

## **CHARACTERISATION OF PROBIOTIC PROPERTIES OF LACTOBACILLUS AND DEVELOPMENT OF OAT MILK BASED YOGURT**

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### **Abstract**

**Introduction** Oats have also recently been considered suitable for the diet of celiac patients. Owing to their high nutritional value, oat-based food products like bread, biscuits, cookies, probiotic drinks, breakfast cereals, flakes, and infant food are gaining increasing consideration. (Pelinescu et al.,2009; Malek et al.,2012) Research and development on oat and its products may be helpful in combating various diseases known to mankind. This paper provides an overview of the nutritional and health benefits provided by oats as whole grains and their value-added products. It is designed to provide insight into the processing of oats and their effect on their functional properties. (Mital et al. 1974; Scalabrini et al., 1998). **Materials and Methods** Isolation of lactic acid bacteria. **Biochemical Test** Identification Indole Test Negative Methyl red Test Positive Voges-Proskauer Test Negative Citrate Utilization Test Negative Catalase Test Negative Oxidase Test Negative Carbohydrate fermentation test;The observation of a colour change from red to yellow indicates positive lactose, maltose, and sucrose fermentation was there. Litmus milk test ;The litmus milk test was done and the change of colour change of violet to pink indicates the fermentation of lactose and it is acidic. **Molecular identification of the isolated strain using 16SrRNA sequencing** The isolated strain was identified using a 16SrRNA sequence using Helini purefast DNA Bacterial genomic DNA minis pin prep kit (Helini biomolecules,

Chennai, India). The strain was identified as *Lactobacillus Salivarius*. Safety Assessment Haemolytic activity and DNase activity was done that is safety assessment tests and Colonies were streaked on blood agar plates and DNase agar were incubated for 24 hours and there is no haemolytic activity was observed. Bile tolerance was done and the culture was tolerant of bile. Acid tolerance was done and the culture was tolerant to acid. Antagonistic activity was done. Nutritional evaluation of oat yogurt Estimation of carbohydrate Carbohydrate was estimated by anthrone method and the result was found to be 46%. Estimation of protein; Protein was estimated by lowryes method and the result was found to be 11% Estimation of aminoacid; Amino acid was estimated by ninhydrin method and the result was found to be 0.1 mg/100ml. Summary And Conclusion In this study the contents of oat milk yogurt were estimated, but no validated through the experimental studies and animal studies. For the further or future study, the work has to be validated for the experimental analysis and animal studies for identify the cholesterol control ability of oat milk fortified yogurt. Probiotic oats yogurt was produced successfully. Oat milk is obtained from oat flour and contains antioxidant substances that counteract cellular aging and fibres that improve and facilitate digestion. Factors in Favor: having a low sugar content, oat milk is highly recommended for people with diabetes. Compared to traditional milk, it has a high quantity of fibre. Like soy milk, oat milk also contains a good percentage of carbohydrates and vitamin E.

**Keywords:** Probiotic , oats milk ,Oat yogurt .

## **Introduction**

Oats have also recently been considered suitable for the diet of celiac patients. Owing to their high nutritional value, oat-based food products like bread, biscuits, cookies, probiotic drinks, breakfast cereals, flakes, and infant food are gaining increasing consideration. (Pelinescu et al., 2009; Malek et al., 2012) Research and development on oat and its products may be helpful in combating various diseases known to mankind. This paper provides an overview of the nutritional and health benefits provided by oats as whole grains and their value-added products. It is designed to provide insight into the processing of oats and their effect on their functional properties. (Mital et al. 1974; Scalabrini et al., 1998).

Purpose Interest in probiotic food products has constantly increased due to the awareness of the importance of gut microbiome; an increasing demand has encouraged the development of other

matrices such as cereals, vegetable, and fruit juices to deliver probiotics. The purpose of this paper is to standardize and evaluate a ready-to-serve probiotic oats milk drink fermented with microencapsulated *Lactobacillus plantarum* to be further used as a therapeutic module. (Coeuret et al., 2003) Research limitations/implications Analysis of all the parameters was conducted only with three samples; this was the potential limitation identified in this study as a large sample size always be a better representative of the results. Practical implications Spice and strawberry flavoured non-dairy oats milk drink facilitated to be a suitable carrier for microencapsulated *L. plantarum* with good sensory attributes, low fat, moderate calorie, high fibre content, antioxidant potential, and a shelf life of two-week period at 4°C. Originality/value The developed ready-to-serve, spice, and strawberry flavoured non-dairy oats milk drink with compactly packed functional components inclusive of beneficial probiotic organisms,  $\beta$ -glucan and antioxidants can be prescribed as therapeutic food for many clinical conditions and would serve as a good probiotic option for vegans. (Miremadi, Sherkat & Stojanovska et al., 2016).

Yogurt is a milk-based product manufactured by *lactic acid* fermentation enabled by symbiotic yogurt cultures. Yogurt is largely considered to be a health product, and it is employed to deliver probiotics and prebiotics to the consumer (Marsh et al., 2014; Kamal et al., 2018). However, not all yogurts are probiotics, and neither are they all functional products. There is increasing demand for health-promoting beverages, which is prompting the dairy industry to develop functional probiotic yogurts to meet the demand. However, there seems to be a scarcity of reviews providing critical information on regulatory frameworks in regions of the world, clinical trial outcomes, and methodological approaches for enumerating multi-probiotic strains in yogurt (Cutrim et al., 2016; Kamal et al., 2018). This review, relating to functional probiotic yogurt, covers the newest information on the topic for the period mostly between 2014 and 2019. Probiotic yogurt is a popular functional food product around the world. Delivering an appropriate number of viable probiotic bacteria is critical in determining the health-improving properties of yogurt. The viability of probiotics in yogurt can be affected by food components (e.g., sugars, proteins, fat, vitamins, minerals, flavoring agents, antioxidants, and amino acids), processing-related factors (e.g., heat treatments, homogenization, and fermentation temperature) and also microbiological factors (e.g., type of strains and inoculum level). (Bisanz et al., 2014; Ray and Joshi, 2015; Moineau-Jean et al., 2019). This work is

for the production of probiotic oat milk-based yogurt production from *Lactobacillus Salivarius*. Therefore, our objectives of this study were; to explore the use of oats in producing oat milk and production of oat yoghurt by fermentation of oat milk with *Lactobacillus salivarius* isolated from oat milk.

## Materials and Methods

### Isolation of lactic acid bacteria

De Man, Rogosa, and Sharpe (MRS) Broth were prepared and 1g of curd was put into it and kept at incubation for 48 hours at 37°C. MRS Agar was prepared and culture from MRS Broth was streaked on it and kept for incubation for 24 hours at 37°C. White glistening colonies on the MRS Agar plate were observed. Plating out technique was used for isolation of *Lactobacillus* spp. The selected colonies were purified using streak plate technique. Finally, the single colony of bacteria was isolated by observing their colonial morphology and some physiological tests (Gram staining, indole, methyl red, Voges-Proskauer Test, Citrate Utilization Test, catalase reaction and oxidase reaction).

Biochemical Test	Identification
Indole Test	Negative
Methyl red Test	Positive
Voges-Proskauer Test	Negative
Citrate Utilization Test	Negative
Catalase Test	Negative
Oxidase Test	Negative

### Carbohydrate fermentation test

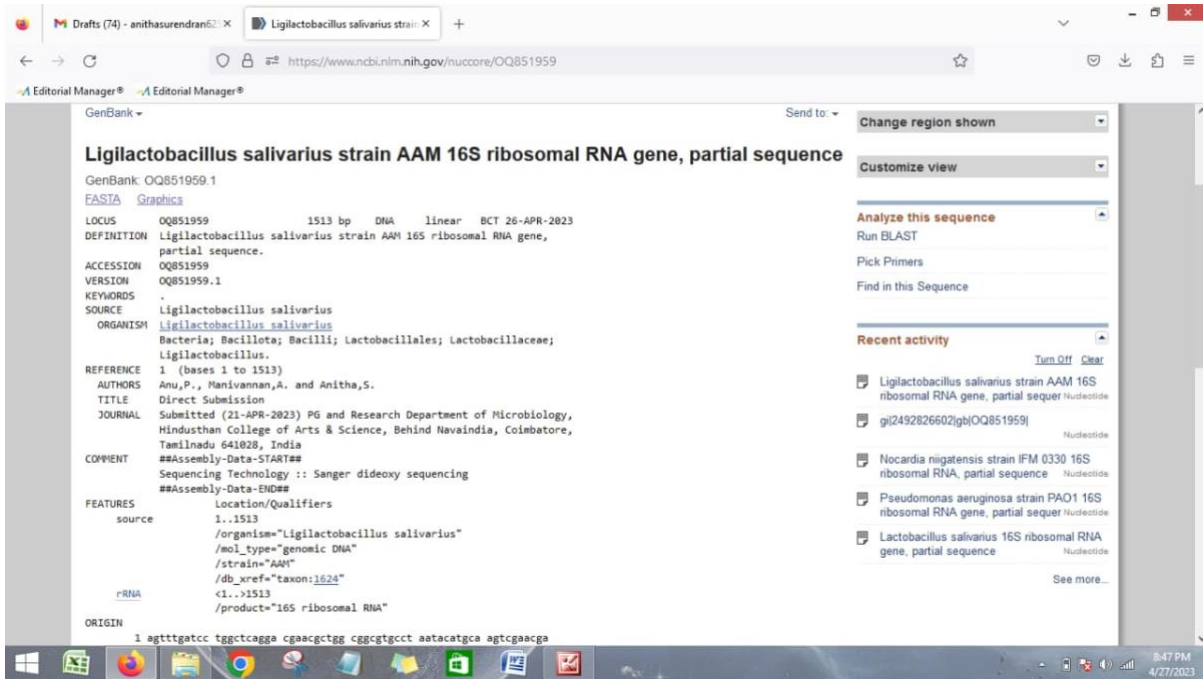
The observation of a colour change from red to yellow indicates positive lactose, maltose, and sucrose fermentation was there.

### Litmus milk test

The litmus milk test was done and the change of colour change of violet to pink indicates the fermentation of lactose and it is acidic.

### Molecular identification of the isolated strain using 16SrRNA sequencing

The isolated strain was identified using a 16SrRNA sequence using Helini purefast DNA Bacterial genomic DNA minis pin prep kit (Helini biomolecules, Chennai, India). The strain was identified as *Lactobacillus Salivarius*.



### Safety Assessment

Haemolytic activity and DNase activity was done that is safety assessment tests and Colonies were streaked on blood agar plates and DNase agar were incubated for 24 hours and there is no haemolytic activity was observed.

### Bile Tolerance

Bile tolerance was done according to Tsai *et al.*, (2007). MRS Agar with 0.3% bile salt was prepared and the pour plate method was used for the estimation of the survivability of *Lactobacillus salivarius*. And the culture was tolerant of bile.

### Acid Tolerance

Acid tolerance was done according to (Hofmann *et al.*, 1994). MRS Agar acidified using 2N HCl was prepared and the organism was pour-plated for the estimation of the survivability of *Lactobacillus salivarius* kept for incubation for 24 hours at 37°C and the culture was tolerant to acid.

### Antagonistic activity

ORGANISM	ZONE OF INHIBITION
<i>Staphylococcus aureus</i>	15mm
<i>Salmonella spp</i>	3mm
<i>E. coli</i>	25mm
<i>Klebsiella pneumoniae</i>	19mm
<i>Pseudomonas oxytoca</i>	14mm
<i>Bacillus spp</i>	1mm

### **Production of oat milk**

Oats soaking in distilled water and add alpha amylase for breaking down the starch and filter the mixture using a sterile cloth and pasteurized at 72°C for 15 sec and the result was oat milk

### **Production of oat milk yogurt**

Addition of probiotic bacteria and mixed well after the incubation of 37°C for 24 hours the oat yogurt was collected.

### **Nutritional evaluation of oat yogurt**

#### **Estimation of carbohydrate**

Carbohydrate was estimated by anthrone method and the result was found to be 46%.

#### **Estimation of protein**

Protein was estimated by lowryes method and the result was found to be 11%

#### **Estimation of Fat**

#### **Estimation of aminoacid**

Amino acid was estimated by ninhydrin method and the result was found to be 0.1 mg/100ml.

### **Result and Discussion**

Lactobacillus spp. were isolated by adding 1g of curd into MRS broth, which was identified based on their colony morphologies grown on MRS (Man, Rogosa and Sharpe) media and on the basis of biochemical tests conducted in the laboratory. Based on biochemical characteristics and further screen, these two isolates were selected for probiotic screening and further study. Gram staining, Catalase test, Oxidase test, Sugar fermentation, Bile salt test, and pH tolerance test were done by maintaining pH as 3.5 in this regard. (Md. Ahmadu Islam et al., 2018) Bile tolerance was measured as described by Gilliland et al. (1984). Briefly, growth was measured in MRS broth containing 0.05 to 0.25% bile salt in 7 h by spectrophotometer (OD<sub>600nm</sub>) and bile salt-free MRS was used as control. After the biochemical tests carbohydrate fermentation test was done for detecting the ability of microorganisms to ferment a specific carbohydrate for this test 50 ml of peptone was prepared with 0.2 mg of phenol red and in each of the 3-test tube added 5 ml of peptone broth and in the three-test tube added lactose, maltose, sucrose respectively. And add the Durhams tubes in to all the test tubes without any bubbles and incubated for 24 hours at 37°C. The positive result was observed by colour change from red to yellow. (Farzana Akter et al., 2018) And the results are positive for all the sugars. Litmus milk test was done for determining an organism's ability to metabolize litmus milk. The colour change of violet to pink indicates that the fermentation of lactose and it is acidic. By

comparing with (Atanasova J & Moncheva P et al., 2014). For the results Pinkish-red: Acid reaction, lactose fermentation Blue: Alkaline reaction, no fermentation, organisms attack nitrogenous substances in the medium A clot or curd formation: Milk protein coagulation.

Oats milk was prepared by crushing oats while making the oats milk we are added some alpha amylase into it for breaking down the starch content in to it and then Pasteurization was done by using magnetic stirrer for 72°C for 15 min and keep the milk overnight. and oats yogurt was prepared by addition of probiotic yogurt after one day incubation and incubate it again for 24 hours After incubation the yogurt was cooled at refrigerator for hours and we will get the oats yogurt.

Carbohydrate was estimated to 1g of powdered sample in a refluxing flask add 10mL of cold neutral detergent solution. Add 2mL of decahydronaphthalene and 0.5g sodium sulphite. Heat to boiling and reflux for 60min. Filter the contents through sintered glass crucible (G-2) by suction and wash with hot water. Finally give two washing with acetate. Transfer the residue to a crucible, dry at 100°C for 8h. Cool the crucible in a desiccator and weigh. (Goering, H D and Vansoest, *et al.*, 1975). Then protein was estimated Pipette out protein solution in the range of 0 to 200 mg/1 mL into d/f test tubes. Make up the volume of all the test tubes to 1 mL with water. Add 5 mL of alkaline solution to each test tube, mix thoroughly, and allow to stand at room temperature for 10 minutes. Add 0.5 mL of dilute FC-reagent rapidly with immediate mixing. After 30 minutes read the extinction against the appropriate blank at 650 nm. (Alberts B, Johnson A; *et al.*, 2002). Finally, aminoacid was also estimated to 0.1mL of extract, add 1mL of ninhydrin solution. Make up the volume to 2mL with distilled water. Heat the tube in boiling water bath for 20min. Add 5mL of the diluents and mix the contents. After 15min read the intensity of the purple colour against a reagent blank in a colorimeter at 570nm. The colour is stable for 1h. Prepare the reagent blank as above by taking 0.1mL of 780% ethanol instead of the extract (Moore, S and Stein, W H *et al.*, (1984)). Finally antagonistic activity was performed for this test MRS agar plate was prepared and single streaked our *Lactobacillus spp* and after one day of incubation vertically streak with other organisms e.g.; *Staphylococcus*, *Streptococcus aureus*, *E. coli*, *Salmonella*, *Pseudomonas*, *Bacillus* and identify after one another day of inoculation. The antagonistic ability includes adhesion to the intestine, reduction of pathogenic bacterial adhesion to the intestine, aggregation and coaggregation as well as production of antimicrobial substances such as bacteriocins (Peres et al., 2012; Khan and Kang, 2016; Russo et al., 2017).

### Summary And Conclusion

In this study the contents of oat milk yogurt were estimated, but no validated through the experimental studies and animal studies. for the further or future study, the work has to be validated for the experimental analysis and animal studies for identify the cholesterol control ability of oat milk

fortified yogurt. Probiotic oats yogurt was produced successfully. Oat milk is obtained from oat flour and contains antioxidant substances that counteract cellular aging and fibres that improve and facilitate digestion. Factors in Favor: having a low sugar content, oat milk is highly recommended for people with diabetes. Compared to traditional milk, it has a high quantity of fibre. Like soy milk, oat milk also contains a good percentage of carbohydrates and vitamin E.

## Reference

- Choi AR, Patra JK, Kim WJ, Kang SS. Antagonistic Activities and Probiotic Potential of Lactic Acid Bacteria Derived from a Plant-Based Fermented Food. *Front Microbiol.* 2018 Aug 24; 9:1963. doi: 10.3389/fmicb.2018.01963. PMID: 30197633; PMCID: PMC6117381.
- Aggarwal AC, Sharma S. 1961. A laboratory Manual of Milk Inspection. Asia Publishing House, Calcutta, India.
- Moore, S and Stein, W H (1984) In: *Methods in Enzymol* (Eds. Colowick, S P and Kaplan, N D) Academic Press New York **3** 468
- Misra, P S, Mertz E T and Glower, D V (1975) *Cereal Chem* **52** 844
- Theymoli Balasubramanian and Sadasivam, S (1987) *Plant Food Hum Nutr* 3741.
- Taylor F, Huffman MD, Macedo AF, Moore TH, Burke M, Davey Smith G, Ward K, Ebrahim S. Statins for the primary prevention of cardiovascular disease. *Cochrane Database Syst Rev.* 2013 Jan 31;2013(1):CD004816. doi: 10.1002/14651858.CD004816.pub5. PMID: 23440795; PMCID: PMC6481400.
- Markowiak P, Śliżewska K. Effects of Probiotics, Prebiotics, and Synbiotics on Human Health. *Nutrients.* 2017 Sep 15;9(9):1021. doi: 10.3390/nu9091021. PMID: 28914794; PMCID: PMC5622781.
- Alcorta A, Porta A, Tárrega A, Alvarez MD, Vaquero MP. Foods for Plant-Based Diets: Challenges and Innovations. *Foods.* 2021 Feb 1;10(2):293. doi: 10.3390/foods10020293. PMID: 33535684; PMCID: PMC7912826.
- Rasane P, Jha A, Sabikhi L, Kumar A, Unnikrishnan VS. Nutritional advantages of oats and opportunities for its processing as value added foods - a review. *J Food Sci Technol.* 2015 Feb;52(2):662-75. doi: 10.1007/s13197-013-1072-1. Epub 2013 Jun 25. PMID: 25694675; PMCID: PMC4325078.
- Y. Shimakawa *et al.* Evaluation of *Bifidobacterium breve* strain Yakult-fermented soymilk as a probiotic food *International Journal of Food Microbiology* (2003)
- Amjad Raeisi, Vahid FarshbafDerhami, Ali Hosseini, Sajjad Dehghani sensory evolution



and accessibility of soy yogurt with different grouping of treatment FFNR 12, Volume 3, Issue 1,2017:1-6

- Ammor, S., G. Tauveron, E. Dufour, I. Chevallier, 2005. Food Microbiol., 22, 529-539
- Arai S, Suzuki H, Fujimake M, Sakurai Y (1996) Studies on flavour components in soybean. Part 2. Phenolic acids in defatted soybean flour. Agric Biol Chem 30:364–369
- Bolotin A, Wincker P, Mauger S, Jaillon O, Malarne K, Weissenbach J, Ehrlich SD, Sorokin A. The complete genome sequence of the lactic acid bacterium *Lactococcus lactis* ssp. *lactis* IL1403. Genome Res.2001; 11:731–53.
- Bustos, I., Garcia-Cayuela, T., Hernandez-Ledesma, B., Pelaez, C., Requena, T. & Martinez-Cuesta,
- M.C. (2012). Effect of Flavan-3-ols on the Adhesion of Potential Probiotic Lactobacilli to Intestinal Cells. Journal of Agricultural and Food Chemistry, 60, 9082-9088.
- FAO, Food and Agriculture Organization of the United Nations and World Health Organization (2001) Evaluation of health and nutritional properties of powdered milk and live lactic acid bacteria. Geneva, Switz. Agriculture Organization of the United Nations and World Health Organization Expert Consultation Report.ftp//
- Luca L, Oroian M. Oat Yogurts Enriched with Synbiotic Microcapsules: Physicochemical, Microbiological, Textural and Rheological Properties during Storage. Foods. 2022 Mar 24;11(7):940. doi: 10.3390/foods11070940. PMID: 35407027; PMCID: PMC8998009.
- Raikos V, Juskaite L, Vas F, Hayes HE. Physicochemical properties, texture, and probiotic survivability of oat-based yogurt using aquafaba as a gelling agent. Food Sci Nutr. 2020 Oct 6;8(12):6426-6432. doi: 10.1002/fsn3.1932. PMID: 33312528; PMCID: PMC7723214.
- Paudel D, Dhungana B, Caffè M, Krishnan P. A Review of Health-Beneficial Properties of Oats. Foods. 2021 Oct 26;10(11):2591. doi: 10.3390/foods10112591. PMID: 34828872; PMCID: PMC8625765.
- Abedi E, Hashemi SMB. Lactic acid production - producing microorganisms and substrates sources-state of art. Heliyon. 2020 Oct 12;6(10): e04974. doi: 10.1016/j.heliyon. 2020.e04974. PMID: 33088933; PMCID: PMC7566098.
- F. Mozzi, in Encyclopedia of Food and Health, 2016
- J.A. Narvhus, L. Axelsson, in Encyclopedia of Food Sciences and Nutrition

(Second Edition), 2003

- *Foods* 2021, 10(6), 1304; <https://doi.org/10.3390/foods10061304> Received: 26 April 2021 / Revised: 26 May 2021 / Accepted: 29 May 2021 / Published: 7 June 2021 (This article belongs to the Special Issue Barley and Oats: Chemistry, Health Benefits, Processing and Utilizations)
- Kechagia M, Basoulis D, Konstantopoulou S, Dimitriadi D, Gyftopoulou K, Skarmoutsou N, Fakiri EM. Health benefits of probiotics: a review. *ISRN Nutr.* 2013 Jan 2; 2013:481651. doi: 10.5402/2013/481651. PMID: 24959545; PMCID: PMC4045285.
- Shi LH, Balakrishnan K, Thiagarajah K, Mohd Ismail NI, Yin OS. Beneficial Properties of Probiotics. *Trop Life Sci Res.* 2016 Aug;27(2):73-90. doi: 10.21315/tlsr2016.27.2.6. PMID: 27688852; PMCID: PMC5031164.
- Kim JI, Park TE, Maharjan S, Li HS, Lee HB, Kim IS, Piao D, Lee JY, Cho CS, Bok JD, et al. Soluble RANKL expression in *Lactococcus lactis* and investigation of its potential as an oral vaccine adjuvant. *BMC Immunol.* 2015; 16:71
- Lamartiniere CA, Cotroneo MS, Fritz WA, Wang J, Mentor-Marcel R, Elgavish E. Genistein chemoprevention: timing and mechanisms of action in murine mammary and prostate. *J Nutr* 2002; 132:552S–8S.
- Champagne, C. P., da Cruz, A. G., & Daga, M. (2018). Strategies to improve the functionality of probiotics in supplements and foods. *Current Opinion in Food Science*, 22, 160–166.
- Donkor, O. N., Henriksson, A., Vasiljevic, T., & Shah, N. P. (2005). Probiotic strains as starter cultures improve angiotensin-converting enzyme inhibitory activity in soy yoghurt. *Journal of Food Science*, 70, M375–M381.
- Garrigues C, Loubiere P, Lindley ND, Cocaigh-Bousquet M. Control of the shift from homolactic acid to mixed-acid fermentation in *Lactococcus lactis*: predominant role of the NADH/NAD<sup>+</sup> ratio. *J Bacteriol.* 1997;179:5282–7.
- Najgebauer-Lejko, D., Sady, M., Grega, T., & Walczykca, M. (2011). The impact of tea supplementation on microflora, pH and antioxidant capacity of yoghurt. *International Dairy Journal*, 21, 568-574 of its cholesterol-lowering properties. *Applied and Environmental Microbiology*, 69.
- . Hornung, B., E. Amtmann and G. Sauer,(1994). Lauric acid inhibits the

maturation of vesicular stomatitis virus. J. Gen.Virol., 75: 353-361