A J Journal of Medical Sciences

AJ Journal of Medical Sciences

ORIGINAL ARTICLE

Influence of Probiotic Fermentation on Polysaccharides of *Tamarindus indica* Seeds

D M Pooja[1], Haseena Sheikh[1], B S Gunashree[1,*]

[1]*Department of Studies and Research in Microbiology, Mangalore University, Jnana Kaveri Campus, Chikka Aluvara, Kodagu, 571232, Karnataka, India*

ARTICLE INFO

Article history:
Received 05.04.2025
Accepted 15.06.2025
Published 29.07.2025

* *Corresponding author.*
B S Gunashree
gunashree_bs@yahoo.co.in

https://doi.org/10.71325/ajjms.v2i2.25.22

ABSTRACT

Background: Tamarind seeds are the legumes rich in protein, carbohydrates, lipids and minerals. Tamarind seed polysaccharide (TSP) is a natural polymer isolated from *Tamarindus indica*. It is a by-product of the tamarind pulp industry. Due to the presence of tannins and other pigment compounds in the seed coat (testa), the whole seed is unsuitable for direct consumption. **Methods:** In this present study submerged fermentation using three different Probiotic bacteria such as *Lactobacillus plantarum, Lactobacillus plantarum* S1 and *Lactobacillus helviticus* was carried out to assess the significant production of exopolysaccharide from *Tamarindus indica* seeds. **Results:** The three probiotic bacteria showed different amounts of exopolysaccharide production in water and media- based fermentation with raw and defatted tamarind seed powder as a substrate. The amount of EPS produced by *Lactobacillus plantarum* and *Lactobacillus helviticus* was 23 and 38% respectively in both defatted and raw tamarind seed powder fermented with media. **Conclusion:** *Lactobacillus plantarum* shows the more production of Exopolysaccharides in both water based and MRS media based defatted and raw tamarind seed powder. While adding Probiotic bacteria it increases the even more quantity of EPS.

Keywords: *Tamarindus indica*; *Lactobacillus plantarum*; *Lactobacillus plantarum* S1; *Lactobacillus helveticus*; Exopolysaccharide

INTRODUCTION

Tamarindus indica is a leguminous tree species belonging to the dicotyledonous group of angiosperms. Leguminosae, a native of dry Savanna of tropical Africa. The major areas of tamarind production are in Asian countries like India, Bangladesh, Sri Lanka and Thailand. India is the leading global producer and consumer of *Tamarindus indica*[1]. Tamarind is primarily cultivated for its fruit pulp, which is widely utilized in the preparation of beverages, as well as in the flavoring of confectioneries, curries, and sauces.[2].

Every part of the tree is shown to have some use, either in textile, carpentry, nutritional or medicinal field. Seed is a cheaply available by-product of tamarind pulp industry which is shown to form about 40% of the total weight[3].

Tamarind seeds are a rich source of protein, containing significant amounts of several essential amino acids such as isoleucine, leucine, lysine, methionine, phenylalanine, and valine. Additionally, the seeds provide essential fatty acids and minerals, particularly calcium, phosphorus, and potassium, whose levels are relatively higher compared to other legumes. Tamarind kernel powder (TKP), tamarind seed polysaccharides, and tamarind gum have been found to be highly valuable in various industrial applications, including textiles, paints, and pharmaceuticals. Tamarind kernel powder can be used as a good substitute for pectin for making jelly[4].

Fermentation is the natural process in which various microorganisms like yeast, bacteria and fungi are involved in the exchange of complex substrates into simpler compounds. These compounds are useful to humans on industrial scale[5].

Microbial polysaccharides have interesting characteristics for the food industry, especially when produced by food grade bacteria. Polysaccharides produced by lactic acid bacteria (LAB) during fermentation are extracellular macromolecules that can be categorized as either homopolysaccharides or heteropolysaccharides, based on their chemical composition and structural characteristics. The most prominent exopolysaccharide (EPS)- producing LAB genera include *Lactobacillus, Leuconostoc, Weissella, Lactococcus,* *Streptococcus, Pediococcus*, and *Bifidobacterium*[6].

In the present investigation, raw tamarind seeds were fermented with and without probiotic isolates such as *Lactobacillus helveticus, Lactobacillus plantarum* and *Lactobacillus plantarum* S1. The exopolysaccharides were extracted from all the fermented samples and their estimation was carried out.

MATERIALS AND METHODS

Raw and healthy tamarind seeds were collected from Hassan District, Karnataka State, INDIA. The seeds were washed, dried, powdered and sieved to obtain fine powder and the powdered samples were stored in airtight containers for subsequent use. All chemicals and reagents used in the study were of analytical grade and were procured from SRL (Sisco Research Laboratories, India), India, Hi-Media, India and standards were from SIGMA, USA.

The probiotic bacterial isolates namely *Lactobacillus helveticus, Lactobacillus plantarum* and *Lactobacillus plantarum* S1 obtained from Department of Fermentation Technology and Microbiology, CSIR- CFTRI, Mysore were grown in De Mans Rogosa and Sharpe (MRS) agar medium and maintained in refrigerator at 4°C.

Fermentation

Tamarind seed powder was defatted using Petroleum ether. The raw and defatted tamarind seed samples were fermented using three different probiotic organisms, like *Lactobacillus helveticus, Lactobacillus plantarum* and *Lactobacillus plantarum* S1 for about 3 days in the orbital shaker at 37°C and 200 rpm. Fermentation was conducted using both distilled water and MRS broth as media.

Production of Exopolysaccharide from fermented raw and defatted samples

Exopolysaccharide extraction was carried out by the method of Patil, (2010). The method is as follows: water- based and media- based culture broth of both raw and defatted tamarind seed samples were centrifuged at 4000 rpm for 20 minutes. After centrifugation, 1:1 ratio of cold isopropyl alcohol was added to the supernatant and the mixture was kept overnight in refrigerator for precipitation. After overnight incubation, the mixture was centrifuged at 4000rpm for 15 minutes to collect the pellet and supernatant was discarded. The pellet containing polysaccharide was washed twice with acetone and then kept for drying in hot air oven. The dried exopolysaccharide was weighed and stored at 4°C for further use.

RESULT AND DISCUSSION

Sample collection

Tamarind (*Tamarindus indica*) seed samples were collected and used for the production of exopolysaccharides through submerged fermentation, utilizing probiotic strains such as *Lactobacillus plantarum, Lactobacillus plantarum* S1, and *Lactobacillus helveticus* (Figure 2).

Fig. 1: A) *Tamarindus indica* seeds; B) *Tamarindus indica* seed Powder

Bacterial cultures

The probiotic bacterial isolates *Lactobacillus plantarum, Lactobacillus helveticus, Lactobacillus plantarum* S1 were subcultured on MRS agar plates.

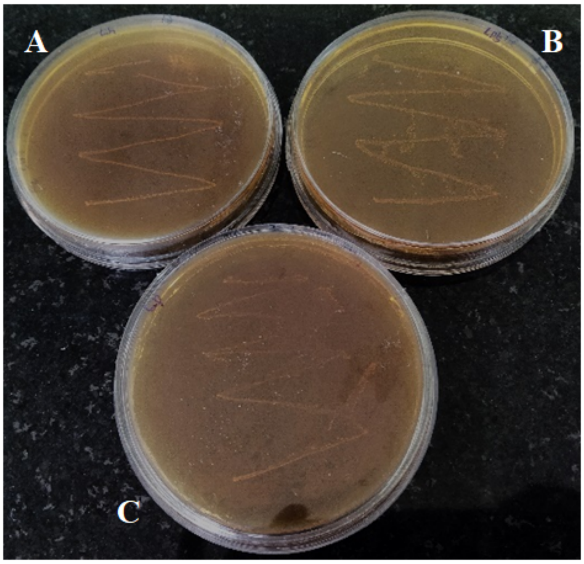

Fig. 2: Pure cultures of *Lactobacillus*species (A) *Lactobacillus helveticus*; (B) *Lactobacillus plantarum* S1; (C) *Lactobacillus plantarum*

Fermentation of raw and defatted samples

Both raw as well as defatted samples were fermented using probiotic bacterial isolates such as *Lactobacillus plantarum*, *Lactobacillus helveticus* and *Lactobacillus plantarum* S1.

Production of EPS from both water and MRS media based fermented raw and defatted Tamarindus indica seeds:

The exopolysaccharide obtained through water- based and MRS media- based fermentation was dried, weighed and stored at 4°C for further analysis.

EPS from water and MRS- based fermented raw Tamarindus indica seeds:

The results showed an exopolysaccharide content of 14.5, 13.5 and 4.5 g/L in water based fermented tamarind seed samples with *Lactobacillus plantarum, Lactobacillus plantarum* S1 and *Lactobacillus helveticus* respectively (Figure 2 and Table 1). Of the three strains used, highest exopolysaccharide content was found in *Lactobacillus plantarum*. Kumar et al., (2011)[7] reported that the polysaccharide from tamarind seeds were purified using water and precipitated with acetone. The percentage yield of polysaccharide was shown to be 78. The results of MRS media- based fermentation of *Tamarindus indica* seeds for exopolysaccharide production is shown in Figure 3 and Table 1. The results indicated an exopolysaccharide content of 4.5, 5.0 and 12.0 g/L in case of *Lactobacillus plantarum, Lactobacillus plantarum* S1 and *Lactobacillus helveticus* respectively where the highest EPS content was in *Lactobacillus helveticus*. Kim and Yun (2005)[8] reported a comparative study on the production of EPS by using two entomopathogenic fungal strains. The maximum concentration of EPS produced in optimal media (MY) was 20.94 g/l.

Table 1: Exopolysaccharide content in fermented water- based and MRS media raw tamarind seeds

Isolate	Water-based (g/L)	MRS-based (g/L)
Control	0.5	1.0
Lactobacillus plantarum	**14.5**	4.5
Lactobacillus plantarum S1	13.5	5.0
Lactobacillus helveticus	4.5	**12.0**

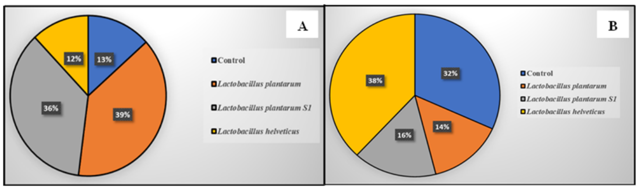

Fig. 3: Exopolysaccharide percentage in fermented raw *Tamarindus indica* seeds. A) Water- based, B) MRS- based

Production of EPS in water and MRS- based fermented defatted Tamarindus indica seeds:

The results of water and MRS- based fermented defatted samples are shown in Table 2 & Figure 4. The result showed a highest exopolysaccharide content in *Lactobacillus plantarum* S1 (4.0 g/l). Followed by this, the other two strains showed same level of exopolysaccharide content (2.6 g/l). The lower level of exopolysaccharide in defatted samples may be due to the involvement of lipids in its synthesis. The exopolysaccharide content was significantly higher with defatted *Tamarindus indica* seed powder as substrate for fermentation using the three test organisms when compared to water- based medium. The results showed were 0.71 g/l in *Lactobacillus plantarum* and 0.68 g/l in both *Lactobacillus plantarum* S1 and *Lactobacillus helveticus* respectively. Balasubramanian et al., (2018) reported the optimization study to enhance the production of exopolysaccharide from *Aspergillus* sp., with central composite designs and showed a maximum yield of 22.2 mg/g.

Table 2: Exopolysaccharide content in water and MRS-based fermented defatted seeds

Samples	Water- based (g/l)	MRS- based (g/l)
Control	2.6	10.2
Lactobacillus plantarum	2.6	**0.71**
Lactobacillus plantarum S1	**4.0**	0.68
Lactobacillus helveticus	26	0.68

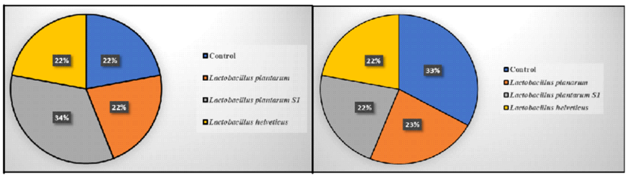

Fig. 4: Exopolysaccharide percentage in fermented defatted *Tamarindus indica* seeds. A) Water-based, B) MRS-based

An earlier study reported on the production of EPS by using *Lactobacillus paracasei* strain from kefir grains. The amount of EPS produced by strains of *L. paracasei* CIDCA 83124 was 1170, 527 and 373 μg with 1×10^8 CFU at 20, 30, and 37°C respectively. Another strain, *Lactobacillus paracasei* CIDCA 83123, exhibited lower exopolysaccharide (EPS) production, showing similar behavior to CIDCA 83124. EPS yields of 390 μg, 309 μg, and 270 μg were observed at 20°C, 30°C, and 37°C, respectively, when cultured at a concentration of 1×10 CFU/mL. This trend highlights a characteristic feature of mesophilic EPS-producing lactic acid bacteria (LAB), where EPS production tends to increase at lower temperatures[9,10].

Effect of fermentation with and without inoculum on EPS production by Tamarindus indica seeds:

The results of EPS content in raw and defatted water based *Tamarindus indica* seeds fermented with and without inoculum is shown in Table 3. The results indicated that fermentation with inoculum had 10 and 3- fold higher yield of EPS when compared to fermentation without inoculum in defatted and raw tamarind seed sample. This shows the importance of using probiotic strains in the production of exopolysaccharides. The amount of EPS produced by *Lactobacillus plantarum* and *Lactobacillus helveticus* was 23 and 38% respectively, using both defatted and raw tamarind seed powder in Media based fermentation. *Lactobacillus plantarum* showed 34 and 39% more production of EPS in both tamarind seed powder through water based fermentation.

Table 3: Water- based *Tamarindus indica* seeds fermented with and without inoculum

Sample	Without inoculum (g/l)	With inoculum (g/l)
Defatted	0.25	2.6
Raw	1.7	5.0

CONCLUSION

Tamarind seeds, the legumes of *Tamarindus indica*, are rich in proteins, carbohydrates, lipids, and essential minerals. Tamarind seed polysaccharide (TSP) is a natural polymer extracted from these seeds and is considered a valuable by-product of the tamarind pulp industry. Although polysaccharides are indigenously present in tamarind seeds, their level significantly increased when raw and defatted samples were fermented with probiotic bacterial strains such as *Lactobacillus plantarum*, *L. plantarum* S1 and *L. helveticus* under submerged fermentation using water and MRS medium. The present finding might find its importance in various industrial application.

Conflict of Interest

None.

Funding

None.

REFERENCES

1. Gitanjali, Sharma V, Jain S. Nutritional Properties of Tamarind (Tamarindus indica) Kernel Flour. *International Journal of Current Microbiology and Applied Science*. 2020;9(5):1359–1364. Available from: https://doi.org/10.20546/ijcmas.2020.905.153.
2. Sarkar BR, Awasthi P, Joshi H. Physical properties and nutritional composition of tamarind kernel powder and quality evaluation of instant chutney mix prepared incorporating tamarind kernel powder. *Journal of Pharmacognosy and Phytochemistry*. 2018;7(6):1342–1346. Available from: https://www.phytojournal.com/archives/2018/vol7issue6/PartX/7-5-74-314.pdf.
3. Shalini P, Murthy KRS. Isolation of tamarind seed protiens, polysaccharides, and cellulase from the seeds of Tamarindus indica. *The Pharma Innovation Journal*. 2017;7(1):372–375. Available from: https://www.thepharmajournal.com/archives/2018/vol7issue1/PartF/7-1-11-976.pdf.
4. Ajayi IA, Oderinde RA, Kajogbola DO, Uponi JI. Oil content and fatty acid composition of some underutilized legumes from Nigeria. *Food Chemistry*. 2006;99(1):115–120. Available from: https://dx.doi.org/10.1016/j.foodchem.2005.06.045.
5. Subramaniyam R, Vimala R. Solid state and submerged fermentation for the production of bioactive substances: a comparative study. *International Journal of Science and Nature*. 2012;3(3):480–486. Available from: http://www.scienceandnature.org/IJSN/IJSN_Vol3(3)S2012/IJSN-VOL3(3)12-1R.pdf.
6. Guérin M, Silva CRD, Garcia C, Remize F. Lactic Acid Bacterial Production of Exopolysaccharides from Fruit and Vegetables and Associated Benefits. *Fermentation*. 2020;6(4):1–21. Available from: https://dx.doi.org/10.3390/fermentation6040115.
7. Kumar GP, Battu GBG, Kotha NS, Raju LRL. Isolation and evaluation of tamarind seed polysaccharide being used as a polymer in pharmaceutical dosage forms. *Research journal of Pharmaceutical, Biological and Chemical Science*. 2011;2(2):274–290. Available from: https://www.rjpbcs.com/pdf/2011_2(2)/35.pdf.
8. Kim HO, Yun JW. A comparative study on the production of exopolysaccharides between two entomopathogenic fungi Cordyceps militaris and Cordyceps sinensis in submerged mycelial cultures. *Journal of Applied Microbiology*. 2005;99(4):728–738. Available from: https://doi.org/10.1111/j.1365-2672.2005.02682.x.
9. Bengoa AA, Iraporda C, Abraham AG, Garrote LG, Llamas MG, Duenas MT. Impact of growth temperature on exopolysaccharide production and probiotic properties of Lactobacillus paracasei strains isolated from kefir grains. *Food Microbiology*. 2018;69:212–218. Available from: https://doi.org/10.1016/j.fm.2017.08.012.
10. Degeest B, Vaningelgem F, Vuyst D, L. Microbiology physiology, fermentation kinetics, and process engineering of heteropolysaccharide production by lactic acid bacteria. *International Dairy Journal*. 2001;11(9):747–757. Available from: https://doi.org/10.1016/S0958-6946(01)00118-2.