Antimicrobial Attributes of Lactic Acid (LA) Bacterial Isolates of Sweet Corn (*Zea mays* L. *saccharata*) against Spoilage Microorganisms of Sweet Corn

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AUTHORS CONTRIBUTION

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Abstract

Sweet corn (Zea mays L. saccharata) a popular cereal, faces the problem of postharvest losses, due to the presence of high moisture and sugar contents. The main cause of spoilage is due to bacterial and fungal contaminations. Lactic acid bacteria (LAB) possess antimicrobial potential and are naturally associated with plant surfaces. LAB are Generally Regarded As Safe (GRAS) and the antimicrobial potential of these LAB can be exploited to eliminate spoilage microorganisms by applying them as biopreservation agents in sweet corn. With this background, experiments were designed to understand the association of beneficial LAB and spoilage microflora in sweet corn. The endophytic and epiphytic LAB (27), spoilage bacteria (10) and spoilage fungi (7) were isolated from spoilt sweet corn kernels cultivated at Rajunkunte (Bangalore north taluk) and Devanahalli (Bangalore rural taluk), Bengaluru district, Karnataka. The highest population of epiphytic and endophytic LA bacterial isolates (1.82 x 10² and 1.23 x 10² CFU g⁻¹ of dry weight, respectively) and the lowest population of spoilage bacteria and fungi (1.71 x 10³ CFU g⁻¹ and 2.09 x 10² CFU g⁻¹ of dry weight, respectively) were found in Rajunkunte samples. The isolates were morphologically and biochemically characterized. All the LA bacterial isolates were screened for their antimicrobial activity against spoilage bacteria and fungi. The isolates LAB-18 and LAB-22 exhibited a substantial inhibitory effect with 3.5 and 3.77 cm² inhibition zone, respectively against spoilage bacteria (SB-7) in the agar well diffusion method and the lowest colony growth of spoilage fungi (SF-3) with 3.05 and 2.64 cm², respectively using agar plug method. Further, the efficient isolates were subjected to molecular characterization using 16S rRNA sequencing. The BLAST search results indicated that LAB-18 and LAB-22 were identified as Lactiplantibacillus pentosus and Lactiplantibacillus plantarum, respectively.

Keywords : Sweet corn, Lactic acid (LA) bacteria, Spoilage microorganisms

CEREALS form an important part of the human diet because of their nutritional properties and beneficial human health effects. They are the sources of carbohydrates, proteins, lipids, vitamins and minerals as well as phytochemicals that are beneficial for human health (Miller and Welch, 2013). As the demand for plant produce continues to rise, premium is being placed on minimizing waste and ensuring safety. Microbial food spoilage is a global issue that results in food shortage and customer dissatisfaction (Snyder and Worobo, 2018). Sweet corn (*Zea mays* L. *saccharata*), is a popular cereal and is generally being harvested when the grain possesses high moisture and soluble sugar contents. However, these two factors make sweet corn a highly perishable product and susceptible to post-harvest spoilage by microorganisms. The microbiota of sweet corn is one of the major problems that has remarkable potential to reduce its shelf life (Saranraj and Geetha, 2012). The bacterial and fungal spoilages are the main causes of economic losses. Fang *et al.* (2023), reported an overall national average post-harvest loss of sweet corn (3.89 %) in USA, primarily attributed to spoilage caused by microorganisms.

Lactic acid (LA) bacteria have a successful history of being used as natural bio-preservative agent in foods (Stoyanova et al., 2012). There are reports mentioning that they also form plant-associated bacterial communities (Lamont et al., 2017) and are naturally associated with a variety of endophytic microorganisms (McInroy and Kloepper, 1995). These LA bacteria are Generally Regarded As Safe (GRAS) and are well known as probiotics due to their strain specific health promoting properties (Reis et al., 2012). The production of various inhibitory compounds such as organic acids, bacteriocins, antibiotics and other products like ethanol, H₂O₂, CO₂, diacetyl, acetaldehyde by LA bacteria mould them as efficient competitors for nutrients (Ouwehand and Vesterlund, 2004). In view of the safety and antimicrobial properties of LA bacteria, they are considered as appropriate candidates for the management of postharvest loss (Dhundale et al., 2018).

The research studies on microorganisms isolated from sweet corn remain scarce with most studies focusing on the plant growth promoting microorganisms of sweet corn (Pande *et al.*, 2020). There are no reports of isolation of lactic acid bacteria and spoilage microorganisms from sweet corn available to date. Hence, this necessitates the study for isolation of LA bacteria and spoilage microorganisms from sweet corn, focusing on isolation and characterization of native beneficial LA bacteria and spoilage microflora of sweet corn and studying the antimicrobial potential of LA bacteria against spoilage microflora.

MATERIAL AND METHODS

Collection of Samples

The samples of sweet corn (Mithaz variety) used for the isolation of lactic acid (LA) bacteria and spoilage microorganisms was collected directly from the farmers' fields of Devanahalli and Rajunkunte, Bengaluru, Karnataka, India. The spoiled sweet corn cobs were used for the isolation of spoilage microorganisms.

Enumeration and Isolation of Spoilage Microorganisms, Endophytic and Epiphytic Lactic Acid (LA) Bacteria

The spoiled sweet corn was employed to isolate microorganisms associated with spoilage. These microorganisms, acquired from the spoiled sweet corn are accountable for the deterioration of the corn. Consequently, the obtained microorganisms were designated as spoilage microorganisms. The standard plate count (SPC) method was followed to isolate spoilage bacteria and fungi from spoilt cobs using Nutrient Agar and Martin's Rose Bengal Agar media, respectively (Kumar et al., 2022). The enumeration and isolation of endophytic and epiphytic lactic acid (LA) bacteria from healthy kernels of sweet corn was carried out by SPC method after surface disinfecting them with 70 per cent ethanol, 2 per cent sodium hypochlorite and sterile distilled water (De Melo Pereira et al., 2012). The efficiency of disinfection procedure was monitored by the method of Hallmann et al. (1997). The thoroughly surface sterilized sweet corn kernels were fully ground in a sterile pestle and mortar. The homogenate (10 g) was plated on de Man, Rogosa and Sharpe (MRS) agar medium (De Man et al., 1960), incubated at 37 °C for 48 hours (Sathe et al., 2007). The healthy samples of sweet corn kernels were used of the isolation of epiphytic LA bacterial isolation using de Man, Rogosa and Sharpe (MRS) agar medium by incubating at 37°C for 48 hours (De Man et al., 1960). A random selection of colonies resembling LA bacteria from both endophytic and epiphytic isolations were further purified.

Morphological and Biochemical Characterization of Spoilage Microorganisms and LA Bacterial Isolates

The spoilage fungi grown on PDA and bacteria on NA media were observed for their colony and cell morphological traits. The various biochemical tests *viz.*, indole production, Methyl Red (MR) and Voges-Proskauer's (VP) and citrate utilization tests were performed for spoilage bacteria (Aneja, 2014). The LA bacterial isolates were examined for their colony and cell morphological characteristics. The various biochemical tests such as Gram staining, catalase test, endospore staining, dextran and exopolysaccharide production, casein and starch hydrolysis, carbohydrate fermentation and gas production profiles were also performed (Cappuccino and Sherman, 1992).

Qualitative Screening for the Growth of Spoilage Microorganisms

The isolates demonstrating robust and rapid growth were identified as the primary spoilage microorganisms, given their evident capability to flourish under *in vitro* conditions. Therefore, the qualitative screening for the growth of spoilage microorganisms was to find the possible phytopathogens of sweet corn. The observations for the growth of spoilage bacteria on NA and fungi on PDA at intervals of 12, 24 and 36 hours were recorded (Kumar *et al.*, 2022).

Screening of Lactic Acid (LA) Bacteria for Antimicrobial Attributes under *in vitro* Conditions

Lactic acid bacteria hinder spoilage microorganisms through a competitive exclusion approach by outcompeting them for resources. Several antimicrobial compounds such as H_2O_2 , organic acids, diacetyls and bacteriocins are produced by LA bacteria (Ibrahim *et al.*, 2021). The agar well diffusion method was employed to evaluate the antibacterial activity of LA bacterial cells against spoilage bacteria (Balouri *et al.*, 2016). The NA medium was seeded with overnight grown spoilage bacteria @ 3 per cent (OD₆₀₀ = 1.0), poured into Petri plates and allowed to solidify. After solidification, agar wells were drilled with cork borer and 50 µL of LA bacterial culture (10⁸ CFU / mL) was transferred into agar well. The agar plates were incubated at 32°C for 48 h. The area of inhibition of spoilage bacterium is calculated using the formula.

Area of inhibition (cm²) = $\pi R^2 - \pi r^2$

Where,

- $\pi = 3.1428.$
- R = Radius of inhibition zone by LA bacterial cells on NA medium.
- r = Radius of well in NA medium (3.5 mm).

The agar plug diffusion method was used to evaluate the antifungal activity of LA bacterial cells against spoilage fungi (Balouri *et al.*, 2016). The spoilage fungi were grown on PDA plates following the single hyphal tip method, incubated at 27°C for 48-72 h. The PDA medium was seeded LA bacterial cells @ 3 per cent (10⁸ CFU /mL). An agar-plug of spoilage fungi from PDA plates was cut using cork borer (five mm diameter) and placed at the centre of LA bacteria seeded PDA plates and incubated at 27°C for 72 h. The area of colony growth of spoilage fungi on PDA medium is calculated using the formula.

Area of inhibition =
$$\pi R^2$$
- πr^2 .

Where,

- $\pi = 3.147$
- R = Radius of colony growth of spoilage fungi on PDA medium.
- r = Radius of fungal plugs on PDA medium (2.5 mm).

DNA Extraction and Sequencing

DNA was extracted following the manufacturer's instructions provided in the JETM DNA isolation kit. The quality and concentration of the extracted DNA were assessed using a NanoDrop 1000 spectrophotometer. Five microliters of DNA samples were submitted to Barcode Biosciences for 16S rRNA gene for bacterial isolates and fungal internal transcribed spacer region (ITS) library preparation and sequencing for fungi. The two primers (22bp forward primer 5'GGAGAGTTA GATCTTGGCTCAG 3' and 20 bp reverse primer 5' AAGGAGGGGATCCAGCCGCA 3') already

reported for 16S rRNA sequences from the NCBI were custom synthesized by Sigma-Aldrich (Sigma, USA) and diluted accordingly for PCR reactions for DNA isolated from bacterial isolates. The two primers (forward primer ITS-1 and reverse primer ITS-4) reported for Internal Transcribed Spacer region (ITS) sequencing were diluted for PCR reactions for fungal DNA isolation. Sequencing was performed on an Illumina MiSeq instrument using the MiSeq Reagent Kit v3 (2×300 bp) and multiplexing was carried out with a dual indexing method. Details regarding the PCR procedures, primers and Illumina sequencing are described by Comeau *et al.* (2016) and Yurgel *et al.* (2017).

Sequence Analysis

The sequence data were subjected to analysis using the online software provided by the National Centre for Biotechnology Information (NCBI), USA. BLAST (Basic Local Alignment Search Tool) search was performed against the NCBI database (http:// www.ncbi.nlm.nih.gov/BLAST/) to identify partial sequence similarities as described by Altschul *et al.* (1990).

RESULTS AND DISCUSSION

The study aimed to investigate lactic acid bacteria and spoilage microorganisms in sweet corn, shedding light on important beneficial and potentially harmful strains, including opportunistic and human pathogens. With this background, the research work included isolation and characterization of these microorganisms, evaluating the antimicrobial capabilities of beneficial lactic acid bacteria against spoilage bacteria. The results are presented and discussed in the below section.

Enumeration and Isolation of Spoilage Microorganisms, Endophytic and Epiphytic Lactic Acid (LA) Bacteria

The research studies related to microbial isolates from sweet corn remain scarce with most studies focusing only on plant growth promoting rhizo microorganisms of sweet corn (Pande *et al.*, 2020). Nevertheless, there are no reports of isolation of LA bacteria and spoilage

microorganisms from sweet corn to date. Hence, isolation of LA bacteria and spoilage microorganisms from sweet corn was carried out. The epiphytic and endophytic LA bacterial isolates (27) were obtained from sweet corn kernels. The highest population of epiphytic and endophytic LA bacterial isolates were encountered from Rajunkunte (1.82 x 10² and 1.23 x 10² log CFU g⁻¹ of dry weight, respectively), compared to Devanahalli (1.76 x 10² and 1.22 x 10² log CFU g⁻¹ of dry weight, respectively) (Fig. 1). Fessard and Remize (2019) isolated 77 LA bacterial isolates from samples of papaya (24), sliced cabbage (47) and tomato (six). The highest LA bacterial population of 8.4 log CFU g⁻¹ was obtained from pickled cabbage samples, compared to tomato (2.9 log CFU g⁻¹) and papaya (5.1 log CFU g⁻¹) samples. Similarly, endophytic LA bacteria namely, Pedicoccus pentosaceus B125, Lactiplantibacillus plantarum B135 and Lactiplantibacillus plantarum Z183 were isolated from acai fruit, signifying açai fruits as potential source of endophytic LA bacteria (Sato et al., 2020). Thus, there are number of reports stating that LA bacteria form a part of natural microbiota of various plants. The highest population of spoilage bacteria and fungi was observed from samples collected from Devanahalli (1.87 x 10³ log CFU g⁻¹ and 2.21 x 10² log CFU g⁻¹ of dry weight, respectively), compared to Rajunkunte (1.71 x 103 CFU g-1 and 2.09 x $10^2 \log \text{ CFU g}^{-1}$ of dry weight, respectively) (Fig. 1). Parida et al. (2020) isolated and identified different spoilage fungi (Alternaria citri, Penicillium digitatum, Aspergillus niger, Fusarium and Mucor spp.) associated with four different varieties of spoilt oranges and wood apples. Evidently, in an investigation for spoilage causing bacterial species, Hasan and Zulkahar (2018) identified four spoilage bacteria, Escherichia sp., Klebsiella sp., Bacillus sp. and Staphylococcus sp. from three types of spoiled fruit samples, including pineapple (Ananas comosus), banana (Musa paradisiaca L.) and papaya (Carica papaya). The outcomes of the population dynamics of LA bacteria and spoilage microorganisms in Rajunkunte and Devanahalli demonstrate that samples with the greatest abundance of LA bacteria displayed the lowest levels of spoilage microorganisms. This



Population dynamics

Fig. 1 : Population of microflora associated with sweet corn

suggests that the LA bacteria are demonstrating antimicrobial effects against spoilage microorganisms, potentially through competitive exclusion or antibiosis mechanisms.

Morphological and Biochemical Characterization of Spoilage Microorganisms and LA Bacterial Isolates

The spoilage bacteria and fungi isolated from spoilt sweet corn were acronymed as SB and SF. The spoilage bacteria (10) were phenotypically characterized based on their colony and cell morphological characteristics and biochemical reactions. The bacterial isolates were identified as described in Bergey's Manual of Systematic Bacteriology (9th edition) (Ludwig et al., 2009). The spoilage bacterial colonies on NA medium exhibited variation in colour, elevation and margin. The isolates ranged in size $(1.94 \text{ x} 1.09 \text{ to} 4.59 \text{ x} 1.96 \mu\text{m})$ and all cells were rod shaped, except for SB-10, had the characteristic coccoid structure. The different cell arrangements of either as independent or chain was observed. The isolates SB-2, SB-6 and SB-7 were positive for Gram's reaction, isolates SB-2 and SB-6 were endospore formers and all the isolates were catalase positive. Basic biochemical characteristics *i.e.*, indole, methyl red, Vogues-Proskauer and citrate utilization tests and different carbohydrate fermentative profile of the spoilage bacterial isolates were studied. The spoilage fungi (seven) isolated from spoiled cobs were morphologically characterized. Based on their growth pattern on PDA medium, the appearance of the mycelium and microscopic examinations of the vegetative and reproductive structures were recorded (Rashmi and Suvarna, 2022).

The LA bacterial isolates were studied for their morphological and biochemical characteristics as mentioned in Bergey's Manual of Systematic Bacteriology (9th edition) (Ludwig et al., 2009). The isolates obtained are named after the place (Devanahalli or Rajunkunte) and region (epiphytic or endophytic) of isolation. Lactic acid (LA) bacterial isolates obtained from Devanahalli are epiphytic (ED1 - ED6) and endophytic (END1 - END5) LA bacterial isolates and Rajunkunte are epiphytic (ER1 - ER7) and endophytic LA bacterial isolates (ENR1 - ENR9). All the LA bacterial isolates were further abbreviated as LAB for easy identification. The genus level identification of LA bacteria was carried out using morphological and biochemical characteristics (Ngouenam et al., 2021). The colonies appeared small, circular to irregular in form with varied pigmentation. All the colonies of the isolates had a smooth entire margin with raised colonies, except for three isolates (ED2, ENR4 and END1). The strains of LA bacteria were phenotypically characterized on the basis of cell morphology, medium to long rod and cocci shaped cells were observed as either independent or short chains.

The LA bacterial isolates were subjected to biochemical characterization for further identification. All the isolates were Gram positive, catalase negative, non-endospore formers and were non motile, signifying the isolates tentatively belonging to the LA bacterial species. Most of the LA bacterial isolates did not produce catalase enzyme as they are anaerobic to microaerophilic in nature (Dacre and Sharpe, 1956). Manalu et al. (2021) investigated the characteristics of LA bacteria isolated from sweet corn. Three LA bacterial strains with rod shape and Gram positive cells were isolated from fermented maize. All three isolates tested positive for triple sugar iron agar (TSIA) findings but negative for catalase, citrate, indole, motility and Voges-Proskauer (VP). The isolates LAB-5 and LAB-14 exhibited positive results for dextran and exopolysaccharide production. The isolates LAB-8, LAB-24 and LAB-6, LAB-20 showed casein and starch hydrolysis, respectively. The ability of LA bacteria to produce either acid or gas from the appropriate inoculated carbon sources were studied. The carbohydrate fermentation profile of LA bacteria aids in grouping of the isolates into homofermentative or heterofermentative and further for the species level identification of the isolates. Patil et al. (2010) reported that on the basis of their morphological, biochemical, physiological, carbohydrate fermentation patterns and 16S rRNA gene sequences, lactic acid bacteria isolated from curd and cucumber M. S. YASHASWINI *et al*.

(*Cucumis sativus*) samples were assigned to the genera of *Lactobacillus*, *Pediococcus* and *Weissella*. The qualitative screening enables us to identify the potential spoilage microorganisms

Screening for Growth of Spoilage Microorganisms

The qualitative screening of spoilage microorganisms was carried out to study the growth potential at different intervals (12, 24 and 36 h). The growth of spoilage bacteria on NA and fungi on PDA media were represented as dense, medium, light, very little and no growth and the observations were recorded. The spoilage bacterial isolates SB-6 and SB-7 displayed dense growth at 12, 24 and 36 hours of incubation. The spoilage fungal isolates SF-1, SF-2 and SF-4 exhibited a growth transitioning from light, to medium and finally to dense growth over the period of 12, 24 and 36 h of incubation. The isolates that exhibited dense and fast growth were regarded as the predominant spoilage microorganisms, as their ability to thrive under *in vitro* conditions was evident.

Screening of Lactic Acid (LA) Bacterial Isolates for Antimicrobial Attributes under *in vitro* Conditions

The antimicrobial capabilities of the 27 LA bacterial isolates were evaluated against ten spoilage bacterial isolates (Fig. 2 and Plate 1). The isolates LAB-1, LAB-







Plate 1 : Inhibition of (A) SB-6 and (B) SB-7 by LA bacterial isolates

2, LAB-4, LAB-18 and LAB-22 isolates displayed the highest area of inhibition against spoilage bacterial isolates. Among these, isolate LAB-22 demonstrated the highest inhibition area measuring 3.77 cm² and 3.42 cm² followed by isolate LAB-18 measuring 3.50 and 2.67 cm² against isolate SB-6 and SB-7, respectively. The lowest inhibition area was displayed by isolates LAB-21 and LAB-20 against all the spoilage bacterial isolates. Similarly, our findings are in accordance with numerous reports. Melia et al. (2018) reported the antibacterial activity of lactic acid (LA) bacteria isolated from bekasam an Indonesian fermented fish food. The crude bacteriocin of LA bacterial isolate MS2 of 9 h incubation time showed the highest inhibition zone of 13.1 mm, 12.7 and 7.3 mm against Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922 and Salmonella sp., respectively. Correspondingly, Taohong *et al.* (2020) studied the bacteriostatic ability of *Lactococcus lactis* strains (HSM-1, HSM-10 and HSM-18) and one *Leuconostoc lactis* strain (HSM-14) against *Staphylococcus aureus*, enterohemorrhagic *Escherichia coli* (EHEC), *Salmonella typhimurium*, *Listeria monocytogenes* and *Aeromonas caviae*.

The application of LA bacteria as antifungal agents to impede fungal growth and detoxify mycotoxins has been reported by plethora of authors (Shehata *et al.*, 2019, Muhialdin *et al.*, 2020 and Abdel-Nasser *et al.*, 2023). The LA bacterial (27) isolates were assessed for their antifungal potential against seven spoilage fungal isolates (Fig. 3 and Plate 2). The LA bacterial isolates LAB-1, LAB-2, LAB-4, LAB-18 and LAB-22 showed the highest inhibition of colony growth.



Fig. 3: Antifungal activity of lactic acid bacterial isolates against spoilage fungi using agar plug method (*Note*: LAB – Lactic acid bacteria; SF – Spoilage fungi)

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Plate 2 : Antifungal activity of LA bacteria (LAB-22) against spoilage fungi (SF) by agar plug method

Isolate code	Organism identified	Accession no.	Closest type strain in the NCBI database	Sequence length (base pairs)	Sequence similarity (%)
LAB-18	Lactiplantibacillus pentosus	OR578510	Lactiplantibacillus pentosus strain 124-2	1460 bp	99.86
LAB-22	Lactiplantibacillus plantarum	OR578511	Lactiplantibacillus plantarum strain JCM 1149	1530 bp	99.67

TABLE 1 Molecular identification of lactic acid bacteria isolated from sweet corn

TABLE 2 Molecular identification of spoilage microorganisms isolated from sweet corn

Isolate code	Organism identified	Accession no.	Closest type strain in the NCBI database	Sequence length (base pairs)	Sequence similarity (%)
SB-6	Bacillus subtilis	OR584245	<i>Bacillus subtilis</i> strain DSM 10	1502 bp	99.47
SB-7	Rhodococcus qingshengii	OR578512	<i>Rhodococcus qingshengii</i> strain djl-6-2	1489 bp	99.76
SF-1	Aspergillus niger	OR600923	<i>Aspergillus niger</i> strain WA-TKA	565 bp	99.89
SF-3	Fusarium subglutinans	OR600924	Fusarium subglutinans strain SF6	574 bp	99.65

The isolate LAB-22 demonstrated the lowest area of colony growth (2.98 and 2.64 cm²) followed by LAB-18 (3.39 and 3.05 cm²) against spoilage fungal isolates SF-1 and SF-3, respectively. The highest colony growth of 38.27 cm² area was exhibited by isolate LAB-23 against SF-5. Khadija *et al.* (2021) reported the antifungal activity of *Lactiplantibacillus plantarum* NRRL B-14768T and *Levilactobacillus*

brevis ATCC 14869T against Fusarium culmorum, Aspergilus niger and Penicillium sp. by plug agar method. There are reports stating that Pectobacterium carotovorum, Streptomyces scabiei, Fusarium oxysporum, F. sambucinum, Alternaria solani, A. tenuissima, A. alternata, Phoma exigua, Rhizoctonia solani and Colletotrichum coccodes were inhibited by Lactococcus lactis, Leuconostoc mesenteroides,

Lacticaseibacillus casei, Lactobacillus acidophilus, Lactiplantibacillus plantarum, Levilactobacillus brevis, Lacticaseibacillus paracasei, Lactiplantibacillus pentosus, Lactobacillus farraginis and Lactobacillus sp. in agar plug diffusion method (Aleksandra et al., 2022).

The cell free supernatants obtained from the efficient LA bacterial isolates were subsequently assessed for their inhibitory effect against spoilage micro organisms. Further, the best isolates were molecularly characterized using16S rRNA sequencing. Based on per cent similarity obtained from the BLAST tool, National Centre for Biotechnology Information database, the LA bacterial isolates (LAB-18 and LAB-22) as Lactiplantibacillus pentosus UASBMIC 18 and Lactiplantibacillus plantarum UASBMIC 22 respectively (Table 1) the spoilage bacterial isolates SB-6 and SB-7 were identified as Bacillus subtilis UASBMIC 6 and Rhodococcus gingshengii UASBMIC 7, respectively; spoilage fungal isolates (SF-1 and SF-3) as Aspergillus niger UASBMIC 1 and Fusarium subglutinans UASBMIC 3, respectively (Table 2). Bacillus subtilis, Aspergillus niger and Fusarium subglutinans have been documented in the spoilage of sweet corn. This is the first report of Rhodococcus qingshengii identified in the spoilage of sweet corn.

Lactic acid bacteria play a role as antimicrobial agent against spoilage microorganisms primarily through competitive exclusion and antibiosis. They produce various antimicrobial substances including hydrogen peroxide, organic acids, diacetyl and bacteriocins. These compounds collectively contribute to biopreservation by effectively impeding the proliferation of spoilage microorganisms. Consequently, the production of these compounds not only benefits lactic acid bacteria but also offers a natural means for food industry to manage harmful microorganisms. This approach reduces the reliance on chemical preservatives in food preservation that happens to be the need of the hour.

Our research uncovers a diverse microflora in sweet corn, ranging from beneficial to spoilage microorganisms. Notably, this is the first study to isolate lactic acid (LA) bacteria and spoilage microorganisms from sweet corn. The beneficial and spoilage microorganisms were subjected to morphological and biochemical analysis. The LA bacteria are recognized for conferring health benefits and for their potential to extend the shelf life of food products. Their production of natural antimicrobial compounds enhance food safety. The isolated LA bacterial strains demonstrated antimicrobial activity against spoilage bacteria. A thorough understanding of beneficial LA bacteria and their antimicrobial potential role against spoilage microorganisms holds a promising role in mitigating the losses associated with sweet corn and thus reducing the health risks linked to spoilage microorganisms. Furthermore, the potential commercialization of these promising LA bacteria as bio preservation agents present a solution to post-harvest losses in sweet corn.

References

- ABDEL-NASSER, A., HATHOUT, A. S., BADR, A. N., BARAKAT, O. S. AND FATHY, H. M., 2023, Extraction and characterization of bioactive secondary metabolites from lactic acid bacteria and evaluating their antifungal and anti-aflatoxigenic activity. *Biotechnol. Rep.*, **38**: 799 - 812.
- ALEKSANDRA, S., ARTUR, K., ILONA, M., JOANNA, B., AGATA, C., WERONIKA, C. W., OKRASA, M., DOROTA, K. AND BEATA, G., 2022, Lactic acid bacteria as biocontrol agents against potato (*Solanum tuberosum* L.) pathogens. *Appl. Sci.*, **12** : 1 - 17.
- Altschul, S. F., Gish, W., Miller, W., Myers, E. W. and Lipman, D. J., 1990, Basic local alignment search tool. *J. Mol. Biol.*, **215** (3) : 403 - 410.
- ANEJA, K. R., 2014, Biochemical test. In: laboratory manual of microbiology and biotechnology. Scientific International Pvt. Ltd., New Delhi, pp. : 30 - 35.
- BALOURI, M., SADIKI, M. AND SAAD, K. I., 2016, Methods for *in vitro* evaluating antimicrobial activity : A review. *J. Pharm. Analy.*, 6 : 71 - 79.
- CAPPUCCINO, J. G., SHERMAN, N., 1992, Biochemical test. In : Microbiology : A Laboratory Manual. Benjamin Cummings Publishing, New york, pp. : 462 - 464.

- COMEAU, A. M., WARWICK, F. V., LOUIS, B. AND CONNIE, L., 2016, Novel chytrid lineages dominate fungal sequences in diverse marine and freshwater habitats. *Sci. Rep.*, **6**: 1 - 6.
- DACRE, J. C. AND SHARPE, M. E., 1956, Catalase production by lactobacilli. *Nat.*, **178** (4535) : 700 - 701.
- DE MAN, J. C., ROGOSA, M. AND SHARPE, M. E., 1960, A medium for the cultivation of Lactobacilli. J. Appl. Bacteriol., 23: 130 135.
- DE MELO PEREIRA, G. V., MAGALHAES, K. T., LORENZETII, E. R., SOUZA, T. P. AND SCHWAN, R. F., 2012, A multiphasic approach for the identification of endophytic bacterial in strawberry fruit and their potential for plant growth promotion. *Microbial Ecol.*, 63: 405 - 417.
- DHUNDALE, V., HEMKE, V., DESAI, D. AND DHUNDALE, P., 2018, Evaluation and exploration of lactic acid bacteria for preservation and extending the shelf life of fruit. *Int. J. Fruit Sci.*, **18** : 355 - 368.
- FANG, R., CHEN, X., ZHANG, X. AND WANG, B., 2023, Comparative transcriptome analyses shed light on the regulation of harvest duration and shelf life in sweet corn. *Food Res. Int.*, **163** : 112 - 128.
- FESSARD, A. AND REMIZE, F., 2019, Genetic and technological characterization of lactic acid bacteria isolated from tropically grown fruits and vegetables. *Int. J. Food Microbiol.*, **301** : 61 - 72.
- HALLMANN, J., QUADT HALLMANN, A., MAHAFFEE, W. F. AND KLOEPPER, J. W., 1997, Bacterial endophytes in agricultural crops. *Can. J. Micribiol.*, **43** : 895 - 914.
- HASAN, N. A. AND ZULKAHAR, I. M., 2018, Isolation and identification of bacteria from spoiled fruits. In : AIP Conference Proceedings. AIP Publishing LLC. 2020 (1) : 73 - 84.
- IBRAHIM, S. A., AYIVI, R. D., ZIMMERMAN, T., SIDDIQUI, S. A., ALTEMIMI, A. B., FIDAN, H., ESATBEYOGLU, T. AND BAKHSHAYESH, R. V., 2021, Lactic acid bacteria as antimicrobial agents : Food safety and microbial food spoilage prevention. *Foods*, **10** (12) : 3131 - 3142.

- KHADIJA, A., ETTOUIL, A., ASMAA, O., ISMAIL, L., SOUAD, H. AND MOHAMMED, O., 2021, Lactic acid bacteria as bio-preservative agents: antifungal activity of *Lactiplantibacillus plantarum* and *Levilactobacillus brevis* against fungi isolated from bioorganic fertilizer. *Plant Cell Biotechnol. Mol. Biol.*, **22** (71): 370 - 377.
- KUMAR, N. B., SUVARNA, V. C., GIRISH, H. C., RASHMI, S. AND SARVANI, B., 2022, Characterization of beneficial and spoilage microflora of onion (*Allium cepa* L.). *Mysore J. Agric. Sci.*, **56** (4) : 105 - 113.
- LAMONT, J. R., WILKINS, O., BYWATER-EKEGARD, M. AND SMITH, D. L., 2017, From yoghurt to yield : Potential applications of lactic acid bacteria in plant production. *Soil Biol. Biochem.*, **111** : 1 - 9.
- LUDWIG, W., SCHLEIFER, K. H. AND WHITMAN, W. B., 2009, Taxonomic outline of the phylum Firmicutes. In: Bergey's Manual of Systematic Bacteriology, Springer, New York, pp. : 15 - 17.
- MANALU, R. T., FEBRIANI, A. AND SYAMSINAR, S., 2021, Isolation and characterization of lactic acid bacteria in fermented sweet corn (*Zea mays*) as antibacterial. *Biosci.*, 5 (2): 141 - 149.
- MCINROY, J. A. AND KLOEPPER, J. W., 1995, Population dynamics of endophytic bacteria in field-grown sweet corn and cotton. *Can. J. Microbiol.*, **41** (10) : 895 - 901.
- MELIA, S., DWI, S. AND YURNALIZA, 2018, Antimicrobial activity of lactic acid bacteria isolated from bekasam against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Salmonella* sp. *ICBSB*, **130**: 1 - 8.
- MILLER, B. D. D. AND WELCH, R. M., 2013, Food system strategies for preventing micronutrient malnutrition. *Food Policy*, 42 : 115 - 128.
- MUHIALDIN, B. J., ALGBOORY, H. L., KADUM, H., MOHAMMED, N. K., SAARI, N., HASSAN, Z. AND HUSSIN, A. S. M., 2020, Antifungal activity determination for the peptides generated by *Lactobacillus plantarum* TE10 against *Aspergillus flavus* in maize seeds. *Food Control*, **109** : 898 - 911.

- NGOUENAM, J. R., KENFACK, C. H. M., KOUAM, E. M. F., KAKTCHAM, P. M., MAHARJAN, R. AND NGOUFACK, F. Z., 2021, Lactic acid production ability of *Lactobacillus* sp. from four tropical fruits using their by-products as carbon source. *Heliyon*, **7** (5) : 79 - 89.
- OUWEHAND, A. C. AND VESTERLUND, S., 2004, Antimicrobial components from lactic acid bacteria. *Food Sci. Technol.*, **139** : 375 396.
- PANDE, A., KAUSHIK, S., PANDEY, P. AND NEGI, A., 2020, Isolation, characterization and identification of phosphate-solubilizing *Burkholderia cepacia* from the sweet corn cv. Golden Bantam rhizosphere soil and effect on growth-promoting activities. *Int. J. Veg. Sci.*, 26 (6): 591 - 607.
- PARIDA, S., SINHA, R. AND MAHALIK, G., 2020, Fruit spoilage fungi associated with orange and wood apple from different market places of Bhubaneswar. *Sustain. Humanosphere*, **16** (1) : 167 - 172.
- PATIL, M. M., PAL, A., ANAND, T. AND RAMANA, K. V., 2010, Isolation and characterization of lactic acid bacteria from curd and cucumber. *Indian J. Biotechnol.*, 9 (2): 166 - 172.
- RASHMI, S. AND SUVARNA, V. C., 2022, Antimicrobial activities of rhizome extracts of mango ginger (*Curcuma amada*) against food spoilage by bacterial isolates of fruits, vegetables and oilseed. *Mysore J. Agric. Sci.*, 56 (1): 1 - 13.
- REIS, J. A., PAULA, A. T., CASAROTTI, S. N. AND PENNA, A. L.
 B., 2012, Lactic acid bacteria antimicrobial compounds
 : Characteristics and applications. *Food Eng. Rev.*,
 4 (2): 124 140.
- SARANRAJ, P. AND GEETHA, M., 2012, Microbial spoilage of bakery products and its control by preservatives. *Int. J. Pharm. Biol. Sci. Arch.*, **3** (1): 38 - 48.
- SATHE, S. J., NAWANI, N. N., DHAKEPHALKAR, P. K. AND KAPADNIS, B. P., 2007, Antifungal lactic acid bacteria with potential to prolong shelf life of fresh vegetables. *J. Appl. Microbiol.*, **103** (6) : 2622 - 2628.
- SATO, S. T. A., MARQUES, J. M., DE FREITAS, A. D. L., PROGENIO, R. C. S., NUNES, M. R. T., DE VASCONCELOS

MASSAFRA, J. M., MOURA, F. G. AND ROGEZ, H., 2020, Isolation and genetic identification of endophytic lactic acid bacteria from the Amazonian açai fruits: probiotics features of selected strains and their potential to inhibit pathogens. *Front. Microbiol.*, **11** : 524 - 536.

- SHEHATA, M. G., BADR, A. N., EL SOHAIMY, S. A., ASKER, D. AND AWAD, T. S., 2019, Characterization of antifungal metabolites produced by novel lactic acid bacterium and their potential application as food biopreservatives. *Ann. Agric. Sci.*, 64 (1): 71 - 78.
- SNYDER, A. B. AND WOROBO, R. W., 2018, The incidence and impact of microbial spoilage in the production of fruit and vegetable juices as reported by juice manufacturers. *Food Control*, **85** : 144 - 150.
- STOYANOVA, L. G., USTYUGOVA, E. A. AND NETRUSOV, A. I., 2012, Antibacterial metabolites of lactic acid bacteria: their diversity and properties. *Appl. Biochem. Microbiol.*, 48 (3) : 229 - 243.
- TAOHONG, C., LELI, W., QINXIN, L., YINGJIE, L., LIN, Y., YIN,
 J., YAN, Z., LE, H., TINGYU, Y., ABBASI, M. N., YANG,
 H., QIYE, W., CONGJIA, T., TAHIR, A. K., LIU, Q., 2020,
 Functional probiotics of lactic acid bacteria from Hu sheep milk. *BMC Microbiol.*, **20** (1) : 1 12.
- YURGEL, S. N., DOUGLAS, G. M., COMEAU, A. M., MAMMOLITI, M., DUSAULT, A. AND PERCIVAL, D., 2017, Variation in bacterial and eukaryotic communities associated with natural and managed wild blueberry habitats. *Phytobioms J.*, 1: 102 - 113.