IJREAT International Journal of Research in Engineering & Advanced Technology, Volume 9, Issue 3, June - July, 2021
ISSN: 2320 – 8791 (Impact Factor: 2.317)
www.ijreat.org

Study of preparation of lassi using *Lacibacillus* species isolated from cabbage paper for International Journal of Research in Engineering and Advanced Technology

Asmita Dulichand Ukey[1], Priyanka Siddharth Muneshwar[2]

[1] Rajlaxmi Foundation College of Agriculture Biotechnology, Madadgaon, Maharashtra, India
[2] MIP College of Food Technology, Aundha (Nagnath), Maharashtra, India

Abstract

The purpose of current investigation is to isolate and characterize *lactobacillus* species from cabbage and to evaluate its probiotic properties by development of lassi. Cabbage is one of the potential source of lactic acid bacteria due to it nutritional composition. In the present findings, *lactobacillus casei*, *lactobacillus delbrueckii* and *lactobacillus acidophilus* were isolated from cabbage. Probiotic properties of *lactobacillus acidophilus* were investigated by development of lassi. The results shown that, all the 10 bacterial isolates shared characteristic of *lactobacillus species.* Based on the physiological and biochemical characterization three isolates were *lactobacillus casei*, two isolates were *lactobacillus delbrueckii* and five isolates were *lactobacillus acidophilus*. On the basis of sensory evaluation score considering all the attributes, the control sample lassi as compared to lassi inoculated with *lactobacillus* species is found best results.

Key words: Lassi, Probiotics, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus delbrueckii*.

1. Introduction

Lactic acid bacteria (LAB) are a broad group of Gram positive, non-spore forming, and catalase-negative, facultative anaerobic and nutritionally fastidious organism. They are widespread in soil, vegetables, meat, milk and the human body. LAB are among the most important groups of microorganisms used in food fermentation where they play an essential role and a wide variety of strains are routinely employed as starter cultures in the manufacture of dairy, meat, vegetable and bakery products (Noopur *et al*., 2010;Hassanzadazar and Ehsani, 2013).

One of the most important contributions of these microorganisms is the extended shelf life of the fermented products. Growth of spoilage and pathogenic bacteria in these foods is inhibited due to competition for nutrients and the presence of starter-derived inhibitors such as lactic acid, hydrogen peroxide, diacetyl and bacteriocins (Noopur *et al*., 2010; Noordiana *et al*., 2013).

Isolation and identification of *lactobacillus* spp. in various food products reveals the indigenous microflora of that region. Isolation of such regional strains helps in identification the best isolates which can be utilized for further study. With the widespread increased interest in biological preservation of food stuffs, we focused on the antagonistic activity of bacteriocin produced by *lactobacillus* spp. that may be utilized in inhibiting the growth of pathogenic and spoilage causing bacteria in order to keep food products healthy and also with increased shelf life.

Lassi is a popular indigenous fermented milk beverage, which is usually prepared by mixing *dahi* and water in required proportions. It is served on very large scale in cold drink shops, bars and restaurants during summer in

almost every state in India. The fermented milk products are prepared by the action of microorganisms by adding starter culture which modify the substrates biochemically and organoleptically in to edible products and are thus, generally palatable, safe and nutritious (Compbell-Platt, 1994).

Lassi is a ready to serve popular and traditional fermented milk beverage of the Indian subcontinent. Good quality Lassi should have creamy consistency, smooth texture, glossy sheen and white colour with yellowish tinge. Milk acidic flavour and sweetish taste of Lassi make it a refreshing soft drink. It is flavoured either with salt or sugar and other condiments or spices like ginger, coriander and mint depending on regional preferences (Aneja *et al.*, 2002).

On the top of the milk products list is the Indian specialty which never fails to please lassi. Lassi having consistency of milk and taste of curd is popular indigenous fermented milk product prepared by mixing dahi and water along with sugar. It is several on a large scale in roadside cafes, cold drink shop, bars, restaurants and hotels in almost all parts of India and outside India (Egypt and Pakistan).

2. Materials and method:

2.1 Collection of Sample –

The cabbage samples were collected randomly from area of Market Yard, Gultekadi, Mahatma Phule Mandai, Pune. The cabbage sample were taken and packaged into sterile plastic containers, and transported to microbiology laboratory of NAFARI, Pune. Experiments were done immediately to prevent deterioration. The leaves of cabbage were washed with sterile distilled water and separated aseptically. Further, cabbage juice was obtained with juice extractor and homogenated using 10% (w/v) phosphate buffer. (Thakkar *et.al. 2015).*

2.2 Isolation of *lactobacillus* species

Lactobacillus species were isolated from homogenated cabbage juice according to Hawaz (2016) and Mohan *et. al.* (2013). The homoginated samples were serially diluted and sample from different dilutions were inoculated by pour plating on selective media (De Man, Rogosa and Sharpe Agar) for isolation of *lactobacillus* species, further it was incubated anaerobically at 37^0C for 48 hrs. The colonies were randomly picked from plates and purified by successive streaking on MRS agar media before being subjected to characterization.

2.3 Physiological and Biochemical Characterization of *lactobacillus* species

Characterization was carried out according to Hawaz (2016). The culture were subjected to various biochemical test such as CO_2 and lactic acid production from glucose was tested in citrate lacking MRS broths media containing inverted Durham tubes. A catalase test was performed by adding 3% of hydrogen per oxide (H_2O_2) in a test tube containing an overnight culture of *lactobacillus* species. The isolates were further characterized by their carbohydrate fermentation pattern using different sugars (lactose, raffinose, sucrose, salicine, cellobiose, gluconate, arabinose, and mellibiose). Gram positive, catalase negative, and bacilli colonies were taken as Lactobacillus species. The culture were further stored and maintained at -20^0C on MRS agar slants supplemented with 10% (v/v) glycerol for further studies.

2.4 Preparation of lassi

Lassi was prepared as suggested by Bhoir *et. al.* (2012) and Ghule *et. al.* (2015). Sample of cow milk was obtained from Katraj Dudh Sangh, Pune and inoculated with *lactobacillus* species isolated from cabbage, treated as treatment 1 (T_1) along with *lactobacillus* species obtained from Microbiology Department of NAFARI, inoculated in control sample (C_0). Distilled was used for preparation of treated and control samples. Level of water and sugar were kept constant for 10 and 8 % respectively and stored at refrigerator temperature (7^0C).

2.5 Organoleptic evaluation of lassi

The lassi samples (Treatment 1 and Control) were subjected to organoleptic evaluation by the panel of six semi-trained judges adopting 9 point Hedonic scale as suggested by Ghule *et.al.* (2015).

2.6 Chemical Analysis of Lassi

The lassi samples (T_1 and C_0) were analyzed for their chemical properties. The percent of acidity was calculated in terms of lactic acid by titrating against 0.1N NaOH according to AOAC (1995) method. The total solid content was estimated by gravimetric method, Protein content was determined by Kjeldahl method as described by Ranganna (2000) for nitrogen estimation, using factor of 6.38 for conversion of nitrogen into protein, The total sugar content was determined by the method as described by Ranganna (2000), Fat content was determined by Gerber centrifuge method, whereas pH will measured by pH meter. Lactose was determine by as per Lane-Eynon's method given in IS:1479 (Part II) 1961. Ash content was estimated as per procedure given in AOAC 1990.

3. Result and Discussion

3.1 Isolation of *lactobacillus* species

10 bacterial colonies were isolated from cabbage juice and were designated as A, B, C, D, E, F, G, H, I and J. All isolates were studied for their morphological characteristics viz., type of colony, colour, margin, elevation, opacity and presence of pigment and the results are shown in Table 4.1. All the isolate colonies appeared as bacilli in their shape. The colour of colonies ranged from off white, shiny white to creamy white. Margins were entire in all the isolates. Elevations of the isolated colonies were convex. The opacity of the isolates was translucent and opaque in nature. Further the colour pigments were absent in all pure colonies of isolates and appeared white to creamish in colour.

Table 1. Morphological Characteristics of Bacterial Strains Isolated from Cabbage

Sr. No.	Isolate No.	Colony Color	Margine	Elevation	Opacity
1.	A	Creamy White, Shiny	Entire	Convex	Opaque
2.	B	Off White	Entire	Convex	Translucent
3.	C	Off White	Entire	Convex	Opaque
4.	D	White	Entire	Convex	Opaque
5.	E	Of White	Entire	Convex	Opaque
6.	F	White	Entire	Convex	Opaque
7.	G	Creamy White, Shiny	Entire	Convex	Translucent
8.	H	Off White	Entire	Convex	Opaque
9.	I	White	Entire	Convex	Opaque
10.	J	White	Entire	Convex	Opaque

3.2 Physiological and Biochemical Characterization of *lactobacillus* species

All the 10 bacterial isolates shared characteristic of *lactobacillus species* which are gram positive, catalase negative, non-motile, non-sporulating, anaerobic and bacilli, produced acid but no gas from fermentation of glucose. All isolates were well identified based on their sugar fermentation profile. According to carbohydrate fermentation, three isolates ferment sugar lactose, sucrose, gluconate and salicine. Two isolates ferment only sucrose and five isolates ferment cellobiose, lactose, sucrose, salicine (Table 4.2). Therefore, based on the physiological and biochemical characterization three isolates were *lactobacillus casei*, two isolates were *lactobacillus delbrueckii* and five isolates were *lactobacillus acidophilus*. This in agreement with Hawaz (2014).

Table 2 Physiological and Biochemical Characteristics of Bacterial Strains Isolated from Cabbage

Isolate No.	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
Gram Reaction	+	+	+	+	+	+	+	+	+	+
Catalase Test	-	-	-	-	-	-	-	-	-	-
Motility Test	-	-	-	-	-	-	-	-	-	-
Spore Formation	-	-	-	-	-	-	-	-	-	-
Aerobic/Anaerobic	An	An	An	An	An	An	An	An	An	An
Cell Shape	Bc	Bc	Bc	Bc	Bc	Bc	Bc	Bc	Bc	Bc
Lactose	+	-	+	+	+	+	+	-	+	+
Raffinose	-	-	-	-	-	-	-	-	-	-
Sucrose	+	+	+	+	+	+	+	+	+	+
Salicine	+	-	+	+	+	+	+	-	+	+
Cellobiose	-	-	-	+	+	+	-	-	+	+
Gluconate	+	-	+	-	-	-	+	-	-	-
Arabinose	-	-	-	-	-	-	-	-	-	-
Mellibiose	-	-	-	-	-	-	-	-	-	-
Strain	*Lc*	*Ld*	*Lc*	*La*	*La*	*La*	*Lc*	*Ld*	*La*	*La*

+ Positive, - Negetive, An – Anaerobic, Bc – Bacillus, *La – Lactobacillus acidophilus*, *Lc – Lactobacillus casei*, *Ld - Lactobacillus delbrueckii.*

3.3 Preparation of lassi

Cow milk was inoculated with *lactobacillus acidophilus* isolated from cabbage and Control sample ware analyzed sub sequentially for organoleptic quality and chemical composition.

3.4 Organoleptic evaluation of lassi

The results for organoleptic quality of lassi samples are presented in table 3 as below

Table 3 Organoleptic Evaluation Score of Lassi

Treatment	**Colour and Appearance**	**Flavour**	**Body and Texture**	**Acidity**
C_0	8.00	7.90	7.40	7.80
T_1	7.80	7.20	7.30	7.50

The highest sensory evaluation score shown for colour and appearance, body and texture and flavour is for control with the score 8.00, 7.90, 7.40 and 7.80. On the basis of sensory evaluation score considering all the attributes, the control sample lassi was liked very much by panal of judges.

3.5 Chemical Analysis of Lassi

The lassi samples (T_1 and C_0) were analyzed chemically for its constituents like fat, protein, lactose, ash content, total sugar, total solids, acidity, pH and results are presented in table 4

Table 4 Chemical Analysis of Lassi

Sr. No.	Chemical Parameter	Treatment C_0			Avg.	Treatment T_1			Avg.
1.	Fat (%)	4.70	4.73	4.69	4.70	3.21	3.25	3.28	3.24
2.	Protein (%)	3.64	3.60	3.62	3.62	3.35	3.38	3.28	3.33
3.	Lactose (%)	4.18	4.20	4.15	4.17	3.99	3.95	3.89	3.94
4.	Ash (%)	0.78	0.82	0.73	0.77	0.68	0.70	0.68	0.68
5.	Total Sugar (%)	14.2	14.1	14.5	14.2	12.6	12.5	12.3	12.46
6.	Total Solid (%)	21.5	21.1	21.4	21.3	25.1	25.3	25.0	25.13
7.	Acidity (%)	0.89	0.95	0.92	0.92	1.02	1.00	0.99	1.00
8.	pH	4.15	4.18	4.21	4.18	4.18	4.15	4.17	4.16

The maximum average fat (4.70%) was observed in the control (C_0) sample of Lassi. Whereas, it was observed average 3.24 % fat in case of Treatment 1 (T_1). Highest average protein (3.62%) content was observed in Control (C_0) sample of Lassi as compared to Treatment 1 (T_1) which has average 3.33% of protein. Average percent of lactose content was also maximum in control (C_0). Whereas, it was observed average 3.94 % lactose content, in case of Treatment 1 (T_1). Ash content was maximum in control (0.77) as compared to Treatment 1 (T_1) which has average 0.68% ash content. Total sugar content is ranged from 14.1 to 14.5 with average 14.2% sugar content in control (C_0) which makes it superior as compared to Treatment 1 (T_1). Maximum total solid (25.13%) was observed in while control (C_0) has 21.3% of total solid. Acidity was increased (1.00%) in Treatment 1 (T_1) whereas control (C_0) has average 0.92% acidity. pH values are slightly variant for control (C_0) 4.18 and Treatment 1 (T_1) 4.16.

Conclusion

10 bacterial colonies were isolated from cabbage juice. All the isolate colonies appeared as bacilli in their shape. The colour of colonies ranged from off white, shiny white to creamy white. Margins were entire in all the isolates. Elevations of the isolated colonies were convex. The opacity of the isolates was translucent and opaque in nature. Further the colour pigments were absent in all pure colonies of isolates and appeared white to creamish in colour.

On the basis of sensory evaluation score and physico-chemical characteristic considering all the attributes, the control sample lassi as compared to lassi inoculated with *lactobacillus* species is found best. The maximum average fat (4.70%) was observed in the control (C_0) sample of lassi. Whereas, it was observed average 3.24 % fat in case of treatment 1 (T_1). Highest average protein (3.62%) content was observed in control (C_0) sample of lassi as compared to treatment 1 (T_1) which has average 3.33% of protein. Average percent of lactose content was also maximum in control (C_0). Whereas, it was observed average 3.94 % lactose content, in case of Treatment 1 (T_1). Ash content was maximum in control (0.77) as compared to treatment 1 (T_1) which has average 0.68% ash content. Total sugar content is ranged from 14.1 to 14.5 with average 14.2% sugar content in control (C_0) which makes it superior as compared to treatment 1 (T_1). Maximum total solid (25.13%) was observed in while control (C_0) has

21.3% of total solid. Acidity was increased (1.00%) in treatment 1 (T_1) whereas control (C_0) has average 0.92% acidity. pH values are slightly variant for control (C_0) 4.18 and treatment 1 (T_1) 4.16.

References

[1] Bhoir K, Gubbawar S, Shelke R, Nage S and Sakate s. (2012). Physico-chemical properties of Lassi sold in Akola market. *Research Journal of Animal Husbandry and Dairy Science3(1):17-20.*

[2] Coeuret Valerie. Egol S,_Dubernet E, Bernardeau M, Gueguen M and Vernoux J. (2003). Isolation, characterisation and identification of lactobacilli focusing mainly on cheeses and other dairy products. Lait 83 (4), pp.269-306.

[3] Forouhandeh H, Vahed S, Hejazi M, Nahaei M and Dibavar M. (2010). Isolation and Phenotypic Characterization of Lactobacillus species from various Dairy Products. *Current Research in Bctriology 3(2):84-88.*

[4] Ghule B, Desale R.J, Gavhane M and Khore M. (2015). Preparation of strawberry *Lassi. Research Journal of Animal Husbandry and Dairy Science 6(1):22-26.*

[5] Hawaz Estifanos. (2014). Isolation and identification of probitic lactic acid bacteria from curd and in vitro evaluation of its growth inhibithion activities against pathogenic bacteria. *African Journal of Microbilogy Research8(13):pp,1419-1425.*

[6] Hassan P. (2013). Characterization and Identification of *Lactobacillus Acidophilus* using Biology Rapid Identification System. *International Journal of Pharmacy and Pharmaceutical Sciences 6(1)*

[7] Hawaz Estifanos. (2016). Isolation and Characterization of Lactobacillus Species from Head Cabbage (Brassica oleracea var. capitate) and its Potential Application as a Probiotic Agent. *East African Journal of Sciences; 10 (1):23-28.*

[8] Jayachitra J and Ramanathan N.(2013). Role of Lactic Acid Bacteria In Fermentation Of Cabbage Juice. *Indian Streams Research Journal 3(7)*

[9] Kumar D. (2014). Isolation and characterization of bacteria from dairy samples of Solan in Himachal Pradesh for identification of Lactobacillus spp. *Int. J. Pharm. Sci. Rev. Res., 25(2).*

[10] Kyung Y, Edward E. Woodams, Yong D. Hang. (2005). Production of probiotic cabbage juice by lactic acid bacteria. *Department of Food Science and Technology, Cornell University, Geneva, NY 14456, United States*

[11] Karthick S. Effect of Media on Bacteriocin Production by *Lactobacillus brevis* and Evaluation of Anti-Bacterial Activity.

[12] Samuel S, Shukla S, Ramteke PW and Shushma. (2016). Isolation and identification of antagonistic *Lactobacillus* spp. isolated from dairy products against selected pathogens. *The Pharma Innovation Journal ; 5(1):08-13*