IRJSE International Research Journal of Science & Engineering
Int. Res. J. of Science & Engineering, Volume 9 (1) 2021
ISSN: 2322-0015
SJIF Impact Factor 6.70
ORIGINAL ARTICLE
OPEN ACCESS

Study on biosurfactant production by beneficial lactic acid bacteria isolated from traditionally fermented Indian foods

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Manuscript Details
Received :07.02.2021
Accepted: 25.02.2021
Published: 28.02.2021

Available online on https://www.irjse.in
ISSN: 2322-0015

Editor: Dr. Arvind Chavhan

Cite this article as:
Ghume Vaishnavi, Shingade Shilpa, Walhe Rajan. Study on biosurfactant production by beneficial lactic acid bacteria isolated from traditionally fermented Indian foods., *Int. Res. Journal of Science & Engineering*, 2021, Volume 9(1): 5-11.

Abstract

Biosurfactants are also produced by various microorganisms which are isolated from traditionally fermented Indian foods. The properties of biosurfactants are same as those of chemical surfactants but biodegradable, less toxic and compatible with the human skin. There are various biosurfactants known such as glycolipids, rhamnolipids, lipopeptides, phospholipids, fatty acids and polymeric biosurfactant, etc. LAB are believed to have positive health effects that are present in fermented foods with probiotic properties. In the present study, we have isolated a total of 56 isolates of LAB from different traditionally fermented Indian foods. The LAB was characterized on the basis of primary tests for probiotics. Out of 56 isolates, 29 isolates were found to be non-hemolytic of that, 20 isolates were able to tolerate acid at pH 2.0, for 120 minutes also able to tolerate bile at 0.3% concentration, 17 isolates showed BSH activity. Selected isolates were screened for biosurfactant activity by using drop collapse assay and oil spreading assay. Three isolates, out of 17 isolates shown primary tests for biosurfactant activity also, the thin layer chromatography (TLC) method was performed.

Keywords: Fermented foods, Lactic acid bacteria, BSH activity, Biosurfactants, Probiotics

1. Introduction

Traditionally fermented Indian foods are rich in lactic acid bacteria (LAB) and reported to have beneficial effects. Fermented foods in several countries make up a main part of diet because it preserve food, offer benefits as well as adds

nutritional value [1, 2]. Also, LAB and their fermented foods has been noted to confer a variety of health benefits in immune modulation, cancer, infectious diseases [3]. LAB are generally recognized as safe microorganisms and are used in foods and pharmaceutical industries. Also biosurfactants fom LAB stated to have anti- adhesive action against pathogens [4].

Probiotic is defined as, live microorganisms that when administered in adequate amount confer health benefits on the host [5]. They can be advantageous by improving the gut flora and are non-pathogenic bacteria that benefits health of host in many ways [6]. Most probiotic microorganisms belong to lactic acid bacteria (LAB) are from genus *Lactobacillus*, *Bifidobacterium* and *Enterococcus* etc. [7].

Biosurfactants are the biological compounds that are produced extracellularly or as a part of the cell membranes by microorganisms [8].

The protein of surface of LAB is the co-aggregation promoting factor (CPF) that mediates co-aggregation with the pathogens. The co-aggregation prevents the adherence of pathogen to the epithelial cells of the host tissue and successfully creates a barrier that prevents colonization of pathogens [9].

The biosurfactants produce from LAB is used as an alternative of chemically syntheized surfactants and carry many advantages such as lesser toxicity, superior biodegradability and they are more firm and effective than chemical surfactant and have shown anti adhesive activity against pathogens [10, 11]. The present study was aimed at exploring the potential of LAB isolated from various fermented foods to study their primary probiotic properties, and further evaluated for biosurfactant production activity.

2. Materials and Method

Isolation of LAB from traditionally fermented Indian foods.

In this study, the isolation of LAB from various traditional fermented food/batter is carried out. Isolation of LAB was performed using de Man Rogosa Sharpe (MRS) medium. Fermented food Samples mixed with sterile distilled water, diluted and streaked on MRS agar plate, plates were incubated at 37°C for 24-48 hours under microaerophilic condition. The morphotyping was done by staining.

Non hemolytic activity Test:

The medium for test was prepared by incorporating 5% blood in sterile MRS agar plates. Each isolates were spot inoculated onto the sterile blood agar plate and incubated under microaerophilic condition at 37°C for 24-48 hours. [12].

Acid Tolerance:

Bacterial cells from MRS cultures were harvested by centrifugation. These cells were washed with Phosphate Buffer Saline (PBS) and resuspended in buffer (pH 2.0, 3.0 and 4.0 and incubated at 37°C for 120 minutes as per Singhal *et al*. [13] with slight modifica-tions. After centrifugation, bacterial cells resuspended in PBS, washed and inoculated in fresh medium. The results were checked after incubation by comparison with suitable positive and negative controls. The positive results showed turbidity and colour change.

Bile Tolerance:

The isolates were tested for bile tolerance as per method described by Wang *et al*. [14] with some changes. The sterile MRS broths at concentration of 0.1%, 0.2%, and 0.3% bile salt (ox gall) were inoculated with fresh growth of isolates and incubated under microaerophilic condition at 37°C for 24-48 hours. The positive results indicated by colour change and turbidity.

Bile Salt Hydrolysis (BSH) activity:

Fresh growth's spot of 10 µl isolates were inoculated on sterile BSH agar (Sterile MRS agar with 0.5% bile salt and $CaCL_2$) plates in triplicates. The plates were incubated under microaerophilic condition at 37°C for 24-48 hours. After incubation, plates were checked for zone of precipitation and noted as average of three with

SD. Isolates were showing zone of precipitation selected for further studies [15].

Screening of isolates for their biosurfactant activity:
Biosurfactant extraction from isolated bacteria were detected by using drop collapse method and oil spreading assay.

Drop collapse assay:
For screening of isolates for biosurfactant production, the capacity to collapse a droplet was seen. Extracted biosurfactant was pipetted as a droplet on the parafilm. Distilled water was used as a negative control and sodium dodecyl phosphate (SDS) was used as a positive control. Methylene blue was added to the water for staining purpose. The flattening of droplet and spreading of the droplet on the plane surface was checked. The droplets were permitted to become dry and diameter of droplet was noted [10].

Oil spreading assay:
Oil spreading test was performed to compare the surface activity among various isolates. Distilled water was added to a petriplate followed by the addition of crude oil to the surface of water. From each LAB supernatant, 20 µL supernatant was placed onto the centre of oil membrane and diameter of clearly oil displaced circles was recorded [16].

Extraction of Biosurfactants:
Isolates were inoculated into medium containing 0.5% (v/v) oil as carbon and energy source. The broth cultures were incubated for 48 hours at 37°C. For the removal of the cells cultures were centrifuged at 6000 rpm for 20 minutes. The supernatant were tested for the extraction of biosurfactants, pH of the supernatant was adjusted to 2, with 1M H_2SO_4 and add equal volume of chloroform: methanol (2:1). Mixture was mixed and kept overnight for evaporation. Sediments obtained after procedure i.e., the biosurfactant as Sharma *et al*. [17] with slight changes.

Identification of Biosurfactants:
Biosurfactant obtained in above method mixed with PBS, were identified with the help of thin layer chromatography (TLC). It was spotted on the silica plate. The biosurfactant was separated using the solvent chloroform: water: methanol in ratio 65: 24: 4. Ninhydrin reagent was sprayed to detect lipopeptide biosurfactant as a red spots and anthrone reagent was sprayed to detect glycolipid biosurfactant as yellow spots [17].

3. Results and Discussion

In all 56 LAB isolates from different fermented food samples were obtained; all were Gram positive rods or cocci (all not mentioned here). Hemolytic activity of isolates was determined and 29 isolates were found to be nonhemolytic and hence selected for study.

Further screening was done on the basis of primary probiotic characters. Before reaching the intestinal tract, LAB must survive the acidic conditions (pH about 2.0) of the stomach and then bile [18] .Out of 29 tested isolates, 20 isolates were able to tolerate pH 2.0, for 120 min. Moreover, these 20 isolates tolerated bile at the concentration of 0.3% (Table 1). Of these,17 isolates were able to show bile salt hydrolase (BSH) activity (Figure 1), thus these isolates have potential of health benefit as this can be considered as cholesterol reducing capacity and colonization factor as well [19]. Isolates showed resistance to low pH levels and to higher bile concentrations. These traits may enable them to survive in the stomach and intestine or even to compete with other bacterial groups in the environment.

Some species of LAB have been found to be biosurfactant producing strains, and one of major advantage of biosurfactants is that they have the negative impact on other microbial species [10]. As seen, three isolates (HAB-1, UB-1 and PD-2) were shown biosurfactant production, on the drop-collapse method (Table 2) and oil-spreading assay (Table 3). PD-2 isolate showed maximum diameter of zone formed by oil-spreading assay. The biosurfactant extracted was characterized (Table 4) using TLC [17] and redish spots were seen with Ninhydrin reagent. This observation among the isolates can demonstrates the probiotic

functionality of the isolates. Further comparing of the Rf value with known standards and other study is needed to make understanding of biosurfactant production ability by these isolates.

Table 1: Acid and bile tolerance data of isolates

Sr. No.	Isolates	Food samples	Acid Tolerance	Bile Tolerance
			pH- 2.0	0.3%
1	MB-3	Moong Batter	++	+++
2	DC-1	*Dahi-1*	++	+++
3	DC-2	*Dahi-1*	-	-
4	DoB-1	*Dosa* Batter	-	-
5	BCD-1	*Dahi-2*	++	+++
6	BCD-2	*Dahi-2*	-	-
7	SW-3	*Surali Wadi-1*	++	+
8	HAB-1	*Anarasa* Batter	+	+
9	HAB-2	*Anarasa* Batter	-	-
10	VB-2	*Varai* Batter	+	+++
11	UB-1	*Udid* Batter	+	+++
12	RDB-3	*RavaDosa*	-	-
13	PD-1	*PohaDosa*	+	++
14	PD-2	*PohaDosa*	+	+++
15	PDB-1	PD Batter	+	+
16	RI-2	*RavaIdali*	+	++
17	MDB-1	Mix *Dhokla*	++	+
18	MDB-2	Mix *Dhokla*	-	-
19	NDB-1	*NeerDosa*	++	+++
20	RIB	RI Batter	+	+++
21	RK-1	*Kadhi*	++	+++
22	HSWB-1	*SuraliWadi- 2*	++	+++
23	HSWB-2	*SuraliWadi- 2*	++	+++
24	HB-1	*Handwa*	-	-
25	HB-3	*Handwa*	+	+
26	HB-4	*Handwa*	-	+
27	UB-2	*Udid Wada* Batter	++	++
28	UB-3	*Udid*Wada Batter	-	-
29	WCB	*Wheat Cheek*	+	+

Legend: + growth, ++ more growth, - no growth

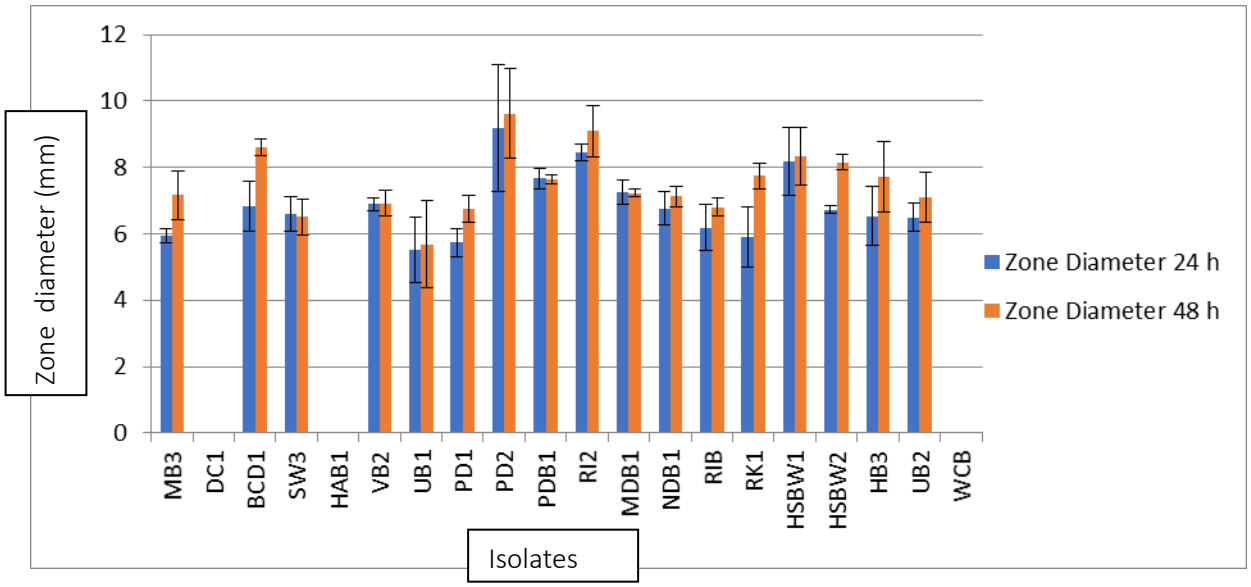

Figure 1: BSH activity of isolates

Table 2: Drop collapse assay of isolates

Sr. No.	Isolate Name	Diameter of test (mm)	Diameter of negative control (mm)	Diameter of positive control (mm)
1	HAB-1	4.72	3.93	5.46
2	UB-1	5.79	3.80	5.73
3	PD-2	5.41	4.03	6.01

Table 3: Oil spreading assay

Sr. No.	Isolate Name	Diameter of test (mm)	Diameter of negative control (mm)	Diameter of positive control (mm)
1	HAB-1	32.92	4.82	80.75
2	UB-1	38.25	4.82	80.75
3	PD-2	58.12	4.82	80.75

Table 4: Result TLC (Rf value) of biosurfactants.

Sr. No.	Isolate name	Distance travelled by compound (cm)	Distance travelled by solvent in (cm)	Rf value
1	HAB-1	4.6	5.4	0.8
2	UB-1	5.3	4.4	1.2
3	PD-2	5.5	4.9	1.1

4. Conclusion

In the present study it is concluded that LAB isolates from traditionally fermented Indian foods can be considered as a putative probiotic candidates having biosurfactant production activity. As these isolates also found to possess BSH activity, along with biosurfactant properties seems to have added value of augmenting beneficial aspects to the food it ferments or supplemented. Since these isolates are obtained from traditionally fermented local foods, would have better adapted the environments and thus have potential to offer benefits efficiently to consumers.

Acknowledgments: Authors thank Principal, Abasaheb Garware College, Pune for the laboratory conveniences.

Conflict of interest

No conflict of interest influenced in this research.

5. References

1. Azam M, Mohsin M, Ijaz H, Tulain UR, Ashraf M A, Fayyaz A. Lactic acid bacteria in traditional fermented Asian foods. Pak. J. Pharm. Sci., 2017; 30 (5): 1803-1814.
2. Satish Kumar R, Kanmani P, Yuvaraj N, Paari K A, Pattukumar V, Arul V. Traditional Indian fermented foods: a rich source of lactic acid bacteria. International Journal of Food Sciences and Nutrition, 2013: *64*(4): 415–428. https://doi.org/10.3109/09637486.2012.746288.
3. Harzallah D, Belhadj H. Lactic Acid Bacteria as Probiotics: Characteristics, Selection Criteria and Role in Immunomodulation of Human GI Muccosal Barrier. In J. M. Kongo (Ed.): Lactic Acid Bacteria - R & D for Food, Health and Livestock Purposes. In Tech. 2013; pp: 197-216. https://doi.org/10.5772/50732.
4. Ghasemi A, Moosavi-Nasab M, Setoodeh P, Mesbahi G, Yousefi G. Biosurfactant Production by Lactic Acid Bacterium Pediococcus dextrinicus SHU1593 Grown on Different Carbon Sources: Strain Screening Followed by Product Characterization. Scientific Reports, 2019. *9*(1). https://doi.org/10.1038/s41598-019-41589-0. Science, 2011, 1–9. https://doi.org/10.1155/2011/201254.
5. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morell, L, Canani RB, Flint H J, Salminen S, Calder PC, Sanders ME. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nature Reviews Gastroenterology & Hepatology,2014; *11*(8): 506–514. https://doi.org/10.1038/nrgastro.2014.66.
6. Arshad FA, Mehmood R, Hussain S, Annus Khan M, Khan MS. Lactobacilli as Probiotics and their Isolation from Different Sources. British Journal of Research;*05*(03).1-11: https://doi.org/10.21767/2394-3718.100043.
7. Mulaw G, Tessema ST, Muleta D, Tesfaye A. *In Vitro* Evaluation of Probiotic Properties of Lactic Acid Bacteria Isolated from Some Traditionally Fermented Ethiopian Food Products. International Journal of Microbiology, *2019*: 1–11. https://doi.org/10.1155/2019/7179514.
8. Behzadnia A, Moosavi-Nasab M, Tiwari BK, Setoodeh P. Lactobacillus plantarum-derived biosurfactant: Ultrasound-induced production and characterization. Ultrasonics Sonochemistry, 2020; *65*, 105037. https://doi.org/10.1016/j.ultsonch.2020.105037.
9. Satpute SK, Kulkarni GR, Banpurkar AG, Banat IM, Mone NS, Patil, RH,Cameotra SS. Biosurfactant/s from Lactobacilli species: Properties, challenges and potential biomedical applications: Biosurfactant/s from Lactobacilli species. Journal of Basic Microbiology, 2016; *56*(11): 1140–1158. https://doi.org/10.1002/jobm.201600143.
10. Kaur S, Kaur P, Nagpal R. In Vitro Biosurfactant Production and Biofilm Inhibition By Lactic Acid Bacteria Isolated From Fermented Food Products. International Journal of Probiotics and Prebiotics, 2015; 10 (1):17-22.
11. Tillawala MH, Shah MN. Screening and Characterisation of Biosurfactant Producing Lactic Acid Bacteria. International Journal of Science and Research, 2018; 7 (3): 1626-1630.
12. Hosseini SV, Arlindo S, Böhme K, Fernández-NC, Calo-Mata P, Barros-Velázquez, J. Molecular and probiotic characterization of bacteriocin-producing Enterococcus faecium strains isolated from nonfermented animal foods: Probiotic E. faecium strains. Journal of Applied Microbiology, 2009; *107*(4): 1392–1403. https://doi.org/10.1111/j.1365-2672.2009.04327.x.
13. Singhal K, Joshi H, Chaudhary BL . Bile and acid tolerance ability of probiotic Lactobacillus strains. Journal of Global Pharma Technology, 2010; 2(12):17-25.
14. Wang SC, Chang CK,Chan SC, Shieh JS, Chiu CK, Duh PD. Effects of lactic acid bacteria isolated from fermented mustard on lowering cholesterol. Asian Pacific Journal of Tropical Biomedicine, 2014: *4*(7): 523–528. https://doi.org/10.12980/APJTB.4.201414B54.
15. Anila K, Kunzes A, Bhalla TC.In vitro cholesterol assimilation and functional enzymatic activities of putative probiotic Lactobacillus sp isolated from

fermented foods/beverages of North West India, J Nutrition and food science, 2016 ;6 (2) :1-5.

16. Alkan Z, Erginkaya Z, Konuray G, Ünal Turhan E. Production of biosurfactant by lactic acid bacteria using whey as growth medium. Turkish Journal of Veterinary and Animal Sciences, 2019; 43(5) :676–683. https://doi.org/10.3906/vet-1903-48.
17. Sharma A, Soni J, Kaur G, Kaur J. A Study on biosurfactant production in *Lactobacillus and Bacillus sp*. 11. International Journal of Current Microbiology and Applied Microbiology, 2014; 3 (11): 723-733.
18. Faye T, Tamburello GE, and Skeie S. Survival of lactic acid bacteria from fermented milks in an in vitro digestion model exploiting sequential incubation in human gastric and duodenum juice. Journal of Dairy Science, 2011; 95 (2): 558-566.
19. Tsai CC, Lin PP, Hsieh YM, Zhang Z, Wu HC, Huang CC. Cholesterol Lowering Potentials of Lactic Acid Bacteria Based on Bile-Salt Hydrolase Activity and Effect of Potent Strains on Cholesterol Metabolism *In Vitro* and *In Vivo*. The Scientific World Journal, 2014: 1-11. doi.org/10.1155/2014/690752.