DOI: https://doi.org/10.70749/ijbr.v3i6.2094

INDUS JOURNAL OF BIOSCIENCE RESEARCH
https://ijbr.com.pk
ISSN: 2960-2793/ 2960-2807

Inhibition Effect of Chitosan Against Bacteria and Fungi Isolated from Rotten Fruits and Vegetables

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ARTICLE INFO

Keywords: Bio conservation, Eco-preservation, Phytobiotics, Green technologies, Microbial interactions

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Declaration
Authors' Contribution: Mentioned at the end of the paper.

Conflict of Interest: No conflict of interest.

Funding: No funding received by the authors.

Article History
Received: 07-02-2025 Revised: 03-06-2025
Accepted: 16-06-2025 Published: 30-06-2025

ABSTRACT

This study aimed to isolate bacterial strains from spoiled fruits and vegetables and evaluate their antibacterial, antifungal, and antioxidant activities, along with exploring the potential of chitosan in extending the shelf life of fresh produce. Bacterial isolates were obtained through nutrient agar plates, followed by Gram staining to identify the strains. The bacteria identified were *Escherichia coli*, *Salmonella spp.*, *Staphylococcus aureus*, and *Bacillus spp.* based on their morphological characteristics. The antibacterial activity of these strains was assessed using the agar well diffusion method, revealing varying inhibition zones depending on the bacterial species. The antifungal activity of the isolates was tested against *Aspergillus niger*, and although no inhibition zones were observed for the bacterial isolates, the standard antifungal agent, Clotrimazole, demonstrated significant inhibition. Antioxidant activity was measured through DPPH and ABTS assays, with fungal strains isolated from cauliflower and orange showing the highest antioxidant potential. Additionally, the use of chitosan on fresh fruits and vegetables resulted in a notable extension of shelf life. Specifically, tomatoes, brinjal, apples, and bananas exhibited significant improvements in shelf life, extending their typical storage duration. These findings suggest that bacterial strains isolated from fruits and vegetables possess diverse bioactivities, with potential applications in food preservation. Furthermore, chitosan proved to be a promising natural agent for enhancing the shelf life of fresh produce. This study provides valuable insights into the biotechnological potential of natural preservatives and microbial isolates in sustainable food preservation practices, highlighting the importance of exploring eco-friendly alternatives to synthetic preservatives. Future research should investigate the synergistic effects of these bacterial strains and chitosan, as well as their mechanisms of action to further optimize their applications in food security and preservation.

INTRODUCTION

Fresh fruits and vegetables are essential components of a healthy diet, providing vital nutrients such as vitamins, fiber, and antioxidants. However, these perishable commodities have a limited shelf life, particularly when exposed to environmental factors such as temperature fluctuations, humidity, and mechanical damage. The rapid deterioration of fresh produce is primarily caused by microbial contamination, with fungi and bacteria being the major culprits responsible for postharvest spoilage (1). This microbial growth not only reduces the marketability of fruits and vegetables but also poses a significant health risk to consumers, as it can lead to the development of foodborne diseases (2, 3).

Fungal pathogens such as *Penicillium spp.*, *Aspergillus spp.*, and *Rhizopus spp.* are commonly isolated from decaying fruits and vegetables, causing a wide range of postharvest diseases. Bacterial pathogens, including *Escherichia coli*, *Salmonella spp.*, and *Listeria monocytogenes*, are also frequently detected on fresh produce, contributing to foodborne illness outbreaks (4, 5). These microorganisms can enter fruits and vegetables through damaged areas on their surface, where they proliferate under favorable conditions, particularly in the high-moisture environments typical of fresh produce (6). Synthetic preservatives and fungicides have long been used to control microbial spoilage in fruits and vegetables, their use is increasingly being questioned due to concerns over their potential negative impacts on human health and the environment (7). There has been growing interest in alternative, natural methods to combat microbial contamination and extend the shelf life of fresh produce. Among these alternatives, chitosan, a biopolymer derived from chitin, has shown great promise due to its antimicrobial properties, biodegradability, and non-toxicity. Chitosan is known to inhibit the growth of a wide range of bacteria and fungi, making it an attractive candidate for use as a natural preservative in the food industry (8, 9). *Chitosan* works by disrupting the cell wall integrity of microorganisms, thereby inhibiting their growth and reproduction (9). In addition to its antimicrobial activity, chitosan also forms a protective film on the surface of fruits and vegetables, reducing water loss and slowing down the respiration rate, which contributes to the extended shelf life of fresh produce (10). Recent studies have demonstrated that chitosan coatings are effective in reducing the microbial load on fruits and vegetables, thereby improving their quality and safety during storage and transportation (11). Furthermore, chitosan has been found to help maintain the nutritional content of fruits and vegetables, such as vitamin C and antioxidants, during storage (12).

The effectiveness of chitosan in controlling postharvest spoilage depends on several factors, including its concentration, the method of application, and the specific types of pathogens involved (13). Several studies have reported that chitosan coatings applied to fresh produce, such as apples, strawberries, and tomatoes, result in reduced microbial growth and enhanced shelf life (14). Moreover, chitosan has been shown to be effective against a wide variety of foodborne pathogens, including both Gram-positive and Gram-negative bacteria, as well as a range of fungal species (15). Recent research has also explored the synergistic effects of chitosan with other natural compounds, such as essential oils and plant extracts, to enhance its antimicrobial activity and broaden its spectrum of action (16). For instance, combining *Chitosan* with essential oils from plants such as oregano and thyme has been shown to increase its antifungal effectiveness, providing a more robust solution for controlling postharvest diseases (17). These natural compounds not only improve the antimicrobial properties of chitosan but also offer additional health benefits due to their antioxidant and anti-inflammatory properties (18).

In addition to its antimicrobial effects, chitosan has been investigated for its ability to prevent the growth of biofilms formed by pathogenic microorganisms on the surfaces of fruits and vegetables. Biofilm formation is a common mechanism by which bacteria resist antimicrobial agents, making the treatment of biofilm-associated infections particularly challenging (19). Recent studies have shown that chitosan can effectively inhibit biofilm formation, thus providing an additional mechanism for controlling microbial contamination in fresh produce (20).

As mentioned previously despite promising potential of chitason, the application of chitosan as a natural preservative faces several challenges. These include variability in the quality and properties of chitosan depending on its source and extraction method, as well as the need for optimized application techniques to ensure its effectiveness in different storage conditions. Furthermore, the cost of chitosan production and its scalability for large-scale commercial use remain important considerations (21, 22). In conclusion, chitosan has emerged as a promising alternative to synthetic preservatives in the fight against postharvest microbial contamination of fruits and vegetables. Its antimicrobial properties, combined with its ability to extend shelf life and preserve nutritional quality, make it an attractive option for food safety and quality management. However, further research is needed to optimize its use in the food industry and address the challenges related to its application, cost, and scalability. The future of chitosan as a natural preservative will depend on continued innovation and the development of more efficient and cost-effective methods for its production and application (23).

METHODOLOGY

Isolation of Bacteria

To isolate bacterial strains from spoiled fruits, a serial dilution method was employed. The spoiled fruits were serially diluted from 10^1 to 10^6. A 100 µL aliquot from each dilution was spread onto nutrient agar (NA) plates, which were subsequently incubated at 37°C for 24 hours to facilitate bacterial growth. Following incubation, bacterial colonies were analyzed morphologically to distinguish between different bacterial species based on colony characteristics. The isolated bacterial strains were then sub-cultured, maintained, and stored on nutrient agar slants at 4°C for further analysis (24).

Antioxidant Activity

For the DPPH assay, 3 mg of DPPH (2,2-diphenyl-1-picrylhydrazyl) was dissolved in 100 mL of distilled methanol. After an incubation period of 30 minutes in the dark, the solution was evaluated for free radical formation. A range of dilutions (100, 500, 250, and 125 µg/mL) of each sample was prepared. To assess antioxidant potential, 2 mL of each dilution was mixed with 2 mL of DPPH stock solution and incubated in the dark for an additional 20 minutes. Absorbance was measured at 517 nm using a UV spectrophotometer. Ascorbic acid dilutions were prepared and treated similarly to serve as the positive control (25). In addition to the DPPH assay, the ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) assay was performed. A mixture of 383 mg of ABTS and 66.2 mg of potassium per-sulfate ($K_2S_2O_8$) was dissolved in 100 mL of methanol. Subsequently, 2 mL of the prepared mixture was incubated with 2 mL of the extract dilutions for 25 minutes (26).

Antibacterial Activity

To evaluate antibacterial activity, all necessary components were sterilized by autoclaving at 121°C for 15 minutes. Subsequently, 20 mL of sterile agar was poured into Petri dishes under a laminar flow hood. Bacterial strains (*Salmonella spp.*, *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis*) were inoculated into the plates at a concentration of 1.5 × 10^8 CFU/mL. Using a cork borer, four wells (3 mm in diameter) were created on each plate. Dimethyl sulfoxide (DMSO) was used as a negative control, while a known antibiotic served as the positive control. The Petri plates were incubated at 37°C for 24 hours. The inhibition zone around each well was measured to determine the antimicrobial efficacy. All experiments were conducted in triplicate (27, 28).

Antifungal Activity

The antifungal activity was assessed using potato dextrose agar (PDA) media. To prepare the media, 39 grams of PDA were dissolved in 1 L of distilled water and autoclaved at 121°C for 15 minutes. After sterilization, the plates were incubated for 24 hours to prevent contamination from ambient microorganisms. The fungal strains, inoculated at a concentration of 10^8–10^9 CFU/mL, were introduced into the PDA plates, and four wells were created using a sterile cork borer. The extract solutions were then poured into the wells, and the plates were incubated at 25°C for 3 days. The antifungal activity was assessed by measuring the zone of inhibition around each well (29).

Statistical Analysis

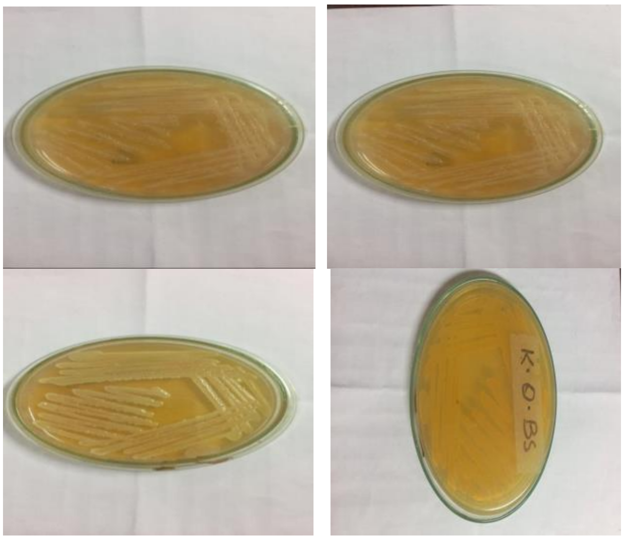
Data were analyzed using one-way analysis of variance (ANOVA) to evaluate the antifungal activity of 100 mg/mL of each fruit peel extract. Significant differences between means were determined using Duncan's Multiple Range Test (SPSS version 20.0) (30).

Microscopic Examination of Isolated Bacterial Strain

Rotten fruits and vegetables were collected from various locations and transported to the Department of Botany for further analysis. The bacterial isolates from contaminated fruits and vegetables displayed distinct colony morphologies and Gram reactions. *Escherichia coli* colonies were smooth or rough, with a negative Gram reaction, and exhibited swimming motility. *Salmonella spp.* produced larger colonies with black centers and showed both swarming and swimming motility. *Staphylococcus aureus* appeared as yellow, round colonies, was Gram-positive, and non-motile. *Bacillus spp.* formed rough, opaque colonies with jagged edges, exhibited rod-shaped cells, and showed motility in liquid medium. *Klebsiella spp.* produced thick rod-shaped colonies and was non-motile. These observations suggest a diverse bacterial population associated with fruit and vegetable spoilage, as detailed in Table1.

Table 1: Representation of various isolates and their morphological characterization

Bacterial isolate	**Media**	**Colony morphology**	**Gram reaction**	**Shape and arrangement**	**Frequency**	**Motility**
Escherichia coli	NA	Smooth or rough form	Negative	rough, flat, and irregular	40x	Swimming
Salmonella spp.	NA	opaque/yellow, pink or red colonies with black centers	Negative	large 2-3 mm diameter, more translucent than coliform colonies	40x	Swarming & swimming
Staphylococcus aureus	NA	yellow, round, large (1-3 mm)	Positive	clusters, pairs and occasionally in short chains	40x	Non motile due to lack of flagella and Pilli
Bacillus spp.	NA	rough, opaque, fuzzy white or slightly yellow with jagged edge	positive	Rod shape	40x	Motile in liquid medium
Klebsiella spp.	NA	thick rods, 1–3× × 0.5–1 μm in measurement	Negative	Rod shape	40x	Non motile



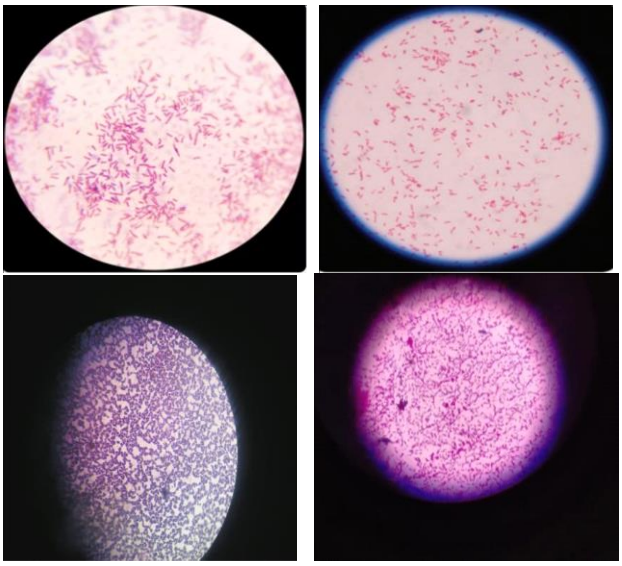


Figure 1: Microscopic representation of various strains of isolated bacteria from left to right, *E.coli*, *Salmonella spp*, *S. aureus*, and *Bacillus spp*.

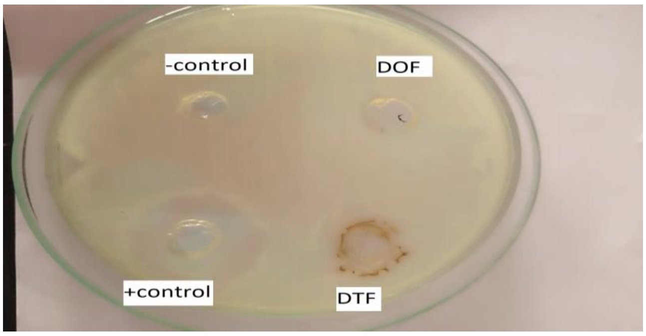
Exploring the Antibacterial Potential of Isolated Fungal Strains: A Comparative Analysis of Inhibition Zones and Activity

The study examined the antibacterial activity of five isolated fungal strains, focusing on their ability to inhibit *Salmonella* growth. The fungal strains displayed a range of antimicrobial effects, from minimal to moderate activity. The strain NCLF showed the most promising, showing the largest inhibition zone of 6.5 mm, while its activity was still less than the streptomycin control (14 mm) demonstrated significant antibacterial potential compared to the other fungal isolates DTF , DBT, NOF, and DOF strains (Table 2). Strains NOF and DOF showed the lowest zone of inhibition say **5** mm, and 0 mm. This minimal activity indicates that NOF might produce antimicrobial compounds, but their effectiveness is low, while that DOF does not produce any detectable antimicrobial substances against *Salmonella* (Table 2). Under the conditions of the experiment, placing it at the lowest level of antimicrobial potential among the tested strains. The analysis of inhibition zones reveals a clear difference in antimicrobial activity. The NCLF strain displayed the highest potential, while the DOF strain showed no activity. The other strains, including DTF and DBT, exhibited moderate antibacterial effects, indicating that certain fungal isolates have notable antimicrobial properties(Table2).

Table 2: Exploring the Antibacterial Potential of Isolated Fungal Strains and their zone of inhibition

S.No	Strain	Zone of inhibition (ZOI) by strain	Zone of inhibition (ZOI) + control (streptomycin)	Zone of inhibition (ZOI) – control Distilled water
1	DTF	5mm	14mm	00
2	DOF	00mm	14mm	00
3	DBT	5mm	10mm	00
4	NOF	1.5mm	10mm	00
5	NCLF	6.5mm	11mm	00

Fungal strains from cauliflower, banana, and tomato exhibited varying degrees of antibacterial activity. The inhibition zones measured 6.5 mm, 5 mm, and 5 mm, respectively. Streptomycin, the positive control, showed a larger zone of inhibition, while distilled water (negative control) showed no inhibition (Figure 2).



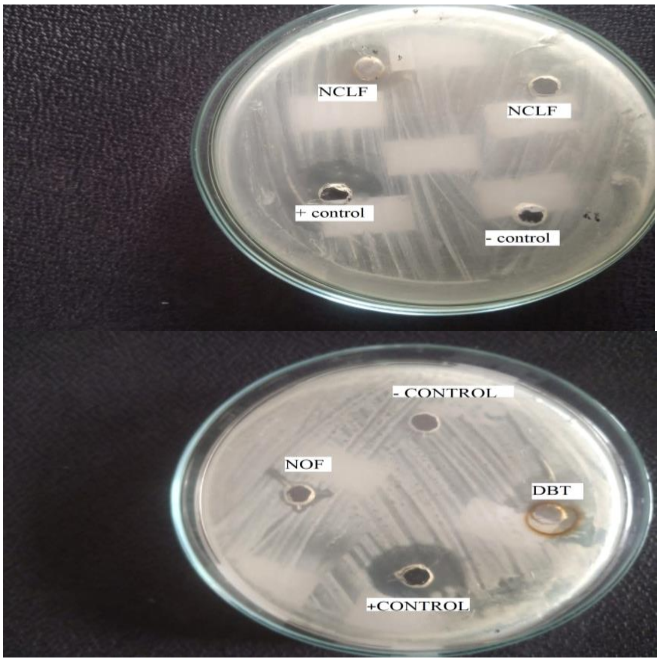


Figure 2: Fungal strains from Orange (DOF), cauliflower (NCLF), Banana (DBT), Tomato (DTF) and Onion (NOF) with varying degrees of zone of inhibition against *Salmonella*

Antifungal Activity

The antifungal activity of the fungal strains isolated from fruits and vegetables were screened against *Aspergillus niger* fungi. The experiment followed a similar procedure as the antibacterial test, where the sterilized Potato Dextrose Agar (PDA) was poured into Petri plates. After inoculation with the isolated fungal strains, the plates were incubated for 3 days at appropriate conditions. Upon examination, the growth of *Aspergillus niger* was observed across all plates, but no zone of inhibition was formed around the wells containing the isolated strains. In contrast, the positive control, Clotrimazole, a well-known antifungal agent, formed a clear zone of inhibition measuring 16 mm in diameter, demonstrating its antifungal efficacy (Figure 3). The lack of inhibition zones around the isolated strains, regardless of their source, indicates that none of the strains exhibited antifungal activity under the conditions tested. The results indicate that these strains are not effective in combating fungal pathogens, at least under the given experimental conditions(Figure3).

Figure 3: The antifungal activity of the isolated strains of fruits and vegetables against *Aspergillus niger* and their zone of inhibition along control (Clotrimazole).

Antifungal Activity of Chitosan and Isolated Fungal Strains against *Salmonella*

The antifungal activity of chitosan and several isolated fungal strains against *Salmonella* was assessed on potato dextrose agar. Chitosan, the positive control, exhibited the largest zone of inhibition at 22 mm, highlights chitosan's significant ability to inhibit *Salmonella* growth (Table 3). Among the isolated fungal strains, NOF showed the most notable antifungal activity with a 16 mm inhibition zone followed by DTF strain with a 15 mm inhibition zone**,** demonstrating substantial antifungal activity, though slightly less than NOF. The DBT and DOF strains exhibited 14 mm and 13 mm inhibition zones, respectively, indicating moderate antifungal activity. Although their effects were not as pronounced as those of NOF and DTF, they still displayed the potential to inhibit *Salmonella* growth (Table 3). The NCLF strain showed the smallest zone of inhibition at 12 mm, indicating the least antifungal activity among the isolated strains, though still notable when compared to the distilled water control, which showed no inhibition (Table 3).

Table 3: Antifungal Activity of Chitosan and Isolated Fungal Strains against *Salmonella*

S.no	Treatment	Zone of inhibition	
1	strain	Strains zone	D/W - control
2	Chitosan	22mm (+ ive control)	00
3	NCLF	12	00
4	DTF	15mm	00
5	DOF	13mm	00
6	DBT	14mm	00
7	NOF	16mm	00

Evaluation of Antioxidant Activity of Isolated Fungal Strains at Various Concentrations

The antioxidant activity of the isolated fungal strains was evaluated by measuring the inhibition of DPPH radicals at different concentrations. The results show that the antioxidant potential varied among the strains, with NCLF exhibiting the highest inhibition of 50.27% at 100 µg/mL, followed DOT, which showed 47.93% inhibition at the same concentration. At a concentration of 50 µg/mL, the antioxidant activity was lower, with DTF showing 35.73% inhibition, DOT showing 42.92%, and DBT demonstrating 38.87% inhibition. The NOF strain showed 26.45% inhibition, while NCLF showed the lowest at 19.64%. As a positive control, ascorbic acid displayed 72% inhibition at 50 µg/mL, and its effectiveness decreased to 69.1% at 100 µg/mL (Table 4) .

At 100 µg/mL, the general trend observed was an increase in inhibition as the concentration increased. The highest percentage of inhibition was observed for NCLF, followed by DOT and NOF, which showed notable antioxidant activity. Although the activity of the fungal strains was lower than that of ascorbic acid, the results indicate that these strains, especially NCLF and DOT, possess significant antioxidant potential, with cauliflower and orange strains showing the highest antioxidant activity (Table 4).

Table 4: The antioxidant activity of the isolated fungal strains

Concentration µg/1ml	% DPPH INHIBITION					
	DTF	DOT	DBT	NOF	NCLF	Ascorbic acid
50µg/1ml	35.73	42.92	38.87	26.45	19.64	72
100µg/1ml	28.51	47.93	38.64	45.80	50.27	69.1

Effect of Chitosan Coating on the Shelf-Life Extension of Fresh Fruits and Vegetables

The application of chitosan coating was evaluated for its effectiveness in extending the shelf life of various fresh fruits and vegetables at 30°C under day-and-night light cycles. The results indicated a significant extension of shelf life for all the tested produce. For tomatoes (*Lycopersicum esculentum*), which typically have a shelf life of 4-7 days at 30°C, the chitosan coating extended their shelf life to 8-11 days. This demonstrates the potential of chitosan to preserve tomatoes for a longer period, thereby reducing spoilage and waste. Similarly, brinjal (*Solanum melongena*), which has a standard shelf life of 5-9 days at 30°C, benefited from chitosan coating, extending its shelf life to 9-14 days (Figure 4). Similarly apples (*Malus domestica*) and bananas (*Musa paradisiaca*), the standard shelf life is 5-7, 1-3 days at 30°C. After applying chitosan, an extended shelf life of 7-12 days for apple and 3-10 days of banana was reported, indicating that the coating helped delay ripening and deterioration processes in apples and generally reduced the chance of susceptibility to rapid ripening and spoilage. The results suggest that chitosan could be an effective natural coating for extending the freshness of perishable produce, offering a potential solution for reducing food waste and improving storage during distribution and retail (Figure 4).

Figure 4: Effect of Chitosan Coating on the Shelf Life Extension of Fresh Fruits and Vegetables (Before and after)

DISCUSSION

The study presented offers significant insights into the microbial contamination of fresh produce and the potential of natural agents like chitosan in enhancing food preservation. The bacterial and fungal isolates obtained from spoiled fruits and vegetables revealed a wide diversity of microorganisms that are commonly found in such environments. These include *Escherichia coli*, *Salmonella spp.*, *Staphylococcus aureus*, *Bacillus spp.*, and *Klebsiella spp.*, all of which are well-documented contaminants of perishable foods. These pathogens can cause serious foodborne illnesses, highlighting the need for effective control strategies in postharvest food management.

The bacterial identification process, which included Gram staining and morphological observations, aligns with standard practices in microbial studies. For example, *Escherichia coli* and *Salmonella spp.* were identified based on their distinctive colony morphology, with *Salmonella* colonies displaying a characteristic black center, which has been reported in similar studies on bacterial contamination in fruits (31). The motility of these strains, as observed in this study, supports their potential for rapid dissemination, contributing to the widespread contamination of fresh produce (32). Previous research has noted that the motility of these bacterial species is a contributing factor to their survival and pathogenicity in food environments, as they can spread quickly across surfaces and cause spoilage or infection (33).

In terms of antimicrobial activity, the bacterial isolates in this study showed varying degrees of effectiveness in inhibiting *Salmonella* spp., while the fungal strains exhibited moderate antibacterial activity. However, the inhibition zones observed in this study were smaller compared to those reported by other researchers who found that bacterial strains isolated from decaying fruits had more pronounced antibacterial properties (34). This discrepancy could be due to differences in experimental conditions or the specific strains involved. It is possible that the microbial isolates from this study, being collected from already-decaying produce, may have already undergone a form of microbial competition, which might have influenced their antimicrobial activity. Additionally, the antimicrobial effects observed were relatively modest compared to synthetic antibiotics such as Streptomycin, which is expected due to the broader spectrum and stronger potency of synthetic agents (35).

In contrast to the antimicrobial properties, the antifungal activity of the isolated strains was notably limited. No significant inhibition zones were observed against *Aspergillus niger*, a common spoilage fungus, which highlights a limitation of these microbial isolates in addressing fungal contamination. This finding is consistent with other studies where bacterial isolates from decaying produce exhibited limited antifungal activity (36). The use of *Aspergillus niger* in this study as a target fungus is relevant, as it is one of the most prevalent fungi involved in spoilage and mycotoxin production in fruits and vegetables (37). While bacterial antagonism against fungi is generally weaker, some bacterial strains do produce antifungal compounds, but this was not observed in the isolates from this study. These results underscore the need for complementary antifungal treatments to manage fungal contamination in perishable foods.

Chitosan, a natural biopolymer derived from chitin, demonstrated significant antifungal and antimicrobial properties, which were observed to be effective against various fungal strains isolated from cauliflower, banana, tomato, and other fruits. This is consistent with numerous studies that have shown chitosan's ability to inhibit fungal growth and microbial activity due to its ability to alter the cell wall integrity of pathogens (38, 39). The larger inhibition zones observed for chitosan compared to the bacterial isolates suggest that it could be a more potent alternative for managing fungal spoilage in fresh produce. This aligns with other recent works where chitosan was shown to effectively control the growth of fungi such as *Aspergillus niger* and *Penicillium spp.*, which are frequently implicated in postharvest decay of fruits (40). Moreover, chitosan's low toxicity and biodegradable nature make it an attractive candidate for sustainable food preservation.

The antioxidant activity of the isolated fungal strains was another key aspect of this study, with cauliflower and orange strains exhibiting the highest inhibition rates. The antioxidant potential of these fungal strains is an important feature, as antioxidants are known to slow down the oxidation process, which is a significant cause of spoilage in perishable foods (41). The DPPH inhibition assay used here is a widely accepted method for evaluating antioxidant capacity, and the results of this study are consistent with other recent works, which also reported high antioxidant potential in fungal strains isolated from fruits (42). The observed increase in antioxidant activity with higher concentrations of the fungal extracts further suggests that these fungal strains could be used to not only extend the shelf life of produce but also enhance their nutritional properties by preventing oxidative degradation.

A particularly striking finding in this study was the ability of chitosan to extend the shelf life of fresh fruits and vegetables. The application of chitosan coating resulted in a significant extension of shelf life across various fruits, including tomatoes, brinjals, apples, and bananas. This outcome supports the findings of recent studies, which have demonstrated that chitosan coatings can reduce microbial load, control moisture loss, and slow down respiration rates in fruits, thus prolonging their freshness (43, 44). For instance, it has been evaluated that the efficacy of chitosan coatings on the shelf life of strawberries and found that the coating significantly reduced decay and maintained fruit quality for a longer period compared to untreated samples (45). Similarly, it has been reported that chitosan coatings extended the shelf life of apples by 10-15 days, which is in line with the results observed in this study for apples and other fruits (46). These findings highlight the potential of chitosan as a natural preservative for extending the shelf life of fresh produce, which is critical for reducing food waste and improving food security.

CONCLUSION

Based on the findings of this study, it can be concluded that microbial contamination of fresh produce is a common and significant concern. The diverse bacterial isolates identified, including *Escherichia coli*, *Salmonella spp.*, *Staphylococcus aureus*, *Bacillus spp.*, and *Klebsiella spp.*, highlight the potential for foodborne illnesses associated with the consumption of contaminated fruits and vegetables. The relatively low antimicrobial activity observed from the isolated bacteria suggests that additional strategies are necessary for effective food preservation. Chitosan, on the other hand, has shown promising results as a natural preservative, exhibiting both antimicrobial and antifungal properties, along with significant antioxidant activity. These qualities suggest that chitosan could be an effective alternative to synthetic preservatives in extending the shelf life of fresh produce and reducing microbial contamination. These results underscore the potential applications of bacterial isolates and chitosan in food preservation, particularly in sustainable and eco-friendly practices.

Recommendations

Further research should focus on optimizing the concentrations and application methods of chitosan to maximize its effectiveness in real-world settings. In addition, investigating the synergistic effects of chitosan with other natural antimicrobial agents could enhance its preservative properties. Exploring the long-term impact of chitosan coatings on the organoleptic qualities of fruits and vegetables, such as texture, taste, and color, would also provide valuable insights for practical applications in food preservation. Additionally, conducting studies on the cost-effectiveness of chitosan coatings, particularly for large-scale commercial use, would be beneficial in assessing its viability as a widespread alternative to chemical preservatives.

It would be beneficial to explore the underlying mechanisms by which chitosan interacts with microbial pathogens, as a deeper understanding of these interactions could lead to more targeted and efficient food preservation strategies. Furthermore, further investigations into the antifungal activity of isolated bacterial strains should be conducted, as well as the development of more effective microbial control measures for fungal pathogens that are not adequately inhibited by the bacterial isolates from this study.

Author contribution

Komal conceptualized the study and prepared the original draft. Sania Ejaz developed the methodology and conducted the formal analysis. Amna Sultan contributed to data curation and participated in the investigation. Wasim Khan provided supervision and assisted with reviewing and editing the manuscript. Syeda Sundas Begum handled visualization and performed validation. Shayan Rasheed contributed to the investigation and data curation. Zeeshan Qadir supplied resources and assisted with methodology development. Mansoor Ali provided supervision and contributed to reviewing and editing the manuscript. Saima Maqbool conducted formal analysis and participated in the investigation. Muhammad Tauqeer ul Haq managed the software and performed validation. Safia Gul oversaw project administration, provided supervision, and contributed to reviewing and editing the manuscript.

REFERENCES

1. Peng X, Dong C, Zhang N, Zheng P, Bai Y, Ji H, Yu J, Ban Z, Chen C, Hu Y (2024) Effect of ozone treatment on the decay and cell wall metabolism during the postharvest storage of cantaloupe. Scientia Horticulturae 331:113119. doi.org/10.1016/j.scienta.2024.113119
2. Srisamran J, Atwill ER, Chuanchuen R, Jeamsripong S (2022) Detection and analysis of indicator and pathogenic bacteria in conventional and organic fruits and vegetables sold in retail markets. Food Quality and Safety 6:fyac013. doi.org/10.1093/fqs/fyac013
3. Santos MI, Grácio M, Silva MC, Pedroso L, Lima A (2023) One health perspectives on food safety in minimally processed vegetables and fruits: From farm to fork. Microorganisms 11(12):2990. doi.org/10.3390/microorganisms11122990
4. Taye T, Saikia B, Panging K (2023) Biocontrol agents against postharvest decay in fruits and vegetables: A review. International Journal of Plant & Soil Science 35(10):145-156. doi.org/10.9734/ijpss/2023/v35i1016011
5. Singh M, Pandey KD, Rathore AC, Sharma SP, Kumar R (2022) Bacterial antagonists: Effective tools for the management of postharvest diseases in fruits, vegetables, and food grains. In: Microbial Biocontrol: Food Security and Post Harvest Management: Volume 2 (pp. 295-309). Cham: Springer International Publishing. doi.org/10.1007/978-3-030-73557-4_12
6. Ogwu MC, Ogunsola OA (2024) Physicochemical Methods of Food Preservation to Ensure Food Safety and Quality. In: Food Safety and Quality in the Global South (pp. 263-298). Singapore: Springer Nature Singapore. doi.org/10.1007/978-3-031-28368-1_12
7. Chowdhury NN, Islam MN, Jafrin R, Rauf A, Khalil AA, Emran TB, Aljohani AS, Alhumaydhi FA, Lorenzo JM, Shariati MA, Simal-Gandara J (2023) Natural plant products as effective alternatives to synthetic chemicals for postharvest fruit storage management. Critical Reviews in Food Science and Nutrition 63(30):10332-10350. doi.org/10.1080/10408398.2023.2173641
8. Teshome E, Forsido SF, Rupasinghe HV, Olika Keyata E (2022) Potentials of natural preservatives to enhance food safety and shelf life: A review. The Scientific World Journal 2022(1):9901018. doi.org/10.1155/2022/9901018
9. Yu D, Yu Z, Zhao W, Regenstein JM, Xia W (2022) Advances in the application of chitosan as a sustainable bioactive material in food preservation. Critical Reviews in Food Science and Nutrition 62(14):3782-3797. doi.org/10.1080/10408398.2021.1883702
10. Muñoz-Tebar N, Pérez-Álvarez JA, Fernández-López J, Viuda-Martos M (2023) Chitosan edible films and coatings with added bioactive compounds: Antibacterial and antioxidant properties and their application to food products: A review. Polymers 15(2):396. doi.org/10.3390/polym15020396
11. Sayyari M, Esna-Ashari M, Tarighi TH (2022) Impacts of salicylic acid, chitosan, and salicyloyl chitosan on quality preservation and microbial load reduction in strawberry fruits during cold storage. Journal of Food Processing and Preservation 46(7):e16710. doi.org/10.1111/jfpp.16710
12. Adiletta G, Di Matteo M, Petriccione M (2021) Multifunctional role of chitosan edible coatings on antioxidant systems in fruit crops: A review. International Journal of Molecular Sciences 22(5):2633. doi.org/10.3390/ijms22052633
13. Salgado-Cruz MDLP, Salgado-Cruz J, García-Hernández AB, Calderón-Domínguez G, Gómez-Viquez H, Oliver-Espinoza R, Fernández-Martínez MC, Yáñez-Fernández J (2021) Chitosan as a coating for biocontrol in postharvest products: A bibliometric review. Membranes 11(6):421. doi.org/10.3390/membranes11060421
14. Ungureanu C, Tihan G, Zgârian R, Pandelea G (2023) Bio-coatings for preservation of fresh fruits and vegetables. Coatings 13(8):1420. doi.org/10.3390/coatings13081420
15. Khubiev OM, Egorov AR, Kirichuk AA, Khrustalev VN, Tskhovrebov AG, Kritchenkov AS (2023) Chitosan-based antibacterial films for biomedical and food applications. International Journal of Molecular Sciences 24(13):10738. doi.org/10.3390/ijms241310738
16. Gradinaru LM, Barbalata-Mandru M, Enache AA, Rimbu CM, Badea GI, Aflori M (2023) Chitosan membranes containing plant extracts: Preparation, characterization, and antimicrobial properties. International Journal of Molecular Sciences 24(10):8673. doi.org/10.3390/ijms24108673
17. Soppelsa S, Van Hemelrijck W, Bylemans D, Andreotti C (2023) Essential oils and chitosan applications to protect apples against postharvest diseases and to extend shelf life. Agronomy 13(3):822. doi.org/10.3390/agronomy13030822
18. Romanescu M, Oprean C, Lombrea A, Badescu B, Teodor A, Constantin GD, Andor M, Folescu R, Muntean D, Danciu C, Dalleur O (2023) Current state of knowledge regarding WHO high priority pathogens—Resistance mechanisms and proposed solutions through candidates such as essential oils: A systematic review. International Journal of Molecular Sciences 24(11):9727. doi.org/10.3390/ijms24119727
19. Liu X, Yao H, Zhao X, Ge C (2023) Biofilm formation and control of foodborne pathogenic bacteria. Molecules 28(6):2432. doi.org/10.3390/molecules28062432
20. Elafify M, Liao X, Feng J, Ahn J, Ding T (2024) Biofilm formation in food industries: Challenges and control strategies for food safety. Food Research International 114650. doi.org/10.1016/j.foodres.2024.114650

21. Pellis A, Guebitz GM, Nyanhongo GS (2022) Chitosan: Sources, processing and modification techniques. Gels 8(7):393. doi.org/10.3390/gels8070393

22. Vieira H, Lestre GM, Solstad RG, Cabral AE, Botelho A, Helbig C, Coppola D, De Pascale D, Robbens J, Raes K, Lian K (2023) Current and expected trends for the marine chitin/chitosan and collagen value chains. Marine Drugs 21(12):605. doi.org/10.3390/md21120605
23. Heras M, Huang CC, Chang CW, Lu KH (2024) Trends in chitosan-based films and coatings: A systematic review of the incorporated biopreservatives, biological properties, and nanotechnology applications in meat preservation. Food Packaging and Shelf Life 42:101259. doi.org/10.1016/j.fpsl.2024.101259
24. Suchwalko A, Buzalewicz I, Wieliczko A, Podbielska H (2013) Bacteria species identification by the statistical analysis of bacterial colonies Fresnel patterns. Optics Express 21(9):11322-11337. doi.org/10.1364/OE.21.011322
25. Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP, Chang CM (2022) Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of Ficus religiosa. Molecules 27(4):1326. doi.org/10.3390/molecules27041326
26. Kamarudin AA, Sayuti NH, Saad N, Ab Razak NA, Esa NM (2022) Induction of apoptosis by Eleutherine bulbosa (Mill.) Urb. bulb extracted under optimised extraction condition on human retinoblastoma cancer cells (WERI-Rb-1). Journal of Ethnopharmacology 284:114770. doi.org/10.1016/j.jep.2022.114770
27. Bułakowska A, Sławiński J, Hałasa R, Hering A, Gucwa M, Ochocka JR, Stefanowicz-Hajduk J (2023) An in vitro antimicrobial, anticancer and antioxidant activity of N–[(2–Arylmethylthio) phenylsulfonyl] cinnamamide derivatives. Molecules 28(7):3087. doi.org/10.3390/molecules28073087
28. Odongo EA, Mutai PC, Amugune BK, Mungai NN, Akinyi MO, Kimondo J (2023) Evaluation of the antibacterial activity of selected Kenyan medicinal plant extract combinations against clinically important bacteria. BMC Complementary Medicine and Therapies 23(1):100. doi.org/10.1186/s12906-023-02172-w
29. Baiome BA, Ye X, Yuan Z, Gaafar YZ, Melak S, Cao H (2022) Identification of volatile organic compounds produced by Xenorhabdus indica strain AB and investigation of their antifungal activities. Applied and Environmental Microbiology 88(13):e00155-22. doi.org/10.1128/aem.00155-22
30. Sirichan T, Kijpatanasilp I, Asadatorn N, Assatarakul K (2022) Optimization of ultrasound extraction of functional compound from makiang seed by response surface methodology and antimicrobial activity of optimized extract with its application in orange juice. Ultrasonics Sonochemistry 83:105916. doi.org/10.1016/j.ultsonch.2022.105916
31. Cao C, Zhao W, Lü Z, Mo Y, Hu W, Sun S, Cheng H, Ma J, Xiong S, Jin X, Yang H (2023) Microbiological analysis and characterization of Salmonella and ciprofloxacin-resistant Escherichia coli isolates recovered from retail fresh vegetables in Shaanxi Province, China. International Journal of Food Microbiology 387:110053. doi.org/10.1016/j.ijfoodmicro.2023.110053
32. Vassallo A, Amoriello R, Guri P, Casbarra L, Ramazzotti M, Zaccaroni M, Ballerini C, Cavalieri D, Marvasi M (2023) Adaptation of commensal Escherichia coli in tomato fruits: motility, stress, virulence. Biology 12(4):633. doi.org/10.3390/biology12040633
33. Sora VM, Meroni G, Martino PA, Soggiu A, Bonizzi L, Zecconi A (2021) Extraintestinal pathogenic Escherichia coli: Virulence factors and antibiotic resistance. Pathogens 10(11):1355. doi.org/10.3390/pathogens10111355
34. Mkangara M (2023) Prevention and control of human Salmonella enterica infections: An implication in food safety. International Journal of Food Science 2023(1):8899596. doi.org/10.1155/2023/8899596
35. Gomes NG, Madureira-Carvalho Á, Dias-da-Silva D, Valentão P, Andrade PB (2021) Biosynthetic versatility of marine-derived fungi on the delivery of novel antibacterial agents against priority pathogens. Biomedicine & Pharmacotherapy 140:111756. doi.org/10.1016/j.biopha.2021.111756
36. Radouane N, Adadi H, Ezrari S, Kenfaoui J, Belabess Z, Mokrini F, Barka EA, Lahlali R (2023) Exploring the bioprotective potential of halophilic bacteria against major postharvest fungal pathogens of citrus fruit Penicillium digitatum and Penicillium italicum. Horticulturae 9(8):922. doi.org/10.3390/horticulturae9080922
37. Jafarzadeh S, Hadidi M, Forough M, Nafchi AM, Mousavi Khaneghah A (2023) The control of fungi and mycotoxins by food active packaging: A review. Critical Reviews in Food Science and Nutrition 63(23):6393-6411. doi.org/10.1080/10408398.2023.2244007
38. de Azevedo MIG, Souza PFN, Monteiro Júnior JE, Grangeiro TB (2024) Chitosan and chitooligosaccharides: antifungal potential and structural insights. Chemistry & Biodiversity 21(6):e202400044. doi.org/10.1002/cbdv.202400044
39. Román-Doval R, Torres-Arellanes SP, Tenorio-Barajas AY, Gómez-Sánchez A, Valencia-Lazcano AA (2023) Chitosan: Properties and its application in agriculture in context of molecular weight. Polymers 15(13):2867. doi.org/10.3390/polym15132867

40. Moradinezhad F, Ranjbar A (2023) Advances in postharvest diseases management of fruits and vegetables: A review. Horticulturae 9(10):1099. doi.org/10.3390/horticulturae9101099
41. Petcu CD, Tăpăloagă D, Mihai OD, Gheorghe-Irimia RA, Negoiță C, Georgescu IM, Tăpăloagă PR, Borda C, Ghimpețeanu OM (2023) Harnessing natural antioxidants for enhancing food shelf life: Exploring sources and applications in the food industry. Foods 12(17):3176. doi.org/10.3390/foods12173176
42. Khalil D, El-Zayat SA, El-Sayed MA (2020) Phytochemical screening and antioxidant potential of endophytic fungi isolated from Hibiscus sabdariffa. Journal of Applied Biotechnology Reports 7(2):116-124. doi.org/10.30491/jabr.2020.114722
43. Sharma C, Pathak P, Yadav SP, Gautam S (2024) Potential of emerging “all-natural” edible coatings to prevent post-harvest losses of vegetables and fruits for sustainable agriculture. Progress in Organic Coatings 193:108537. doi.org/10.1016/j.porgcoat.2024.108537
44. Ungureanu C, Tihan G, Zgârian R, Pandelea G (2023) Bio-coatings for preservation of fresh fruits and vegetables. Coatings 13(8):1420. doi.org/10.3390/coatings13081420
45. Priyadarshi R, El-Araby A, Rhim JW (2024) Chitosan-based sustainable packaging and coating technologies for strawberry preservation: A review. International Journal of Biological Macromolecules 134859. doi.org/10.1016/j.ijbiomac.2024.134859
46. Soppelsa S, Van Hemelrijck W, Bylemans D, Andreotti C (2023) Essential oils and chitosan applications to protect apples against postharvest diseases and to extend shelf life. Agronomy 13(3):822. doi.org/10.3390/agronomy13030822
47. Liao W, Li Z, Zhou W, Liu J, Zhang D, Zhang X (2023) Biopreservation potential of chitosan and its derivatives: Current trends and perspectives in agriculture. Molecules 28(5):1941. doi.org/10.3390/molecules28051941