



Isolation of *Lactobacillus acidophilus* from Yakult and its application in Probiotic Ice-cream

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Abstract:

Our aim of the research is to isolate the *Lactobacillus acidophilus* bacteria from Yakult which is a dairy product and the application of the organism in probiotic ice-cream. The duration of the research is 4-5 months. For the research, around 20-25 research papers were referred. Probiotics can be defined as live microorganisms (bacteria or yeast) that can bring health benefits to humans or animal bodies, usually the maintenance and the improvement of the microbial balance of the intestinal environment. Ice cream is a dairy product with good potential to act as a food carrier for probiotic bacteria. The growing interest of consumers in therapeutic cultures into Ice cream to result in dietetic Ice cream. Lactobacilli are gram positive and catalase negative bacteria. Most of the dairy industry use Lactic acid bacteria as a starter culture for the manufacturing of fermented products like yoghurt, cheese and Yakult, etc. The purpose of our research is to manufacture a probiotic ice cream by inoculating *Lactobacillus acidophilus* in the ice cream and then to check the colony forming unit per ml on MRS medium and check the viability of the bacteria after frozen storage and to compare the prepared ice cream with the commercial ice cream. Among the most used organisms are those belong to the general of *Lactobacillus* and *Bifidobacterium* which are believed to have beneficial effects on human health, some of the benefits of probiotic foods are promotion of growth and digestion, setting effect on bowel movement, suppression of cancer, catering to lactose intolerance and lowering blood cholesterol levels, etc

Key words: *Lactobacillus acidophilus*, Probiotics, ice cream, health benefits, Rogosa medium

Introduction:

Live microorganisms (bacteria and/or yeasts) that can alter the microbial balance of the gut environment and provide health advantages to people and animals are known as "probiotics." (7) Due to consumers' growing understanding of how food affects their health, the demand for functional foods is fast increasing around the world. (8) Ice cream is a dairy product that has a good chance of serving as a probiotic bacteria food carrier. Since ice cream is consumed by the general public and contains healthy ingredients like dairy raw materials, vitamins, and minerals in addition to being a functional healthy food, adding probiotic bacteria to it is highly useful. Moreover, ice cream promotes significantly increased vitality of probiotic strains during manufacture and especially storage when compared to fermented milks as a carrier. (7)

Among the most popular microorganisms are those from the genera *Lactobacillus* and *Bifidobacterium*, which are thought to improve human health. (6) *Lactobacilli* and *Bifidobacterium* are the most commonly used and studied probiotic organisms. (1) The use of probiotic bacteria in food products, especially probiotic

dairy products such fermented milks, ice cream, and other kinds of cheese, has significantly increased in modern society. Probiotic ice cream is becoming more and more popular among probiotic dairy products since it can be kept for a long time without losing any of its qualities and is a highly sought-after item globally. (7) Ice cream is a popular frozen dairy product around the world because it is tasty, healthy, and nutritious. Ice cream contains nutritional value but has no medicinal effects. Probiotic cultures have been added to ice cream to create dietetic ice cream as a result of customers' increased interest in therapeutic products. (8) Probiotic foods have been linked to a variety of physiological and nutritional advantages, including the promotion of growth and digestion, setting effect on the gastro intestinal tract, enhancing bowel movement, suppressing cancer, accommodating lactose intolerance, lowering blood cholesterol levels, and more. (6) A high rate of bacterial survival during production and throughout the product's shelf life is necessary to retain consumer confidence in probiotic products. (7) Benefits include bacterial pathogen suppression, lowered serum

cholesterol, decreased incidence of constipation, diarrhoea, and colon cancer, enhanced lactose tolerance, improved calcium absorption, increased vitamin synthesis, and immune system stimulation. (3) Our study is to isolate *Lactobacillus acidophilus* from Yakult and its application in probiotic ice-cream.

Material and methods:

Sample preparation:

Yakult was bought from dairy store. Small amount was taken in a test tube.

Isolation of probiotic:

Loopful of sample was streaked on Rogosa Agar Medium. Plates were kept in anaerobic condition at room temperature for 48 hours

Biochemical tests:

Gram's staining, Catalase test, Indole test, Methyl red test, Voges Proskauer test, Citrate test, Urease test and Sugar Fermentation tests were performed for the isolated organism to check if it is *Lactobacillus acidophilus*

Preparation of ice-cream:

Homogenised toned milk, sugar, stabilizer, milk powder, fresh cream, and flavour, all these were mixed in a container and kept at 80°C for 5 minutes. The mixture was cooled at 4°C. Ripening was done at 4°C for 24 hours in the fridge.

Fermentation:

Fermentation of ice-cream was done at 37°C until pH 5.5

Addition of probiotic:

After taking the O.D of the culture suspension, the probiotic was added into the prepared ice-cream mixture. It was cooled at 4°C and then stored at -20°C.

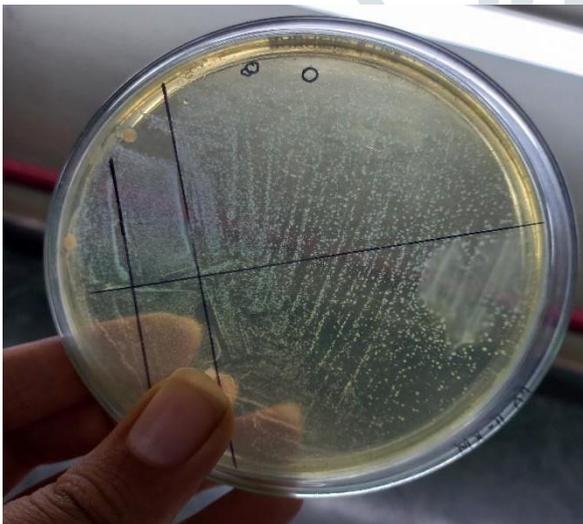
Checking the viability of the probiotic:

Serial dilutions were performed and last 3 dilutions were pour plated using Rogosa Agar Medium

Result and Discussion:



Yakult sample was streaked on Rogosa agar medium. Rogosa medium is used because it is a selective medium for Lactobacillus organism. It supports the growth of lactobacillus spp. The plates were kept at room temperature for 48 hours under anaerobic condition. Lactobacillus is an anaerobic organism. A desiccator was used to create anaerobic condition.



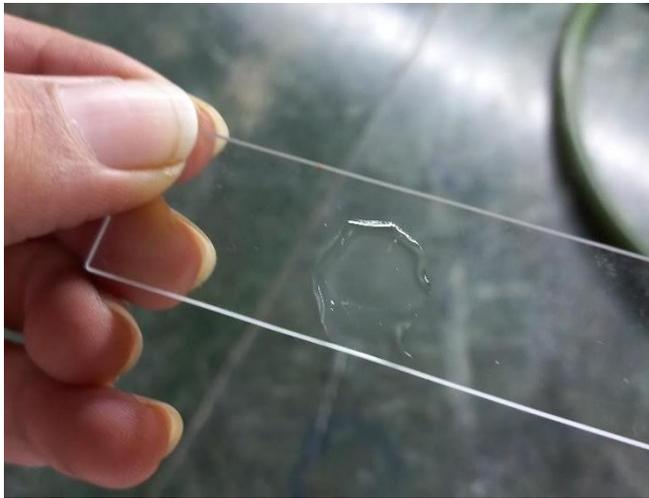
After 48 hours of incubation the plates were observed for the growth of probiotic and colony characteristics were observed.

Table 1: Colony characteristics of the isolated probiotic

Colony characteristics	Observation
Colour	White
Size	Pinpoint
Shape	Circular
Margin	Entire
Elevation	Flat
Opacity	Translucent
Gram's nature	Gram positive rods

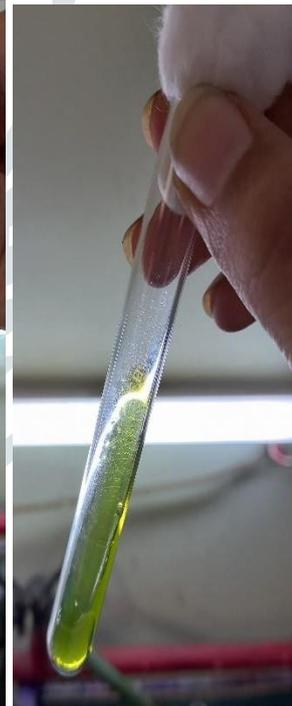
Biochemical tests were performed. Catalase test was done by taking 1 ml of hydrogen peroxide and mixed it with culture which was observed for the effervescence. Gram's staining was done and the isolate was found to be gram positive rods under microscope. Next methyl red test was performed by taking MR-VP broth and adding culture into it. After incubation at RT under anaerobic condition, methyl red reagent was added and was observed for a colour change. Indole test was done by taking tryptone broth and adding

culture into it and observed for ring formation after the incubation period. VP test was done by taking MR-VP broth and adding culture into it. After incubation KOH and alpha naphthol was added and observed for a colour change. Urea test was performed by taking urea broth and adding culture into it and after incubation, phenol red was added to check for any colour change. Next citrate utilization test was performed by streaking the culture suspension on citrate slant and to see if it changes its colour after the incubation period. Lastly Sugar fermentation tests were performed to check if the isolated organism utilizes the sugar and produces gas.



Catalase test

Microscopic slide



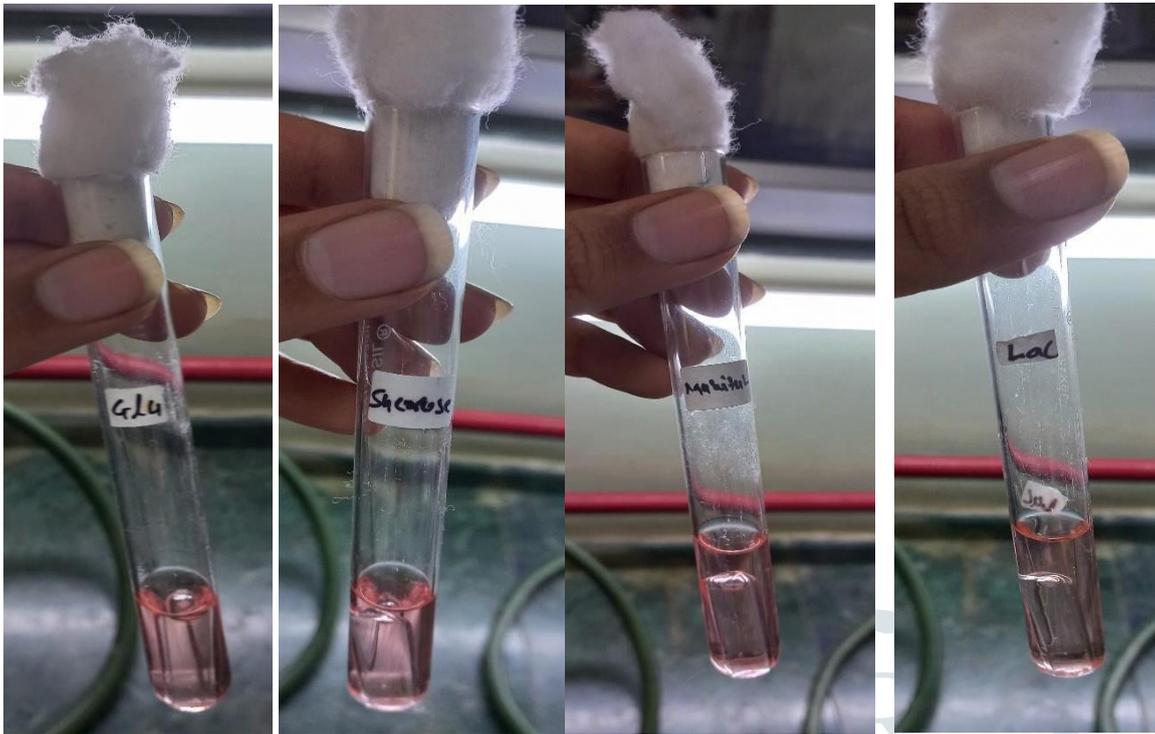
VP test

MR test

Indole test

Citrate test

Urease test



Sugar Fermentation tests

Table 2: Biochemical tests

Tests	Result
Catalase	Negative
Gram's staining	Gram positive rods
MR test	Negative
VP test	Negative
Indole test	Negative
Citrate test	Negative
Urease test	Negative
Lactose	Positive (sugar fermentation and gas production)
Mannitol	Positive (sugar fermentation and gas production)
Sucrose	Positive (sugar fermentation and gas production)
Glucose	Positive (sugar fermentation and gas production)

The isolated organism showed no effervescence which means it is catalase negative. It showed no color change in MR-VP, Indole, Citrate, Urease tests hence they all are negative. The isolated probiotic showed positive results for sugar fermentation tests as we could observe the color change in the broth from colorless to pink which means the organism utilised the sugar and produced gas. These are all the characteristics of *Lactobacillus acidophilus* hence we can say that the isolated probiotic is *Lactobacillus acidophilus*.



The isolate was added into the prepared ice-cream. We mixed all the ingredients, milk, sugar, stabilizer, cream, milk powder and flavor into the container and pasturized it at 80°C for 5 mins. After this the mixture was cooled at 4°C and ripening was done for 24 hours in refrigerator. Later, fermentation was done at 37°C until pH 5.5 and culture was inoculated into the ice-cream. The mixture was then cooled at 4°C and then stored at -20°C. (2)



Table 3: Weekly colony forming units

Weeks	Cfu/ml
Week 1	4.51x10 ⁸
Week 2	6.94x10 ⁸
Week 3	1.03x10 ⁹
Week 4	1.53x10 ⁹

From the observation we can say that the probiotic culture survived the frozen storage condition and was growing properly and that ice-cream acted as the perfect for the lactobacillus to grow. This increased the therapeutic quality of ice-cream as the consumer can have health benefits by consuming this icecream in their diet. 10^8 - 10^9 cfu per ml is considered to be the perfect amount to be consumed as the minimum requirement is 10^6 .

Conclusion:

Probiotic culture was isolated from Yakult. Yakult sample was streaked on rogosa plates, pinpoint translucent colonies were taken to prepare the culture suspension. Gram staining was done and was found to be gram-positive bacilli. The outcome of catalase test was negative. Other biochemical tests like sugar fermentation showed positive results as color change and gas production was observed. The organism showed citrate, urease, MR-VP and indole tests negative. The isolated organism was inoculated into the prepared ice-cream and was checked for cfu/ml and whether the ice-cream supports the growth of bacteria. It maintained the shelf life by having the count of minimum 10^8 - 10^9 cfu/ml without affecting the taste of the ice-cream. The regular commercial ice-cream doesn't have any medicinal effect on human body whereas the probiotic ice-cream provides us with various health benefits like improving digestion, bowel movement, improving gut bacteria, etc. Ice cream can serve as an excellent environment to deliver probiotics into the human intestine. Bacterial cultures maintained at levels high enough to provide the reported therapeutic effects, even after being stored frozen in the products. Probiotic bacteria supplementation has been shown to have a negligible impact on the flavour or composition of ice cream. We can conclude that it is preferable to go for probiotic ice-cream over regular commercial ice-cream.

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