Int.J.Curr.Microbiol.App.Sci (2025) *14*(07): 101-108

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 14 Number 7 (2025)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2025.1407.013

Isolation of Natural Microflora from Mulberry (*Morus spp.*) Fruits and Detailed Characterization of Lactic Acid Bacteria

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Keywords

Mulberry fruit, isolation, microflora, lactic acid bacteria, purification, probiotics

Article Info

Received:
12 May 2025
Accepted:
22 June 2025
Available Online:
10 July 2025

A B S T R A C T

This study investigated the natural microflora, including lactic acid bacteria (LAB), associated with fresh mulberry (*Morus spp.*) fruits collected from various regions of Tamil Nadu. Standard microbiological methods were used to enumerate bacteria, fungi, yeast, and LAB. Bacterial populations ranged from 6.09 to 7.29 log cfu g^{-1}, fungal counts from 2.65 to 2.87 log cfu g^{-1}, and yeast populations from 3.12 to 3.35 log cfu g^{-1}. LAB counts on MRS agar ranged between 4.72 to 6.87 log cfu g^{-1}. Four morphologically distinct LAB colonies were isolated, all Gram-positive and catalase-negative. Most isolates were rod-shaped, while 20% were cocci. The dominant colony type round, creamy, and raised with entire margins accounted for 54.42% of the isolates. These findings indicate that mulberry fruits harbour a diverse microflora, including LAB strains with potential applications in probiotic development and functional food fermentation.

Introduction

Fruits and vegetables acts as a vital source of nutrients to human beings as they provide necessary vitamins, fats, minerals, and oil in the right proportion for human growth and development. Generally, the kind and number of different fruit associated microorganisms varied with nutritional and physical parameters of the fruit. The mulberry fruits are edible and have been widely used in medicinal syrups, jam, marmalade, frozen desserts, juice, paste, ice cream, wine, distillates, natural food colorant and liquor (Singhal *et al.,* 2003). Isolation of microorganism including lactic acid bacteria (LAB) from fruits and vegetables have been frequently reported (Bae *et al.,* 2006) however, studies on ripened mulberries associated microorganisms remains scarce. The lactic acid bacteria (LAB) present on the fruit surface depicts a positive role in the fermentation process. The isolation and screening of microorganism from natural raw material has always been the most powerful means for obtaining useful and stable strains for industrial production of important products.

Lactic acid bacteria (LAB) play an essential role in food preservation and contribute to the nutritional, sensory, and health-promoting properties of various food products. They are involved in the formation of yoghurt,

cheese, pickles, whey, soybean sauce, fermented soybean, and silage (de Vos and Hugenholtz, 2004). LAB produce lactic acid, which protects fermented food products from microbial spoilage.

Several commercial strains, such as *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus brevis*, and *Leuconostoc mesenteroides*, are widely used individually or in combination. Mulberry fruits, rich in sugars and functional compounds, are suitable for lactic fermentation.

For instance, Zheng *et al.,* (2014) reported that fermenting mulberry with *Leuconostoc mesenteroides* enhances its probiotic value while reducing sugar content. Lee and Hang (2010) developed functional yoghurts using mulberries and multiple lactic strains. Karaca *et al.,* (2011) reported high sensory scores for yoghurts produced with mulberry addition.

Despite these studies, reports on LAB diversity in mulberry fruits remain limited. Hence, this study aimed to isolate, purify, and phenotypically characterize LAB from mulberry fruits.

Materials and Methods

Sample Collection

Fruits of four popular mulberry cultivars (V1, S13, MR2, and S36) were harvested during the fruit-bearing season (September to November) from various mulberry-growing districts of Tamil Nadu. Fully ripened fruits were collected aseptically in sterile zip-lock polythene bags and stored at 4°C until analysis.

Isolation of Microorganisms and Lactic Acid Bacteria (LAB)

The native microflora including bacteria, fungi, yeast, and LAB was enumerated using the plate count method (Hoben and Somasegaran, 1982). 10 grams of each fruit sample was homogenized using a sterile pestle and mortar in 100 ml of sterile distilled water. Serial dilutions up to 10^4 were prepared in sterile test tubes, and 1 ml of each dilution was plated onto appropriate media.

For total bacteria, fungi, and yeast, nutrient agar, potato dextrose agar (PDA), and yeast extract peptone dextrose agar (YEPD) were used, respectively. The plates were incubated at room temperature (28 ± 2°C) and colony-forming units (cfu) were recorded as log cfu g^{-1} of wet fruit weight.

For LAB isolation, serial dilutions were plated on de Man, Rogosa, and Sharpe (MRS) agar and incubated at 37°C for 24–48 hours (Kamble and Pathade, 2010). Morphologically distinct colonies were selected and purified by repeated streaking on MRS agar. Pure cultures were maintained on MRS slants at 4°C for further characterization.

Phenotypic Characterization of LAB

The purified LAB isolates were subjected to phenotypic characterization. Colony morphology including shape, size, color, elevation, and margin was documented. Gram staining and catalase tests (using 3% hydrogen peroxide) were performed. Cell morphology was examined under a microscope (Harrigan, 1998).

Results and Discussion

Isolation of Microorganisms from Mulberry Fruits

Fruits and vegetables are naturally associated with a wide range of microorganisms, including bacteria, parasites, protozoans, and viruses (Beuchart *et al.,* 1998). The microbial count for different cultivars of mulberry fruits is presented in Table 1.

The bacterial population ranged from 6.09 to 7.29 log cfu g^{-1}, fungal counts from 2.65 to 2.87 log cfu g^{-1}, and yeast colonies between 3.12 to 3.35 log cfu g^{-1} (Plate 1). Similar studies have reported microbial loads ranging between 10^3 to 10^9 cfu g^{-1} (Zagory, 1999).

The diversity and abundance of fruit-associated microorganisms generally vary depending on nutritional and physical attributes of the fruit. In tomato, for instance, bacterial populations ranged between 1.9×10^6 to 5.0×10^6, mould counts were approximately 5.0×10^4, and total yeast counts were around 3.5×10^3 cfu g^{-1} (Mbajiuka *et al.,* 2014). Similarly, fungal counts in tomatoes ranged between 1.3×10^3 and 2.0×10^3 cfu g^{-1} (Samuel and Orji, 2015).

Isolation and Purification of LAB

Lactic acid bacteria (LAB) were isolated from mulberry

fruits are exhibited in Plate 2. The mean population of lactic acid bacteria counts ranging from 4.72 to 6.87 log cfu g^{-1} (Table 1).

LAB is commonly dominant in fermented food products due to their lactic acid and bacteriocin production, which inhibit spoilage organisms (Askari *et al.,* 2012).

Previous studies have reported LAB counts of 1.48 to 3.77 log cfu ml^{-1} in watermelon (Chen *et al.,* 2016) and 5.74 to 16.68 × 10^3 cfu g^{-1} in kokum fruits (Dushyantha *et al.,* 2010), which are consistent with the values observed in this study.

Phenotypic Characterization

Four morphologically distinct LAB colonies were identified from the mulberry fruits (Table 2). All isolates were Gram-positive and catalase-negative (not shown). Most isolates were rod-shaped, while 20% were cocci arranged in chains (Plate 4). The dominant phenotype was round, creamy, raised with an entire margin (Plate 3) comprising 54.42% of the total LAB isolates. The highest LAB diversity was observed in fruits of the MR2 cultivar collected from the Palladam region.

Table.1 Microbial population of mulberry fruits collected from different locations

Cultivars	Location	Population (log cfu.g^{-1} wet weight of mulberry fruit)			
		Bacteria	Fungi	Yeast	Lactic acid bacteria
V1	Coimbatore	6.23 ±1.08	2.65 ±0.13	3.22±0.50	5.63 ±2.61
	Salem	7.29±0.88	2.80 ±0.47	3.27±0.40	5.90±0.58
	Avinashi	6.26±0.12	2.71 ±0.23	3.20 ±1.03	6.65±4.23
S13	Erikodi	6.28 ±089	2.79 ±0.30	3.12 ±3.18	4.72 ±1.65
MR2	Dharmapuri	7.26 ±1.53	2.74 ±0.03	3.27 ±0.84	5.82 ±2.83
	Palladam	6.09 ±0.26	2.87 ±0.67	3.12±0.83	6.87 ±0.80
	Udumalpet	6.20±1.17	2.71±0.23	3.35 ±0.95	5.74 ±3.72
S36	Coimbatore	7.26 ±0.15	2.74 ±0.03	3.21 ±0.85	4.89 ±5.45
Mean		6.62	2.75	3.22	5.72
SEd		0.79	0.26	0.71	2.86
CD(p=0.05)		1.66	0.55	1.50	6.01

Data mean ± Standard deviation

Table.2 Morphological and Microscopic Characteristics of Lactic Acid Bacteria Isolated from Mulberry Fruits

S. No	Colony characteristics	Cell morphology	Gram reaction	Percent occurrence
1.	Round, creamy, raised and entire	Rod, more than 3 cells in chain	+	54.42
2.	Round, medium sized, white, raised and entire	Circular, Diplococci	+	18.72
3.	Irregular, raised, creamy, opaque, raised	Rod, more than 3 cells in chain	+	20.05
4.	Round, small, white, raised, opaque and entire	Rod, more than 3 cells in chain	+	6.80

Plate.1 Microflora of mulberry fruits

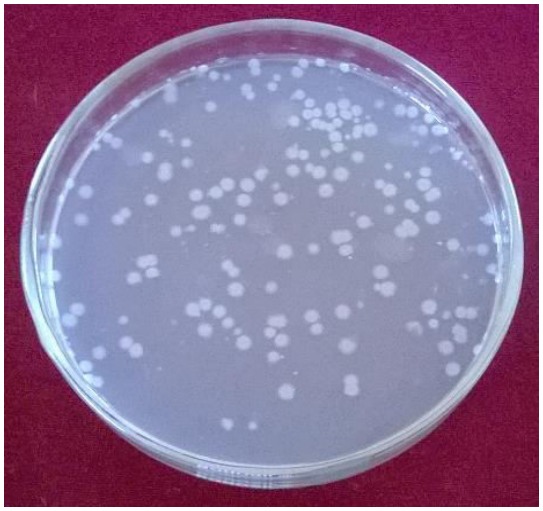
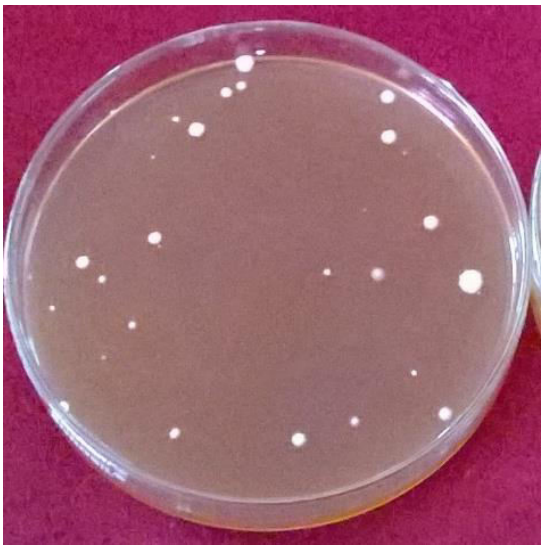
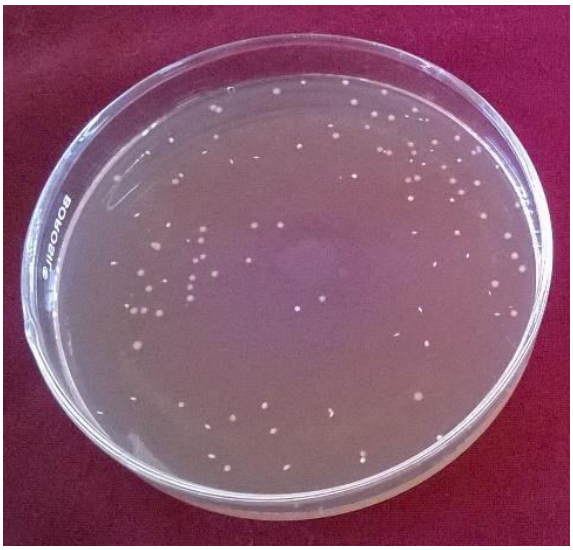
Bacteria	Fungi
	
Yeast	**Lactic acid bateria**
	

Plate.2 Population of lactic acid bacteria in mulberry fruits

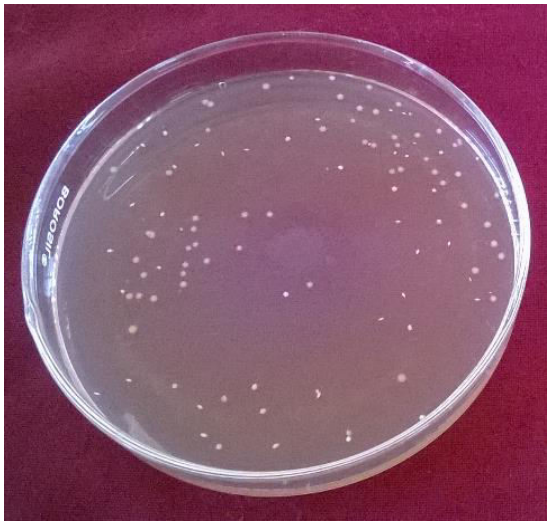
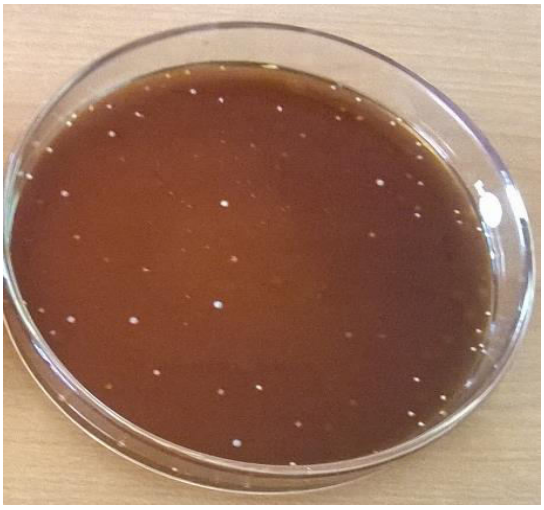
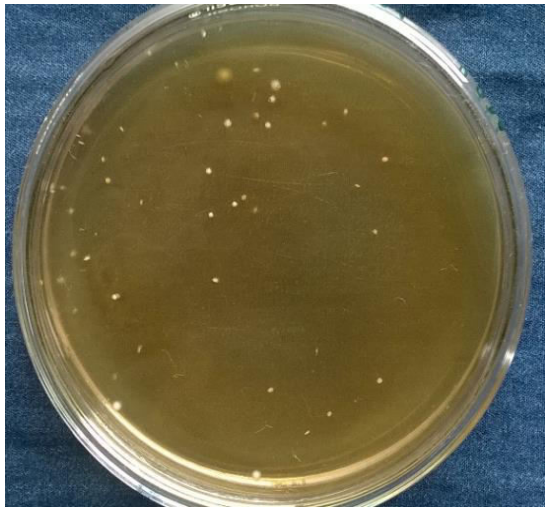
(a)	(b)
	
(c)	**(d)**
	

Plate.3 Dominant Colony Morphology of Lactic Acid Bacteria Isolated from Mulberry fruits

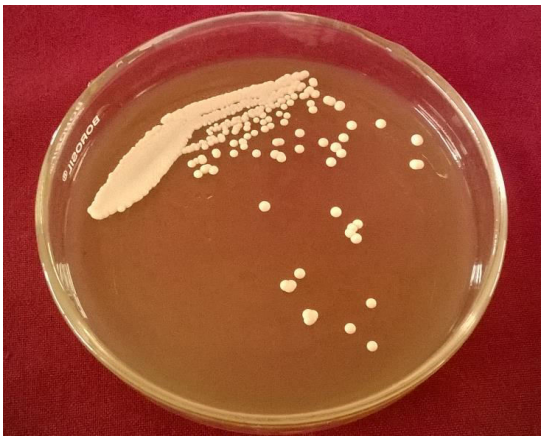
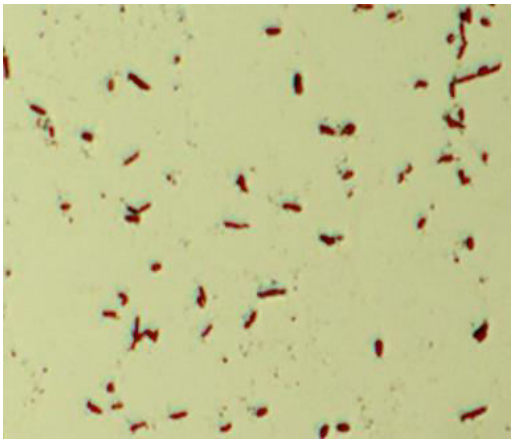
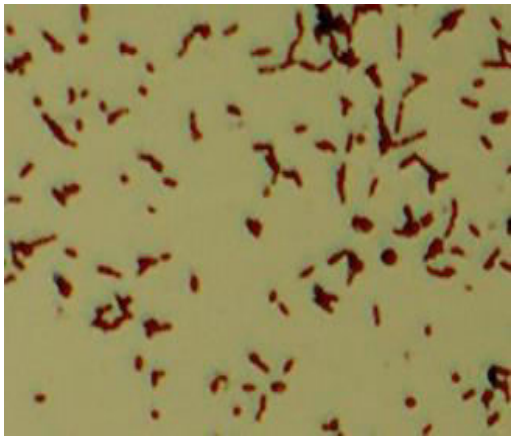
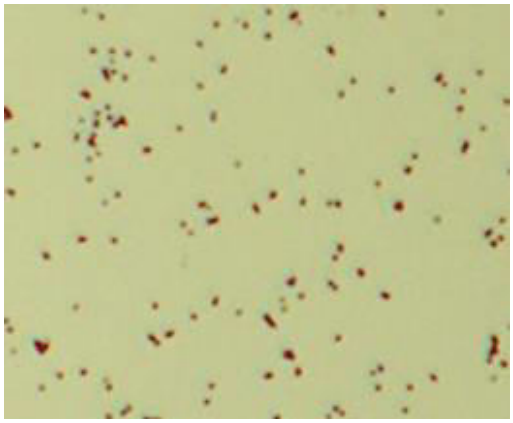
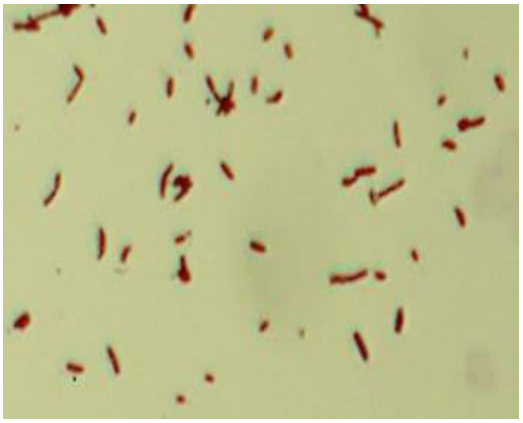


Plate.4 Microscopic Morphology of Native LAB Isolates (40×)

(a)	**(b)**
	
(c)	**(d)**
	

The most abundant species identified was *Weissella cibaria*, followed by *Lactobacillus plantarum*, which corresponds with findings by Di Cagno *et al.,* (2009), who reported these species as frequently occurring in plant environments.

This study revealed that mulberry (*Morus spp.*) fruits harbor diverse natural microflora, including significant populations of lactic acid bacteria (LAB). Four morphologically distinct LAB isolates were identified, with *Weissella cibaria* and *Lactobacillus plantarum* being predominant. These results suggest that mulberry fruits are a natural source of beneficial LAB with potential use in probiotics and food fermentation.

Author Contributions

S. Menaka: Investigation, formal analysis, writing—original draft. R. Kalpana: Validation, methodology, writing—reviewing. M. Sabarish:—Formal analysis, writing—review and editing. M. Mithilasri: Investigation, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

References

Askari GA, *et al.,* (2012). Screening of lactic acid bacteria isolated from dried fruits and study of their antimicrobial activity. Middle-East Journal of Scientific Research, 11:209-215.

Bae, S., G.H. Fleet and G.M. Heard. 2006. Lactic acid bacteria associated with wine grapes from several Australian vineyards. Journal of Applied Microbiology, 100:712-727. https://doi.org/10.1111/j.1365-2672.2006.02890.x

Beuchat, L. R. (1998). Surface decontamination of fruits and vegetables eaten raw: A review. Food Safety Unit, World Health Organization. WHO/FSF/FOS/98.2.

Chen PN, *et al.,* (2016). Lactic acid bacteria associated with fresh fruits. International Journal of Advanced Biotechnology Research, 63:5864-5868.

de Vos WM, Hugenholtz J. (2004). Engineering metabolic highways in lactococci and other lactic acid bacteria. Trends Biotechnol., 22:72-79. https://doi.org/10.1016/j.tibtech.2003.11.011

Di Cagno R, *et al.,* (2009). Effect of autochthonous LAB starters on health-promoting and sensory properties of tomato juice. J. Food Microbiol., 128:473-483. https://doi.org/10.1016/j.ijfoodmicro.2008.10.017

Dushyantha DK, *et al.,* (2010). Native LAB isolates of kokum for preparation of fermented beverage. European Journal of Biological Science, 2:53-56.

Harrigan WF. (1998). Laboratory Methods in Food Microbiology. 3rd ed. Academic Press.

Hoben, H.J., and P. Somasegaran. 1982. Comparison of the pour, spread and drop plate methods for enumeration of Rhizobium spp. In inoculants made from presterilized peat. Applied and Environmental Microbiology., 5: 1246-1247. https://doi.org/10.1128/aem.44.5.1246-1247.1982

Kamble RD, Pathade GR. (2010). Potential application of Lactobacillus isolates for preparation of soft drink. Int. J. Adv. Biotechnol. Res., 1:5-10.

Karaca OB, *et al.,* (2011). Physicochemical, mineral and sensory properties of set-type yoghurts produced with molasses. International Journal of Dairy Technology, 65:111-117. http://dx.doi.org/10.1111/j.1471-0307.2011.00731.x

Lee AC and Hang YH. (2010). Development of functional yoghurts with mulberries. Korean J. Food Sci. Ani. Resour., 30:649-654. http://dx.doi.org/10.5851/kosfa.2010.30.4.649

Mbajiuka, Chinedu, Enya and Emmanuel. 2014. Isolation of microorganism associated with deterioration of tomato and pawpaw fruits. Int. J. Curr. Microbiol. App. Sci., 3: 501-512.

Samuel, O. and M.U. Orji. 2015. Fungi associated with the spoilage of post-harvest fruits sold in major

markets in Awka, Nigeria. Universal Journal of Microbiology Research, 3: 11-16. http://dx.doi.org/10.13189/ujmr.2015.030201

Singhal B.K., A. Dhar, B.B. Bindroo, P.M. Tripathi S.M.H. Qadri and M. M. Ahsan. 2003. Medicinal utilities of mulberry and non-mulberry food plants of the silkworm. In: Recent Progress in Medicinal Plants, Vol. 8, Phytochemistry and Pharmacology II. Studium Press LLC, USA, pp. 477-500.

Zagory, D. (1999). Washing fresh fruits and vegetables: A review. In J. S. Smith & Y. H. Hui (Eds.), Food processing: Principles and applications (pp. 33–53). Technomic Publishing.

Zheng X, *et al.,* (2014). Changes of α-glucosidase content and characteristics in mulberry juice fermented with Leuconostoc mesenteroides. Acta Alimentaria, 43:668-675. http://dx.doi.org/10.1556/AAlim.43.2014.4.17

How to cite this article:

Menaka, S., R. Kalpana, M. Sabarish and Mithilasri, M. 2025. Isolation of Natural Microflora from Mulberry (*Morus spp.*) Fruits and Detailed Characterization of Lactic Acid Bacteria. *Int.J.Curr.Microbiol.App.Sci.* 14(07): 101-108. **doi:** https://doi.org/10.20546/ijcmas.2025.1407.013