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Investigation of cellulosic packets impregnated with silver nanoparticles for enhancing shelf-life of vegetables

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ABSTRACT

Losses due to postharvest spoilage are major factors in food industries. Microbial contamination from field, cold storage and at consumer's place is one of the main causes for food quality loss and shelf life reduction. This report demonstrates the usefulness of low cost and eco-friendly cellulosic packets impregnated with silver nanoparticles for storage of vegetables. *Aeromonas* sp. was isolated from rotten vegetables (tomatoes and cabbage) and was designated as CTM. Biochemical and microbiological tests together with 16S rDNA sequencing confirmed the isolate as *Aeromonas hydrophila.* Silver nanoparticles showed bactericidal effect on bacterial isolate CTM. Minimum Inhibitory Concentration (MIC) value of nanoparticles against CTM was 15.3 μg/ml. Packets impregnated with nanoparticles exhibited significant antimicrobial property. Periodic evaluation of stored vegetables in these packets demonstrated enhanced shelf life with no significant changes in nutritional values whereas vegetables stored in packets without nanoparticles impregnation demonstrated decreased in values. Moisture content was also maintained which makes the vegetables looks fresh. Thus, the developed food packets will be helpful in preventions of microbial growth at varied conditions and will enhanced the shelf life of vegetables.

1. Introduction

The appearance of food is one of the major determinants of its appeal to consumers and subsequently, sales of the product. Microbial contamination is one of the major causes that determine food quality loss and shelf-life reduction (Barth, Hankinson, Zhuang, & Breidt, 2009; Fung, 2006; Lund, Baird-Parker, & Gould, 2000; Stratev, Daskalov, & Vashin, 2015). Prevention of food spoilage microorganisms is commonly achieved by chemical preservatives (Rawat, 2015). However, the increasing demand for minimally processed, extended shelf-life foods and reports of preservatives having potential toxicity demand food manufactures to find alternatives means of preservation. Advancements in nanotechnologies promise to bring a range of benefits to the food chain, in terms of new processes, materials and applications for efficient food production, less use of agrochemicals; hygienic food processing; improved food tastes and textures; less use of chemical preservatives; improved absorption of nutrients and supplements and innovative packaging concepts (Duncan, 2011; Garrido, Vitas, & Garcia-Jalon, 2009; Tiede et al., 2008; Weir, Westerhoff, Fabricius, Hristovski, & von Goetz, 2012). A major area for current nanotechnology applications in the food sector is for food packaging. The new nanoparticle-polymer composites can offer a number of improvements in mechanical performance as well as certain functional properties, such as antimicrobial activity to protect the packaged foodstuffs, biosensor to detect microbial presence, gas barrier, vapour barrier and bio-compatible and environment friendly materials (Abreu et al., 2015; Jong-Whan, Hwan-Man, & Chang-Sik, 2013).

Focusing on the preservation and packaging methods of vegetables, there are many different ways of packaging and preservation of different vegetables. Preserving these vegetables by freezing keep them fresh but some time they may also contain those bacteria that preferably can grow in cold environments, making the vegetables harmful for health or may cause spoilage even in cold preservation (Barth et al., 2009). Bacterial pathogens that can survive in cold temperature include *Listeria monocytogenes, Yersinia enterocolitica, Aeromonas hydrophilia* or wild type spoilage bacteria *Psuedomanas fragi* or *Brochothrin thermosphacta* (Beuchat, 1996).

Among these, *A. hydrophila* is the most potent pathogen which

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has been associated to two dreadful human diseases: septicemia and gastroenteritis (Elhariry, 2011). About 34% of vegetables including leafy vegetables were found to be contaminated with *Aeromonas* species. The major cause of food deterioration includes growth of microorganisms which can be transmitted from field, water, mis-handling of vegetables, insects, parasites, rodents, etc. temperature change, moisture and dryness, air and light (Beuchat, 1996). There has been an ongoing effort to control the food borne pathogen in vegetables and in processing facilities. Research performed which aimed at developing new and improved methods to prevent the survival and growth of bacteria that spoiled the vegetables in transportations so that the shelf life of the vegetables is extended and have less adverse effect on human health. Several studies are being conducted that utilize various preservation techniques and most of them aim at achieving food safety without compromising the sensory and nutritional qualities of foods.

As silver nanoparticles is one of the common antimicrobial materials, it is widely used in preservation and packaging of fruits and vegetables. Interpretation of the role of silver nanoparticles in fighting bacterial infection is important for the progress in the field of antimicrobial compounds. Silver nanoparticles (AgNPs) work by interfering in bacterial metabolism, and thus inhibit synthesis of functional enzymes and proteins or deregulating cellular activities like DNA replication, cell division, etc (Singh, 2016). It also weakens the progress of biofilm formation on surface of food materials.

Silver Nanoparticles coated food packets have been designed and are available in the market. The cost of such packets is quite high and unaffordable by common citizens. In the present study, AgNPs impregnated into cellulosic paper was used for packaging for vegetables storage. The developed packets provide an effective solution by improving the shelf-life of the vegetables (cabbages and tomatoes) and controlling microbial growth during storage at room temperature (25 ± 2 °C).

2. Materials and methods

2.1. Materials

Silver nitrate (>99.9% pure) was purchased from Sigma Aldrich, 2,3,5-Triphenyl tetrazolium chloride (TTC), Safranin, bacterial growth media and all other reagents used in this study were procured from Himedia, India. All reagents provided were of analytical grade. Fresh vegetables (cabbage and tomato) procured locally from the local market at Haldia, West Bengal. Cellulosic paper was procured from a local vendor.

2.2. Vegetable collection and shelf life study

Cabbage and tomato procured locally from the market at Haldia, were thoroughly washed with running tap water (2–3 times) followed by autoclaved double distilled water. Surface water on vegetables was soaked with sterile tissue papers. These vegetables were incubated at room temperature aseptically. Daily monitoring was done to check the progress of spoilage and microbes presence. The parameters which were studied were freshness, odour, pigment and surface microbial growth.

2.3. Isolation of microbes from spoiled vegetables

The growth of microbes along with foul odors was the indication of vegetables spoilage. From the half rotten vegetables (tomatoes and cabbage), surface microbes from each vegetable were isolated. Bacterial isolations were carried out in nutrient agar medium by streaking the bacteria collected from the vegetables and incubating at room temperature overnight. After completion of incubation period, distinct colonies were isolated and cultured in enrichment broth medium.

2.4. Characterization of the isolated bacteria

The selected bacterial strains were characterized by optical microscope (Olympus), biochemical assays and molecular characterization using 16S rDNA sequencing.

Standard Gram's staining procedure was followed for differentiation of isolated bacteria according to their staining property. The morphology of the bacteria was observed under microscope and was recorded. The isolated bacterial strains were characterized by using various standard biochemical assays (Saha & Santra, 2014).

The PCR amplified product of 16S rDNA was sequenced at Chromous Biotech Pvt. Ltd., Bangalore using ABI Prism 377 DNA Sequencer (Perkin Elmer). The primers pairs 27F forward (5′AGAGTTTGATC(AC)TGGCTCAG) and 1100R reverse (5′GGGTTGCGCTCGTTG3′) was used in this study. The sequences amplified using primer pairs (forward or reverse) were aligned using Expasy server and phylogenetic analysis was performed.

2.5. Synthesis and characterization of silver nanoparticles

Synthesis of silver nanoparticles (AgNPs) was described in Goswami, Sahareen, Singh, and Kumar (2015). Briefly, 1.0 ml of tea decoction was added to 9 ml of freshly prepared 1 mM aqueous Silver nitrate ($AgNO_3$) with constant stirring with magnetic stirrer at 50 °C for 4 h in a Teflon container. The change in color (colorless to yellowish brown) shows the reduction of silver ions into nanoparticles. The synthesized AgNPs was characterized using UV–visible spectroscopy (Shimadzu UV spectrophotometer, PharmaSpac, UV-1700), Transmission Electron Microscopy (JEOL, JEM 2100 electron microscope) and Fourier transformed infrared spectroscopy (FTIR Shimadzu, IR Prestige 21).

2.6. In vitro antimicrobial efficacy of the silver nanoparticles

In vitro antimicrobial activity of the AgNPs was screened against the isolated bacteria. Bacterial strain, CTM stock cultures were maintained at 4 °C on nutrient agar medium. Active cultures were prepared by inoculating fresh nutrient broth medium with a loopful of cells from the stock cultures at 37 °C for overnight. To get desirable cell counts for bioassays, overnight grown bacterial cells were subculture in fresh nutrient broth at 37 °C.

Minimum inhibitory concentration of AgNPs against CTM was determined using the growth indicator Tetrazolium/formazan test (Triphenyl Tetrazolium Chloride)). In the presence of viable bacteria, TTC is reduced to purple formazan and thus the change from colourless to red colour indicates the viability of the bacterial cells. All bacterial strains were grown in 10 ml of LB with shaking at for overnight (37 °C). 25 μl of bacterial suspension was added to each Eppendorf tubes and then a different concentration of AgNPs (25 μl, 50 μl, 75 μl and 100 μl) was added into each eppendorf tube. The final volume of each eppendorf was adjusted to 1 ml by LB medium. All set of eppendorf tubes were incubated at 37 °C overnight. 25 μl of sterile TTC (5 mg/ml) was put in each eppendorf tube and then were incubated at 37 °C again. After overnight incubation, the minimum inhibitory concentration (MIC) of AgNPs against CTM was determined by observing the colour change in each eppendorf.