratory starter culture and fermentative starter culture

Materials and Methods

Bacterial strains and media

The strain *Lactobiclus* Gjr used in this study was isolated from *Dahi*. Strain was identified to the genus level using a polyphasic approach (Morphological identification, catalase test, sugar profiling) and maintained as glycerol stock at -20 °C and routinely propagated in MRS broth (HiMedia), for 16 h at 37 °C.

Preparation of heme & menaquinone supplemented broth

The screening of culture for aerobic respiration was carried out using heme (HiMedia) & menaquinone (Sigma Aldrich) supplemented MRS broth (MRS-H). The heme stock solution (2.5 mg/ml) was prepared by weighing heme and dissolving in 0.05 M NaOH solution. The menaquinone stock solution (1 mg/ml) was prepared by weighing menaquinone and dissolving in ethanol. An appropriate volume of heme & menaquinone stock solution was added to MRS broth to achieve a final heme concentration of 2.5 µg/ml & menaquinone concentration of 1 µg/ml of the MRS broth.

Screening of isolates for aerobic respiration ability

Lactobacillus Gjr was screened for aerobic respiration ability. A volume of 25 ml MRS-H (treatment) was dispensed in 250 ml volume flask (cultures in 250 ml conical flask with cotton plug) and control MRS medium also dispensed in 250 ml flask (cultures in 250 ml conical flask with cotton plug). The prepared medium were autoclaved at 121 °C for 20 min. Inoculate culture @ 2% in both control and treatment sample. The treatment and control sample were incubated in static and shaking (rotary shaker at 120 rpm) condition. The respiration in cultures was ascertained by increase in OD_{600} in MRS-H as compared with MRS as determined by spectrophotometer (Model: UV-Vis 119, Systronics Pvt. Ltd. Ahmedabad, India).

Optimization of heme and menaquinone concentration in MRS broth

Lactobacillus Gjr culture was screened for aerobic respiration ability, MRS broth with various combinations of heme and menaquinone were prepared and culture was inoculated @ 2%. Inoculated flasks were incubated at 37 °C for 24 h. After incubation the growth was measured spectrophotometrically by taking culture OD_{600}. All trails were run in triplicate. Combination which provide higher cell biomass was used for achieving higher cell biomass, various combination of heme and menaquinone used in the optimization study are shown in Fig.1

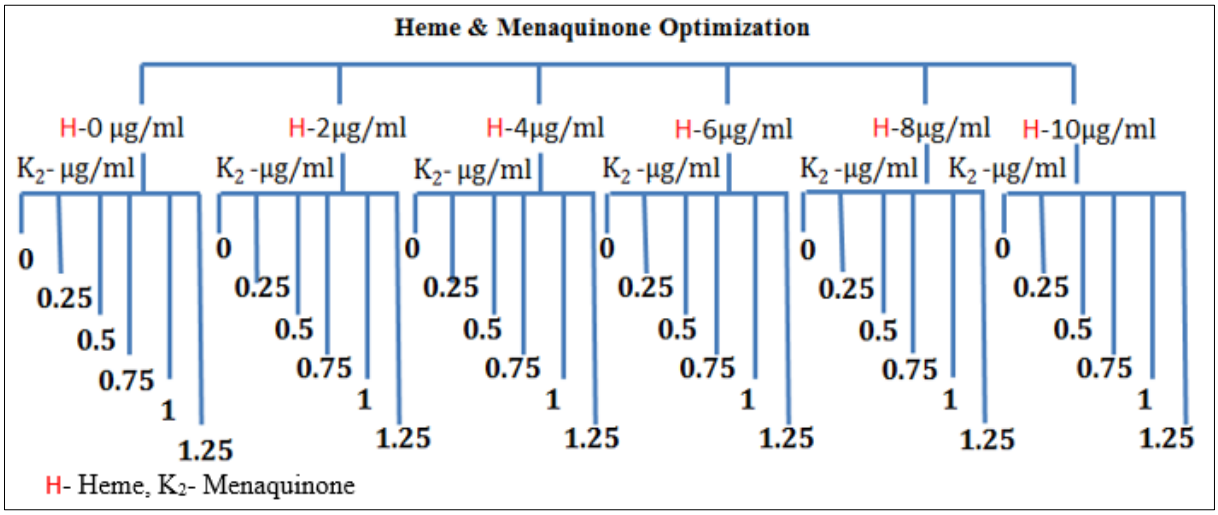

Fig 1: Various combinations of heme and menaquinon used for aerobic respiration

Preparation of starter culture

Optimized concentration of heme and menaquinone was used for starter culture preparation. The *Lactobacillus* Gjr was inoculated @ 2% in optimized heme and menaquinone supplemented 250 ml MRS broth (MRS-H) and incubated it at 37 °C after 24 h of incubation, centrifuged at 10,000 rpm in 4 °C for 10 minute for cell pellet and harvested cell pellet was washed and suspended into 50 ml sterilized reconstituted skim milk. There was one control starter culture also prepared by same culture using non heme and menaquinone supplemented MRS broth.

Evaluation of starter activity

The starter culture activity was checked by determining the rate of acid production in both control starter culture & respiratory starter culture the cultures were inoculated @ 2% into reconstituted skim milk, incubated at 37 °C for 12 h and checked for the acidity at 1 h intervals. Acidity was measured by titration with 0.1 N sodium hydroxide solution using phenolphthalein indicator (Lampert, 1947) [7].

Result and Discussion

Aerobic respiration ability in *Lactobacillus* Gjr

Respiration was induced in cultures using MRS-H broth. The growth in shaking and static condition of *Lactobacillus* Gjr was compared. *Lactobacillus* Gjr showed OD_{600} difference (0.662) in control and treatment samples in static conditions and thus was found to be respiration competent.

Table 1: Screening of *Lactobacillus* Gjr for aerobic respiration ability

Culture name	Shaking		Static	
	Optical Density (OD)			
	Control	Treatment (H+M)	Control	Treatment (H+M)
Lactobacillus Gjr	0.558±0.002	0.874±0.026	0.485±0.002	1.147±0.006

Result are expressed as Mean± SE (n=3)

(Brooijmans *et al.*, 2009) [1] analyzed genome of lactobacilli for components of electron transport chain and checked the heme induced respiration in lactobacilli and concluded that a *cydABCD* operon that encodes a *bd*-type cytochrome was found in the genome of *Lb. plantarum* WCFS1 They proposed that some lactobacilli contain a branched electron transport chain capable of using oxygen or nitrate as an extracellular electron acceptor. The electron transport chain requires activation by the addition of heme Co-factor and a menaquinone pool in the form of vitamin K2.

Optimization of heme & menaquinone concentration with respect to aerobic respiration in *Lactobacillus* Gjr

Based on screening result heme and menaquinone concentrations was optimized with respect to enhanced aerobic respiration in MRS-H. The results of growth of L*actobacillus* Gjr at different heme & menaquinone concentrations are shown in table. 2 a progressive increase in OD_{600} of *Lactobacillus* Gjr till heme concentration of 4 µg/ml was observed, beyond which a decrease in growth was observed. Highest OD_{600} value was observed at 4µg/ml heme & 1 µg/ml menaquinone (1.632±0.006). Most workers have reported the aerobic respiration in lactobacilli at a heme concentration of 2.5 µg/ml and menaquinoe concentration of 1.0 µg/ml. (Brooijmans *et al.*, 2009) [1] (Guidone *et al.*, 2013) [4] (Zotta *et al.*, 2014) [12] (Ricciardi *et al.*, 2014) [10] (Ianniello *et al.*, 2015) [5] they reported that respiration induces profound changes in *Lactobacillus* metabolism and long-term survival. Heme concentration for bacterial samples need to be delicately controlled in growth medium since it has a very narrow range between activity and toxicity. Higher heme concentration (beyond 4 µg/ml) caused rapid decrease in growth. Menaquinone being a vitamin is required at low concentration ranges and was not found to exhibit any toxicity at tested levels. However no effect on supporting aerobic respiration was observed beyond 1.0 µg/ml menaquinone level.

Preparation of Starter Culture using Aerobic Respiration System in *Lactobacillus* Gjr

Lactobacillus Gjr was used for preparation of respiratory and fermentative starter culture. The respective cultures were prepared by growing the cells in optimized conditions in MRS and H-MRS followed by harvesting the biomass and suspending it in reconstituted skim milk (11% TS). The activity of both the starter culture was monitored by rate of acid production each hours measured by titrable acidity (% LA). The comparison of cultures with respect to acid production ability is shown in table 3 and Figure 2. The fermentative culture showed a slower rate of acid production as compared to respiratory culture. Also, the final acidity developed using respiratory culture was higher. The high acidification rate of starter culture prepared using aerobic respiration could be attributed to enhanced biomass obtained as a result of respiration conditions. Also, since the aerobic respiratory cells were resistant to acidic conditions, the final acidity obtained in this condition was more as compared to fermentative conditions.

Table 2: Heme & menaquinone optimization for *Lactobacillus* Gjr

Vit-K_2 Heme	K_2 0 µg/ml	K_2 0.25 µg/ml	K_2 0.5 µg/ml	K_2 0.75 µg/ml	K_2 1 µg/ml	K_2 1.25 µg/ml
10 µg/ml	0.245 ±0.002^{a1}	0.239±0.005^{a2}	0.224 ±0.0319^{a3}	0.309 ±0.002^{a4}	0.511 ±0.004^{a5}	0.301 ± 0.004^{a6}
8 µg/ml	0.267 ±0.003^{b1}	0.221 ±0.005^{b2}	0.215 ±0.007^{b3}	0.633±0.003^{b4}	0.607±0.005^{b5}	0.600±0.007^{b6}
6 µg/ml	0.256 ±0.003^{c1}	0.335±0.003^{c2}	0.566 ±0.001^{c3}	1.313± 0.007^{c4}	1.560±0.004^{c5}	1.487 ±0.004^{c6}
4 µg/ml	0.325 ±0.005^{d1}	0.366±0.002^{d2}	0.820 ±0.002^{d3}	1.578±0.006^{d4}	1.632±0.006^{d5}	1.589 ±0.004^{d6}
2 µg/ml	0.523 ±0.003^{e1}	0.587 ±0.003^{e2}	0.529 ±0.004^{e3}	0.901±0.004^{e4}	1.029±0.007^{e5}	1.115±0.001^{e6}
0 µg/ml	0.512 ±0.004^{f1}	0.532 ±0.003^{f2}	0.511 ±0.002^{f3}	0.498 ±0.002^{f4}	0.598 ±0.008^{f5}	0.578 ±0.004^{f6}

Table 3: ANOVA (Analysis of variance)

Source of Variation	SS	DF	MS	F	P-value	F crit
Rows	2.588948	5	0.51779	7.389229	0.000226	2.602987
Columns	2.634602	5	0.52692	7.519533	0.0002	2.602987
Error	1.751839	25	0.070074			
Total	6.975388	35				

Table 4: Rate of acid production

Time (h)	Acidity (% Lactic acid)	
	Fermentative Starter	Respiratory Starter
0	0.131	0.131
1	0.211	0.231
2	0.252	0.341
3	0.312	0.537
4	0.421	0.621
5	0.511	0.732
6	0.632	0.889
7	0.761	0.943
8	0.861	1.198
9	0.931	1.233
10	1.123	1.342
11	1.243	1.431
12	1.221	1.444

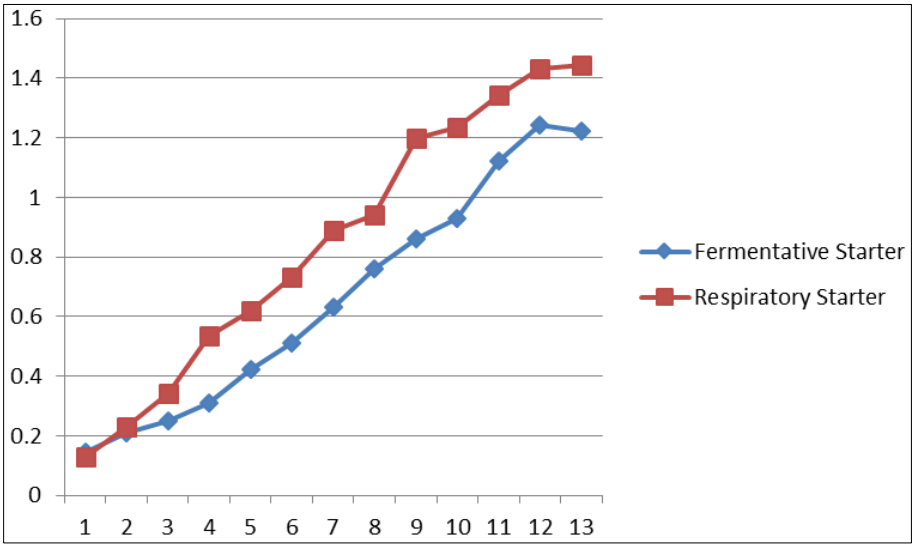

Fig 2: Rate of acid production

Conclusion

This study demonstrated the benefits of respirative starter culture in production of fermented milk product. Respiration in some Lactobacilli culture was triggered by addition of heme, & menaquinone with small amount of oxygen in growth medium. The present study was a pivotal work on the use of aerobic respiration system of lactobacilli for preparation of high viability liquid starter cultures. The prepared starter culture could be potentially used for manufacture of different types of fermented dairy products with reduced fermentation time and improved flavor profile.

References

1. Brooijmans R, de Vos WM, Hugenholtz J. Electron transport chains of lactic acid bacteria - walking on crutches is part of their lifestyle. F1000 Biology Reports. 2009;1:34. http://doi.org/10.3410/B1-34
2. Caplice E, Fitzgerald GF. Food Fermentations: Role of Microorganisms in Food Production and Preservation. International Journal of Food Microbiology. 1999;50(1):131-149.
3. Gaudu P, Vido K, Casselin B, Kulakauskas S, Tremblay J. Respiration Capacity and Consequences in *Lactococcus lactis*. Anotnie van Leeuwenhoek. 2002;82(5):263-69.
4. Guidone A, Ianniello RG, Riccardi A, Zotta T, Parente E. Aerobic Metabolism and Oxidative Stress Tolerance in the *Lactobacillus plantarum* Group. World Journal Microbial Biotechnology. 2013;29(2):1713-1722.
5. Ianniello RG, Ricciardi A, Parente E, Tramutola A, Reale A, Zotta T. Aeration and Supplementation with Heme and Menaquinone affect Survival to Stress and Antioxidant Capability of *Lactobacillus casei* strains. Food Science and Technology. 2015;78(3):817-824.
6. Klaenhammer T, Altermann E, Arigoni F, Bolotin A, Breidt F, Broadbent J, *et al*. Discovering Lactic Acid Bacteria by Genomics. Antonie van Leeuwenhoek. 2002;82(3):29-58.

7. Lampert LM. Milk and dairy products, their composition, food value, chemistry, bacteriology and processing. Chemical publishing Co., Inc., Brooklyn, N.Y.; c1947. p. 242.
8. Marth EH, Steele JL. Applied Dairy Microbiology. 2nd Edition. Marcel Dekker, Inc., New York, Basel; c2001. ISBN: 0-8247-0536-X.
9. Pedersen MB, Iversen SL, Sorensen KL, Johansen E. The Long and Winding Road from the Research Laboratory to Industrial Applications of Lactic acid Bacteria. FEMS Microbiology Reviews. 2005;29(3):611-624.
10. Ricciardi A, Morelli MAC, Ianniello RG, Parente E, Zotta T. Metabolic Profiling and Stress Response of Aerobic Respiratry Cultures of *Lactobacillus plantarum* C17 Grown in a Chemically Defined Medium. Annual Microbiology. 2014;65(5):1639-1648.
11. Yammamoto Y, Poyart C, Cuot TP, Lamberet G, Gruss A, Gaudu P. Respiration Metabolism of Group B *Streptococcus* is activated by Environmental Haem and Menaquinone and Contributes to Virulence. Molecular Microbiology. 2005;56(7):525-34.
12. Zotta T, Riccardi A, Ianniello RG, Parente E, Reale A, Rossi F, *et al*. Assessment of Aerobic and Respiratory Growth in the Lactobacillus *casei* Group. PLOS one. 2014;9(6):1-12.