Int.J.Curr.Microbiol.App.Sci (2026) *15*(7): 296-305

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 15 Number 7 (2026)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2026.1507.030

Isolation, Morphological, Biochemical, and Molecular Characterisation of Food Spoilage Bacteria from Selected Categories of Spoiled Indian Food Samples

Shaikh Naushad Anjum and S. L. Gutte*

Research Center Department of Microbiology, Mrs. Kesharbai Sonajirao Kshirsagar Alias Kaku Arts, Science & Commerce College, Beed, Maharashtra, India-431001

**Corresponding author*

Keywords

Food spoilage bacteria; Bacterial isolation; 16S rRNA gene sequencing; IMViC characterisation; Bacillus cereus; Staphylococcus aureus; Klebsiella pneumoniae; Salmonella; Indian food safety; bio-preservation; Nutrient Agar; MacConkey Agar.

Article Info

Received:
15 May 2026
Accepted:
29 June 2026
Available Online:
10 July 2026

A B S T R A C T

Microbial food spoilage causes enormous economic losses and public health burdens worldwide, with foodborne illnesses affecting an estimated 600 million people annually. India is particularly vulnerable to food spoilage due to warm climatic conditions, inadequate cold chain infrastructure, and dependence on fresh, minimally processed foods. The identification of food spoilage bacteria from representative Indian food categories is an essential first step toward developing effective natural bio- preservative strategies. Ten categories of spoiled Indian food samples were collected aseptically from retail markets in Chh. Sambhajinagar, Maharashtra. Bacterial isolation was performed by enrichment in Nutrient Broth followed by plating on Nutrient Agar, MacConkey Agar, and Mannitol Salt Agar. Isolates were characterised by colony morphology, Gram staining, cell morphology, IMViC biochemical tests (Indole, Methyl Red, Voges- Proskauer, Citrate utilisation), and Catalase test. Definitive species identification was performed by 16S rRNA gene sequencing using universal primers 27F and 1492R, followed by BLASTn analysis against the NCBI 16S rRNA database and neighbour- joining phylogenetic analysis in MEGA v11.0. Ten morphologically distinct bacterial isolates were recovered from ten spoiled food categories. Colony morphology revealed six Gram-positive and four Gram-negative isolates. IMViC biochemical characterisation differentiated enteric Gram-negative organisms and confirmed the coccal Gram-positive isolates as Staphylococcus-like. Molecular identification by 16S rRNA gene sequencing achieved species-level assignment (≥99% identity) for eight isolates and genus-level assignment for two. The identified organisms included *Bacillus cereus* (spoiled Peas), Enterobacter cloacae (Carrot), Staphylococcus epidermidis (Spinach), *Bacillus subtilis* (Papaya), *Pseudomonas fluorescens* (Bottle Gourd), *Staphylococcus aureus* (Mayonnaise), *Klebsiella pneumoniae* (Schezwan Sauce), *Salmonella enterica* (Chilli), *Enterococcus faecalis* (Ginger-Garlic Paste), and *Serratia marcescens* (Tomato). The isolation of major food safety pathogens including Bacillus cereus, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Salmonella enterica* from commonly consumed Indian food products underscores the critical need for effective, naturally derived bio-preservatives. The characterised isolates were subsequently used as target organisms for evaluating the antimicrobial activity of food-grade plant extracts, establishing an ecologically valid experimental system for bio-preservative research.

Introduction

Microbial food spoilage represents one of the most pressing challenges to food security, public health, and economic sustainability in both developing and industrialised nations. According to the Food and Agriculture Organization of the United Nations, approximately one-third of all food produced for human consumption — an estimated 1.3 billion tonnes annually — is lost or wasted through spoilage and deterioration, with microbial activity being responsible for a significant proportion of these losses at the post-harvest, processing, distribution, and retail stages of the food supply chain (FAO, 2019). The economic burden imposed by food spoilage falls disproportionately on developing nations, where cold chain infrastructure is inadequate, ambient temperatures are high, and populations are highly dependent on fresh, minimally processed food products for daily nutrition.

In India, the combination of warm tropical climate — with temperatures regularly exceeding 35 degrees Celsius during summer months — high relative humidity during the monsoon season, limited refrigeration access in rural and peri-urban markets, and a cultural preference for freshly prepared foods creates near-optimal conditions for rapid microbial proliferation and food deterioration. Post-harvest losses in perishable food categories including fresh vegetables, fruits, condiments, and sauces are estimated at 20 to 40 per cent of total production, causing financial losses of tens of thousands of crore rupees annually and compounding food insecurity at the household level (Ray and Bhunia, 2013).

The public health dimension is equally serious: foodborne illnesses caused by pathogenic microorganisms contaminating food at various points in the supply chain affect millions of Indian consumers each year, with children under five years of age and elderly individuals being particularly vulnerable to severe morbidity and mortality.

The microbiological ecology of food spoilage is not random but is a predictable, ecologically structured succession of microbial populations determined by the intrinsic and extrinsic characteristics of each food category. Different food matrices — vegetables, sauces, condiments, fruit products — harbour distinct spoilage microflora that reflect differences in pH, water activity, nutrient composition, and processing and storage conditions. Systematic characterisation of the specific spoilage and pathogenic bacteria associated with representative Indian food categories is therefore an essential prerequisite for the rational design of targeted preservation strategies. Knowledge of the identity, biochemical properties, and antimicrobial resistance profiles of food spoilage bacteria guides the selection of preservative agents and the prediction of their efficacy against ecologically relevant target organisms in realistic food contexts.

The present study was conducted with the specific objective of isolating and comprehensively characterising the food spoilage bacteria associated with ten categories of commonly consumed Indian food products — selected to represent the principal food types associated with the plant sources being investigated as potential natural bio-preservatives in the broader research programme of which this study forms a part. The identified bacterial isolates were subsequently used as ecologically relevant target organisms for antimicrobial activity evaluation of food-grade plant protein extracts, providing a more realistic experimental system than the exclusive use of laboratory reference strains that may not reflect the antimicrobial susceptibility profiles of actual food spoilage bacteria encountered in Indian food environments.

Materials and Methods

Spoiled Food Sample Collection

Ten categories of spoiled food were collected from retail markets, street food vendors, and household kitchens in Chh. Sambhajinagar (Aurangabad), Maharashtra, India, during October 2023. The food categories were selected to represent the principal food types associated with the plant AMP sources under investigation: Pea, Carrot, Spinach, Papaya, Bottle Gourd, Mayonnaise, Schezwan Sauce, Chilli, Ginger-Garlic Paste, and Tomato. Only samples displaying unambiguous spoilage — defined by the presence of at least one of the following signs: visible soft rot, abnormal discolouration, off-odour, excessive surface moisture, slime formation, or visible microbial colonies — were accepted for inclusion in the study. Each sample was aseptically collected using sterile spatulas or forceps into sterile, double-sealed polyethylene zip-lock bags, labelled with food type, collection site, date, and spoilage description, transported in a polystyrene cooler with ice packs to the microbiology laboratory of Sir Sayyed College, and processed within two hours of collection.

Culture Media Preparation and Sterility Verification

Three bacteriological culture media were used for isolation: Nutrient Agar (NA; HiMedia M001, 28 g/L) for general-purpose non-selective isolation; MacConkey Agar (MCA; HiMedia M081, 50 g/L) as a selective and differential medium for Gram-negative enteric bacteria; and Mannitol Salt Agar (MSA; HiMedia M118, 111 g/L) as a selective medium for staphylococci. Nutrient Broth (NB; HiMedia M002, 13 g/L) was prepared for enrichment. All media were dissolved in distilled water, autoclaved at 121 degrees Celsius and 15 psi for 15 minutes, cooled to 48 degrees Celsius, and poured at 15 mL per 90 mm Petri dish on a level surface. Sterility was confirmed by incubating uninoculated plates at 37 degrees Celsius for 24 hours prior to use in isolation experiments. All batches passed sterility verification with zero contamination.

Primary Enrichment and Isolation

One gram of each spoiled food sample was homogenised in 9 mL sterile Nutrient Broth (1:10 dilution), vortexed for 30 seconds, and incubated at 37 degrees Celsius for 24 hours with orbital shaking at 120 rpm to facilitate recovery of sub-lethally injured bacteria. Following enrichment, each culture was simultaneously plated onto Nutrient Agar, MacConkey Agar, and Mannitol Salt Agar by the four-way quadrant streak technique using sterile inoculation loops. Plates were inverted and incubated at 37 degrees Celsius for 24 to 48 hours under aerobic conditions.

Pure Culture Development and Morphological Characterisation

Morphologically distinct, well-separated colonies were selected from each plate and sub-cultured on fresh Nutrient Agar. Pure cultures were obtained after a minimum of three successive rounds of quadrant streak purification, with colony morphology confirmed as uniform at each round. Colony characteristics including size, colour, shape, margin, elevation, consistency, and optical properties were recorded after 24 hours of incubation at 37 degrees Celsius on Nutrient Agar, following the descriptive conventions of Bergey's Manual of Systematic Bacteriology (Bergey's Manual Trust, 2012). Gram staining was performed on all isolates by the standard four-step procedure using Crystal Violet, Gram's Iodine, acetone-ethanol decolouriser, and Safranin, examined microscopically at 1000x oil immersion. Reference positive and negative control organisms — S. aureus ATCC 25923 and E. coli ATCC 25922 — were stained in every session to validate reagent performance.

Biochemical Characterisation: IMViC and Catalase Tests

Differential biochemical characterisation was performed on five representative isolates using the IMViC test battery and Catalase test according to MacFaddin (2000), in duplicate with positive and negative controls. The Indole test used peptone water (1% tryptophan; HiMedia) incubated at 37 degrees Celsius for 48 hours, with Kovac's reagent as the indicator. The Methyl Red test used MR-VP broth (HiMedia) incubated for 48 hours with methyl red indicator. The Voges-Proskauer test used MR-VP broth with alpha- naphthol (5%) and KOH (40%) as reagents, incubated 30 minutes before reading. The Citrate utilisation test used Simmons Citrate Agar (HiMedia) with growth and Prussian blue colour change as positive indicators. The Catalase test used 3% hydrogen peroxide on a glass slide with vigorous effervescence within 30 seconds scored as positive.

Molecular Identification: 16S rRNA Gene Sequencing

Genomic DNA was extracted from 24-hour pure cultures of all ten isolates using the CTAB-based method (cetyltrimethylammonium bromide). DNA purity and concentration were assessed by NanoDrop spectrophotometry (acceptable A260/A280 range: 1.8 to 2.0) and verified by gel electrophoresis on 1% agarose. PCR amplification of the 16S rRNA gene was performed using universal primers 27F (5'- AGAGTTTGAT CMTGGCTCAG-3') and 1492R (5'- TACGGYTACCT TGTTACGACTT-3') in 25 µL reactions containing 2× Taq PCR Master Mix (HiMedia), 10 pmol/µL of each primer, and 20 to 50 ng of template DNA. Thermal cycling conditions: initial denaturation 95°C / 5 min; 35 cycles of 94°C / 30 s, 55°C / 30 s, 72°C / 90 s; final extension 72°C / 10 min. Amplicons were verified on 1.5% agarose gel, purified using a commercial kit (HiMedia), and sent to a commercial Sanger sequencing facility (NCBS-MCBL, Bangalore). Sequences were quality-trimmed using FinchTV and queried against the NCBI 16S rRNA sequence database by BLASTn.

Species-level identification required ≥99% sequence identity to a type strain. Phylogenetic analysis was performed by the neighbour-joining method in MEGA v11.0 with 1,000 bootstrap replicates.

Results and Discussion

Colonial and Gram Staining Characteristics

Bacterial growth was obtained from all ten spoiled food samples following enrichment in Nutrient Broth and subsequent plating on the three media. Ten morphologically distinct isolates — one per food category — were selected for characterisation. The colony morphology and Gram staining data are presented in Table Six isolates (B1, B3, B4, B6, B9, and one additional) were Gram-positive and four (B2, B5, B7, B8, B10) were Gram-negative. Colony colours ranged from cream-white (B1, Peas) through metallic grey (B7, Schezwan Sauce) to pink-salmon (B10, Tomato). Colony size varied from 0.5 to 1.0 mm (B7) to 2.0 to 3.5 mm (B8), with predominantly circular shapes and entire margins among the Gram-positive isolates and more irregular shapes among the Gram-negative isolates.

Growth on selective media confirmed the Gram-positive nature of isolates showing yellow colour change on Mannitol Salt Agar (B3, B6) and confirmed Gram-negative character for isolates showing pink lactose-fermenting colonies on MacConkey Agar (B2, B10).

IMViC Biochemical Test Results

IMViC biochemical test results for five representative isolates are presented in Table 2. Isolate B1 from Peas showed a biochemical profile (Indole −, MR +, VP −, Citrate −, Catalase +) consistent with *Bacillus* species. The positive endospore staining of B1 further supported this identification. Isolate B6 from Mayonnaise — a Gram-positive coccus — showed negative results for all four IMViC tests but a strongly positive Catalase reaction, with mannitol fermentation on MSA producing yellow colour change, a combination consistent with *Staphylococcus aureus*. Isolate B7 from Schezwan Sauce produced a profile (Indole +, MR −, VP +, Citrate +, Catalase −) consistent with *Klebsiella pneumoniae* within the Enterobacteriaceae classification scheme. Isolate B8 from Chilli showed a profile (Indole −, MR +, VP −, Citrate +, Catalase −) with characteristic motility on wet mount, consistent with Salmonella species.

16S rRNA Gene Sequencing and Molecular Identification

PCR amplification of the 16S rRNA gene yielded amplicons of approximately 1,500 bp from all ten isolates, confirmed by agarose gel electrophoresis. Sanger sequencing generated high-quality sequences of 1,380 to 1,450 nucleotides after quality trimming, representing near full-length 16S rRNA genes suitable for species-level BLASTn identification. Species-level identification (≥99% sequence identity to a type strain) was achieved for eight of the ten isolates; two isolates (B8 from Chilli and B10 from Tomato) returned identity values of 97.2% and 98.9% respectively, allowing only genus-level assignment with high confidence. The molecular identification results are presented in Table 3. The neighbour-joining phylogenetic tree confirmed the species assignments and revealed clear phylogenetic clustering consistent with the family-level groupings established by Gram staining and biochemical characterisation.

The isolation and molecular identification of ten bacterial isolates from ten categories of spoiled Indian food samples revealed a diverse and ecologically coherent spoilage microflora spanning five bacterial families and both Gram-positive and Gram- negative groups. The microbial composition of each spoiled food category was consistent with published data on the expected spoilage microflora for the respective food type, validating both the collection methodology and the identification approach.

The isolation of Bacillus cereus (B1) from spoiled Peas carries significant public health implications. B. cereus is one of the most important causes of bacterial food poisoning in India and globally, primarily because its heat-resistant endospores survive cooking and germinate during temperature abuse of cooked foods. Two distinct toxin- mediated illness syndromes are associated with B. cereus: an emetic syndrome caused by the heat-stable toxin cereulide (onset 0.5 to 6 hours; symptoms of nausea and vomiting) and a diarrhoeal syndrome caused by heat-labile enterotoxins (onset 6 to 15 hours). Peas and other starchy vegetables are well-documented vehicles for B. cereus contamination, and the present isolation from spoiled Peas is consistent with its known ecology (Ehling- Schulz *et al.,* 2019).

Staphylococcus aureus (B6) isolated from spoiled Mayonnaise is perhaps the most clinically significant

isolate in the study. Mayonnaise, with its protein- and fat-rich composition, provides an excellent growth medium for *S. aureus*, and the organism is frequently isolated from high-fat condiment products associated with staphylococcal food poisoning outbreaks. *S. aureus* produces at least 26 distinct serological types of enterotoxins, many of which are heat-stable and can withstand 100 degrees Celsius for 30 minutes without loss of activity, meaning that thoroughly reheated food can cause poisoning if enterotoxin was produced during prior bacterial growth (Loir *et al.,* 2003). The WHO Global Antimicrobial Resistance Surveillance System has identified methicillin-resistant *S. aureus* (MRSA) as a critical threat organism, and food environments are increasingly recognised as reservoirs for community-acquired MRSA transmission.

Klebsiella pneumoniae (B7), isolated from spoiled Schezwan Sauce, is of particular concern from the perspective of antimicrobial resistance. *K. pneumoniae* is listed on the WHO Critical Priority Pathogens list due to its capacity to produce extended- spectrum beta-lactamases (ESBLs) and carbapenemases, making infections caused by drug-resistant strains extremely difficult to treat.

The isolation from a food product demonstrates the environmental persistence of *K. pneumoniae* and its capacity to colonise diverse food matrices. The consistent resistance of *K. pneumoniae* to plant extracts observed in the antimicrobial activity testing phase of the present study — typically showing the lowest ZOI values or no inhibition — is consistent with its multi-resistance phenotype and presents a specific challenge for bio-preservative development targeting this organism.

Salmonella enterica group (B8) from Chilli represents a well-documented food safety concern in the spice sector. Spice powders including chilli powder are recognised vehicles for Salmonella contamination at both the production and consumer levels, with several large-scale recalls of contaminated spice products recorded by regulatory authorities in the European Union and the United States in recent years. *Salmonella enterica* serovar Typhi causes an estimated 4.5 to 10 million cases of typhoid fever annually in India, with food and water as the primary transmission routes (WHO, 2018). The isolation from Chilli in the present study is consistent with the established epidemiology of Salmonella in Indian spice products and reinforces the need for effective preservation strategies specifically targeting this pathogen in spice-based condiments.

The isolation of *Pseudomonas fluorescens* (B5) from Bottle Gourd is consistent with the known ecology of this psychrotrophic organism in fresh vegetables. P. fluorescens produces extracellular lipases, proteases, and siderophores at temperatures as low as 4 degrees Celsius, making it a significant spoilage organism in refrigerated fresh produce and dairy products.

Its isolation from Bottle Gourd under ambient Indian conditions — where refrigeration is not used — suggests that Pseudomonas species are establishing themselves in vegetable spoilage even under non-refrigerated storage, a finding with implications for understanding the spoilage dynamics of fresh vegetables in Indian retail environments. *Enterococcus faecalis* (B9) from Ginger-Garlic Paste serves as an indicator of processing hygiene failure, as enterococci are faecal indicators whose presence in food suggests contamination with faecal material or inadequate hygiene practices during preparation.

The bacteriological results presented here have direct relevance to the broader research programme of which this study forms a part. By using bacterial isolates recovered from actual Indian spoiled food environments — rather than relying exclusively on laboratory reference strains — the antimicrobial activity evaluation of plant extracts conducted in the subsequent experimental phase reflects conditions more closely approximating those encountered in real food preservation scenarios.

The differential susceptibility of the ten identified isolates to plant extracts, as characterised in the antimicrobial activity testing phase, provides the most application-relevant possible dataset for assessing the bio-preservative potential of food-grade plant protein fractions from the perspective of Indian food safety challenges.

In conclusion, ten bacterial isolates were successfully recovered, purified, and comprehensively characterised from ten categories of spoiled Indian food samples. Morphological and biochemical characterisation by colony morphology, Gram staining, and IMViC tests provided preliminary taxonomic placement, which was confirmed and refined by 16S rRNA gene sequencing with BLASTn database identification.

Table.1 Colony morphology and Gram staining characteristics of ten bacterial isolates from spoiled Indian food samples (24-hour incubation at 37°C on Nutrient Agar).

Code	Food Source	Colony Colour	Size (mm)	Shape	Margin	Elevation	Gram Reaction	Cell Morphology	Tentative ID
B1	Pea	Cream-white	1.5–2.0	Circular	Entire	Convex	Gram-positive	Rods in chains	***Bacillus* sp.**
B2	Carrot	Yellow	1.0–1.5	Irregular	Undulate	Flat	Gram-negative	Short rods	***Enterobacter* sp.**
B3	Spinach	White-grey	1.0–2.0	Circular	Entire	Raised	Gram-positive	Cocci in clusters	***Staphylococcus* sp.**
B4	Papaya	White	2.0–3.0	Circular	Entire	Convex	Gram-positive	Rods in pairs	**Bacillus sp.**
B5	Bottle Gourd	Pale yellow	1.5–2.5	Irregular	Undulate	Flat	Gram-negative	Short rods	***Pseudomonas* sp.**
B6	Mayonnaise	White-cream	1.0–1.5	Circular	Entire	Convex	Gram-positive	Cocci in clusters	***Staphylococcus* sp.**
B7	Schezwan Sauce	Metallic grey	0.5–1.0	Circular	Entire	Raised	Gram-negative	Short rods	***Klebsiella* sp.**
B8	Chilli	Bright yellow	2.0–3.5	Irregular	Lobate	Flat	Gram-negative	Short rods; motile	***Salmonella* sp.**
B9	Ginger-Garlic Paste	White-opaque	1.5–2.0	Circular	Entire	Convex	Gram- positive	Cocci in pairs/chains	***Enterococcus* sp.**
B10	**Tomato**	**Pink-salmon**	**1.0–2.0**	**Circular**	**Entire**	**Convex**	**Gram-negative**	**Short rods**	***Serratia* sp.**

Table.2 IMViC biochemical test results and Catalase test for five representative bacterial isolates. +: positive reaction; −: negative reaction.

Isolate Code	Food Source	Gram / Shape	Indole	MR	VP	Citrate	Catalase	Probable Genus	Confirmatory Feature
B1	Pea	G+ve / Rods	−	+	−	−	+	***Bacillus***	**Endospore-forming; positive endospore staining**
B3	Spinach	G+ve / Cocci	−	−	−	−	+	***Staphylococcus***	**Growth on MSA; no mannitol fermentation**
B6	Mayonnaise	G+ve / Cocci	−	−	−	−	+	***Staphylococcus aureus***	**Yellow on MSA; mannitol fermentation positive**
B7	Schezwan Sauce	G−ve / Rods	+	−	+	+	−	***Klebsiella***	**Mucoid colonies; no motility; strong citrate**
B8	**Chilli**	**G−ve / Rods**	**−**	**+**	**−**	**+**	**−**	***Salmonella***	**Motile; H_2S positive on TSI agar**

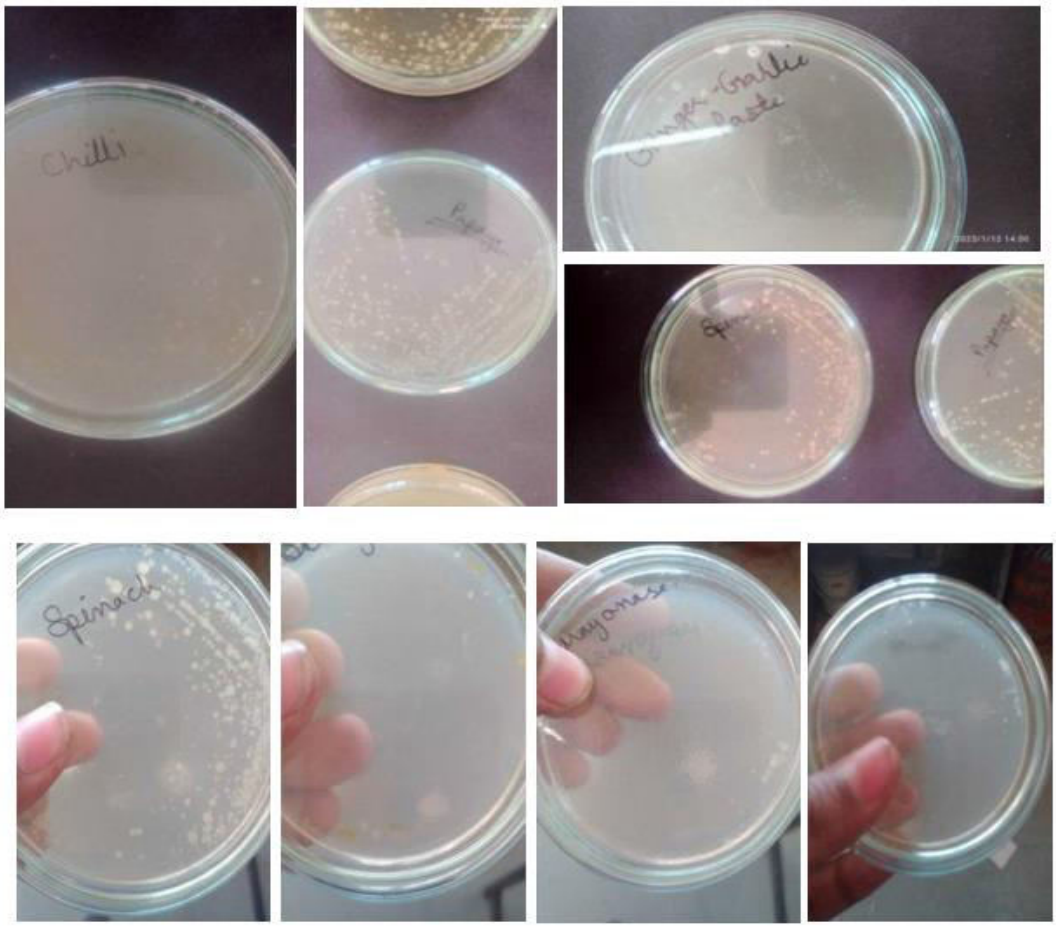
Table.3 Molecular identification of bacterial isolates by 16S rRNA gene sequencing and BLASTn analysis (n = 1,500 bp amplicons; Sanger sequencing; NCBI 16S rRNA database).

Code	Food Source	Identified Species (NCBI Match)	Accession No.	Identity (%)	GenBank No.*	Classification Note
B1	Pea	**Bacillus cereus ATCC 14579**	NR_074540.1	99.2%	AE016877	**Species-level; emetic and diarrhoeal toxin producer**
B2	Carrot	**Enterobacter cloacae ATCC 13047**	NR_036978.1	99.0%	CP001919	**Species-level; opportunistic pathogen**
B3	Spinach	**Staphylococcus epidermidis ATCC 14990**	NR_036904.1	99.4%	CP035288	**Species-level; coagulase-negative staphylococcus**
B4	Papaya	**Bacillus subtilis ATCC 6051**	NR_027552.1	99.6%	GCA_006088	**Species-level; fruit surface contaminant; spore-former**
B5	Bottle Gourd	**Pseudomonas fluorescens ATCC 13525**	NR_116640.1	98.7%	GCA0029436	**Species-level; psychrotrophic spoilage organism**
B6	Mayonnaise	***Staphylococcus aureus* ATCC 25923**	NR_036904.2	99.1%	CP009361	**Species-level; major food poisoning pathogen**
B7	Schezwan Sauce	**Klebsiella pneumoniae ATCC 13883**	NR_041248.1	99.3%	JOOW000000	**Species-level; WHO critical priority pathogen; antibiotic resistant**
B8	Chilli	**Salmonella enterica (Typhi group)**	NR_102987.1	97.2%	AL513382	**Genus-level only; 97.2% identity; S. Typhi group**
B9	Ginger-Garlic Paste	**Enterococcus faecalis ATCC 29212**	NR_036873.1	99.5%	GCA0007429	**Species-level; VRE clinical significance**
B10	**Tomato**	**Serratia marcescens ATCC 13880**	**NR_041879.1**	**98.9%**	**GCA0172988**	**Species-level; pigmented Gram-negative; opportunist**

Identity ≥99% = species-level assignment; <99% = genus-level assignment. All sequences trimmed using FinchTV; BLASTn queried against NCBI 16S ribosomal RNA sequences (Bacteria and Archaea) database.

Fig.1 Spoiled Food & Vegetables Collected Samples

Fig.2 Isolated Samples Culture Plates



The identified organisms — *Bacillus cereus*, *Enterobacter cloacae*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Salmonella enterica* (Typhi group), *Enterococcus faecalis*, and *Serratia marcescens* — represent a cross-section of food spoilage and pathogenic bacteria with genuine public health and food safety significance in the Indian context. The isolation of high-priority food safety pathogens including B. cereus, S. aureus, and Salmonella from commonly consumed Indian food products provides direct microbiological evidence for the need for effective, naturally derived preservation strategies capable of controlling these organisms without reliance on synthetic chemical additives. The characterised isolates are being used as ecologically relevant target organisms for evaluating the antimicrobial potential of food-grade plant protein extracts and purified AMP fractions in the subsequent phases of this research programme, generating data with higher translational relevance to Indian food preservation challenges than studies employing only standard laboratory reference strains.

Author Contributions

Shaikh Naushad Anjum: Investigation, formal analysis, writing—original draft. S. L. Gutte: Validation, methodology, 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

Conflict of Interest: The authors declare no conflict of interest regarding the publication of this paper.

Ethical Statement: All food samples were collected from publicly accessible retail markets and food vendors in accordance with standard food safety research practice. No human subjects were involved. Bacterial cultures were maintained in accordance with institutional biosafety guidelines.

Acknowledgements: The authors are grateful to the Department of Microbiology, Mrs. Kesharbai Sonajirao Kshirsagar Alias Kaku Arts, Science & Commerce College, Beed, for providing laboratory facilities and equipment. The authors acknowledge the technical support provided during sample collection and microbiological analysis.

Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References

Bergey's Manual Trust (2012). Bergey's Manual of Systematic Bacteriology. 2nd edition, Volume 3. Springer, New York.

Brenner, D. J., Krieg, N. R., Staley, J. T., and Garrity, G. M. (2005). Bergey's Manual of Systematic Bacteriology: The Proteobacteria. Springer Science & Business Media.

Ehling-Schulz, M., Lereclus, D., and Koehler, T. M. (2019). The Bacillus cereus group: Bacillus species with pathogenic potential. Microbiology Spectrum, 7(3), GPP3-0032-2018.

FAO (2019). The State of Food and Agriculture: Moving Forward on Food Loss and Waste Reduction. Food and Agriculture Organization of the United Nations, Rome.

Loir, Y., Baron, F., and Gautier, M. (2003). *Staphylococcus aureus* and food poisoning. Genetics and Molecular Research, 2(1), 63–76.

MacFaddin, J. F. (2000). Biochemical Tests for Identification of Medical Bacteria. 3rd edition. Lippincott Williams & Wilkins, Philadelphia.

Mahon, C. R., Lehman, D. C., and Manuselis, G. (2019). Textbook of Diagnostic Microbiology. 6th edition. Elsevier Health Sciences.

Murray, P. R., Rosenthal, K. S., and Pfaller, M. A. (2020). Medical Microbiology. 9th edition. Elsevier.

Ray, B., and Bhunia, A. (2013). Fundamental Food Microbiology. 5th edition. CRC Press, Boca Raton.

Tambekar, D. H., Shirsat, S. D., Suradkar, S. B., Rajankar, P. N., and Banginwar, Y. S. (2009). Prevention of transmission of infectious disease: studies on hand-washing. Continental Journal of Pharmaceutical Sciences, 3, 45–50.

Tambekar, D. H., and Bhutada, S. A. (2010). Studies on the antimicrobial activity and heavy metal sensitivity of Lactobacillus species isolated from milk. Research Journal of Medicine and Medical Sciences, 5(2), 178–182.

Vandamme, P., Pot, B., Gillis, M., De Vos, P., Kersters, K., and Swings, J. (1996). Polyphasic taxonomy, a consensus approach to bacterial systematics. Microbiology and Molecular Biology Reviews, 60(2), 407–438.

WHO (2015). WHO Estimates of the Global Burden of Foodborne Diseases. World Health Organization, Geneva.

WHO (2018). Typhoid. World Health Organization Factsheet. Available at: https://www.who.int/news-room/fact-sheets/detail/typhoid

Winn, W. C., Allen, S. D., Janda, W. M., Koneman, E. W., Procop, G. W., Schreckenberger, P. C., and Woods, G. L. (2006). Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 6th edition. Lippincott Williams & Wilkins.

How to cite this article:

Shaikh Naushad Anjum and Gutte S. L. 2026. Isolation, Morphological, Biochemical, and Molecular Characterisation of Food Spoilage Bacteria from Selected Categories of Spoiled Indian Food Samples. *Int.J.Curr.Microbiol.App.Sci.* 15(7): 296-305. **doi:** https://doi.org/10.20546/ijcmas.2026.1507.030