Inventum Biologicum: *An International Journal of Biological Research*
2024, Vol. 4, Issue 3, pp. 29-32

Inventum Biologicum

Journal homepage: www.journals.worldbiologica.com/ib

Research paper

16S rRNA Gene Sequencing and Phylogeny Analysis Revealed the Presence of Beneficial Bacteria in the Rhizosphere of Eggplant (*Solanum melongena*) from Malda (West Bengal, India)

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ARTICLE INFO

Article history

Received 05 September 2024
Revised 12 September 2024
Accepted 14 September 2024
Published 18 September 2024

Keywords

- Rhizosphere soil
- Eggplant
- Lactic acid bacteria
- *Limosilactobacillus fermentum*
- *Bifidobacterium animalis* subsp. lactis

ABSTRACT

Lactic acid bacteria have been used in agricultural practices with promising potential as soil health-promoting microorganisms. Isolation of lactic acid bacteria from the rhizosphere soil of various crops including brinjal (eggplant), *Solanum melongena* has been reported from different parts of the world. No such reports are available in our region (Malda, India). In this study, we have isolated and characterized lactic acid bacteria from *Solanum melongena* rhizosphere. The rhizosphere soils from the eggplant cultivars (Jhiloria, Chandtara, Jotshna) were processed microbiologically, and three lactic acid bacteria were isolated. The16S rRNA gene sequencing and phylogeny analysis confirmed the identities of lactic acid bacteria as *Limosilactobacillus fermentum* isolated from the rhizosphere of Jhiloria and Chandtara varieties of eggplant, while the isolate from the rhizosphere of the Jotshna variety of eggplant was identified as *Bifidobacterium animalis* subsp. lactis. Thus, the presence of beneficial bacteria (*Limosilactobacillus fermentum* and *Bifidobacterium animalis* subsp. lactis) in the rhizosphere of crop plants might help improve soil health thereby enhancing crop production.

1. Introduction

Among the beneficial bacteria, the most reported members of economic importance are *Lactobacillus* and *Bifidobacterium* (Sanders et al., 2018). Due to their health-beneficial activity, various sources, mainly food samples, have been used for their isolation. Curd has been reported as a very good source of probiotic bacteria belonging to the genus *Lactobacillus* and *Bifidobacterium* (Halder, 2017). Various kinds of honey bees honey are also known as excellent sources of probiotic lactic acid bacteria (Roy & Mandal, 2024). Different kinds of non-milk-based fermented foods have been assessed as rich sources of lactic acid bacteria (Sircar & Mandal, 2023).

The rhizosphere is known as the hotspot of bacterial colonization, wherein interactions between plants (via the root systems) and an array of microorganisms take place (Mendes et al., 2013). Such rhizosphere bacteria play an important role in determining soil quality and promoting plant growth and development. Various beneficial bacteria including *Lactobacillus* are known to harbour in the rhizosphere soil, and previous reports have

DOI 10.5281/ib130524

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demonstrated the isolation of lactic acid bacteria from unconventional sources, rhizosphere and bulk soil, and vermicompost-rich in organic matters from different parts of the world (Stephen & Saleh, 2023; Hiranmayee et al., 2023).

Raman et al. (2022) reported on the isolation of beneficial lactic acid bacteria from the rhizosphere and their application in agriculture. Hiranmayee et al. (2023) isolated various rhizosphere bacteria including *Lactobacillus* spp., and explained the plant-probiotic role of the lactic acid bacteria. Bifidobacteria are excellent colonizers of the human gut and can be isolated from animal and environmental sources (Lamendella et al., 2008; Rodriguez & Martiny, 2020; Argentini et al., 2024; Duranti et al., 2020). Isolation of *Bifidobacterium* from the rhizosphere of plants (*Deschampsia antarctica* and *Colobanthus quitensis*) has been reported (Mendes et al., 2013). Earlier we reported on probiotic lactobacilli isolated from curd and honey samples available locally in our region. However, lactic acid bacteria from plant-associated rhizosphere soils have not been studied locally (Malda, India).

2. Materials and Methods

We collected three rhizosphere soil samples associated with the three cultivars of eggplant, *Solanum melongena* (Jhiloria, Chandtara, Jotshna) from around Malda town (West Bengal, India; latitude: 24°40'20" N to 25°32'08" N, longitude: 87°45'50" E to 88°28'10" E), for the current study. We processed the collected soil samples microbiologically, wherein nutrient broth and agar, and MRS (deMan Rogosa and Sharpe)) broth and agar were utilized (Thakur et al., 2017). We finally obtained the pure cultures of bacterial isolates and subjected those to gram-staining and biochemical tests (catalase, oxidase) for non-spore forming non-motile gram-positive bacilli displaying negative test results for catalase and oxidase production. This is in accordance to the previous publication (Hadadji et al., 2005). Subsequently, we performed TSI (triple sugar iron) tests for the pure lactic acid bacteria cultures (Wieczorska et al., 2020).

Confirmation of the identity of the isolated lactic acid bacteria was determined by 16S rRNA gene sequencing (Biokart Pvt. Ltd, India), and phylogeny analysis, as described elsewhere (Roy & Mandal, 2024). Briefly, query nucleotide sequences from lactic acid bacteria and reference sequences from the NCBI database were matched for similarity detection using the basic local alignment search tool, BLAST (Altschul et al., 1990). We used Molecular Evolutionary Genetics Analysis X (Mega X) software (Kumar et al., 2018), to construct phylogenetic trees, by sequence alignment with the help of the top five identical sequences. The procedure has been explained in detail previously (Roy & Mandal, 2024).

3. Results and Discussion

We have isolated three bacterial isolates (LMEM801, LMEM 802 and LMEM 803) from three rhizosphere soil samples collected from three varieties of eggplant Jhiloria, Chandtara and Jotshna (Fig. 1, Fig. 2, Fig. 3 and Fig. 4). The bacterial isolates were non-spore-forming gram-positive rods, and showed negative test results for catalase and oxidase production, thus satisfying the features of lactic acid bacteria. Previously, we utilized local honey bee honey samples for the isolation of lactic acid bacteria and characterized them using phenotypic tests: gram-staining, catalase, oxidase and TSI tests (Collee et al., 1996). In the current study, in TSI agar the lactic acid bacterial isolates from rhizosphere soils fermented lactose, glucose and sucrose, and did not show hydrogen sulphide gas production. Many earlier studies demonstrated the isolation of lactic acid bacteria from the rhizosphere soil of different plants (Singhal et al., 2021; Hiranmayee et al., 2023).

The 16S rRNA gene sequencing and phylogeny analysis are crucial for the identity confirmation of bacterial isolates. Following such molecular method, both the lactic acid bacteria isolated from Jhiloria and Chandtara varieties of eggplant were identified as *Limosilactobacillus fermentum*, and designated as *Limosilactobacillus fermentum* LMEM801 (Fig. 2) and *Limosilactobacillus fermentum* LMEM802 (Fig. 3) strains. Singhal et al. (2021) identified rhizosphere soil isolates of *Lactobacillus plantarum* (by 16S rRNA gene sequencing) and demonstrated the probiotic potential of the isolated lactobacilli.

The rhizosphere of the Jotshna variety of eggplant contained *Bifidobacterium*, which was identified as *Bifidobacterium animalis* subsp. lactis LMEM803 strain (Fig. 4). Previously, Mendes et al. (2013) demonstrated the presence of *Bifidobacterium* in the rhizosphere of *Deschampsia antarctica* and *Colobanthus quitensis* plants. We have confirmed the identity of the isolated bifidobacterial isolate as *Bifidobacterium animalis* subsp. lactis based upon the

100% sequence similarity between the target and the reference sequences (Fig. 4), by 16S rRNA gene sequencing and phylogenetic analysis. Lactic acid bacteria in rhizosphere soil play a role in the improvement of soil structure, enhancement of soil nutrient uptake, the prevention of plant disease (Murindangabo et al., 2023), and production of antimicrobial compounds (Hirpara et al., 2024).

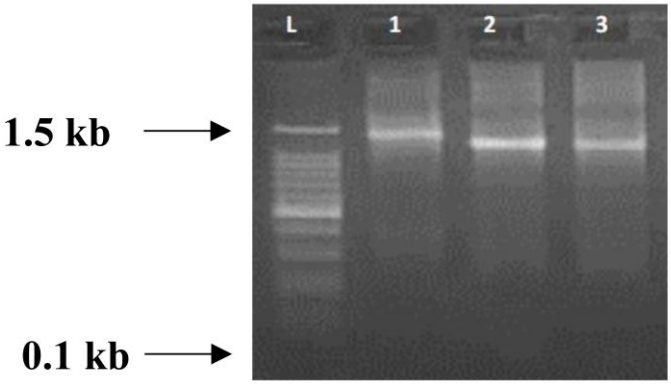

Fig. 1 Agarose gel electrophoresis of 16S rRNA gene amplified by PCR using 100 bp ladder (lane L), displaying ~1.5 kb fragment of *Limosilactobacillus fermentum* LMEM801 (lane 1), *Limosilactobacillus fermentum* LMEM802 (lane 2) and *Bifidobacterium animalis* subsp. lactis LMEM803 (lane 3) isolated from the rhizosphere soil of the Jhiloria variety of eggplant based on sequencing

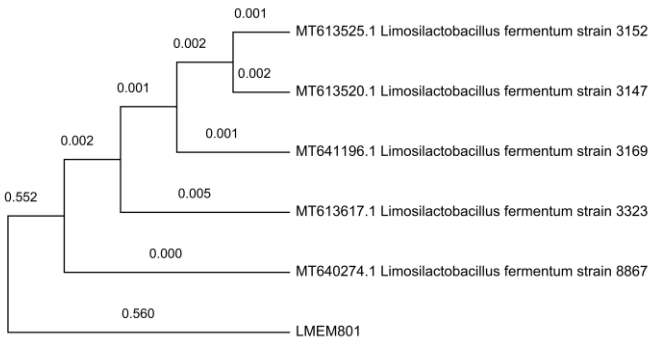

Fig. 2 Phylogenetic tree of *Limosilactobacillus fermentum* LMEM801 isolated the rhizosphere soil of the Jhiloria variety of eggplant based on 16 S RNA gene sequencing

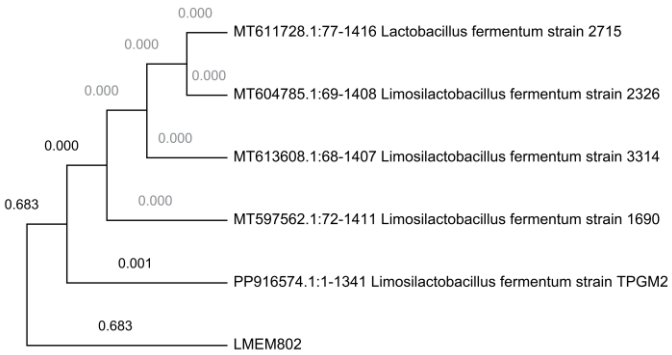

Fig. 3 Phylogenetic tree of *Limosilactobacillus fermentum* LMEM802 isolated the rhizosphere soil of the Chandtara variety of eggplant based on 16 S rRNA gene sequencing

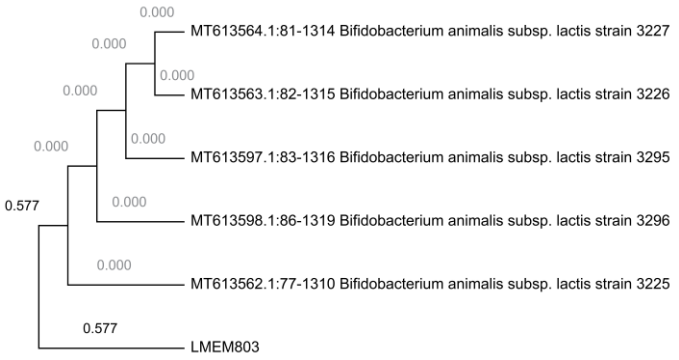

Fig. 4 Phylogenetic tree of *Bifidobacterium animalis* subsp. lactis LMEM803 isolated the rhizosphere soil of the Jotshna variety of eggplant based on 16S rRNA gene sequencing

4. Conclusions

Overall, we demonstrated the isolation of lactic bacteria from the rhizosphere of brinjal cultivars available in our part of the globe (Malda, West Bengal, India), and identified them as *Limosilactobacillus fermentum* and *Bifidobacterium animalis* subsp. lactis, using 16S rRNA gene sequencing and phylogeny analysis. Such lactic acid bacteria have potential health benefits for plants, and they can be applied to soil to increase plant growth and production in addition to preventing plant pathogens.

Author's Contribution

BG (Biswajit Ghosh) collected rhizosphere soil samples for microbiological analysis and molecular identity of isolated bacteria. SM (Shyamapada Mandal) designed the study, discussed, wrote and edited the manuscript.

Conflicts of Interests

The authors declare that there are no conflicts of interests.

Funding Information

We did not receive any funding for the current study.

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