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Combined effect of bacteriocin with gold nanoparticles against food spoiling bacteria - an approach for food packaging material preparation

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Abstract

Here, we report the combined effect of bacteriocin like peptides with gold nanoparticles against food spoiling organism. The peptide of bacteriocin produced by *Lactobacillus plantarum* strain ATM11 was incorporated with gold naoparticles and alone. Similarly, nisin also incorporated with gold nanoparticles and alone. Then, both combined and individual effects were tested against food spoiling organisms and the results revealed the antibacterial activities were increased on combination of bacteriocin with gold nanoparticles and nisin with nanoparticles. Here, our findings reveal that out of four food spoiling organism of *Micrococcus luteus*, *Bacillus cereus* and *Staphylococcus aureus* were found to be most sensitive and interestingly *E. coli* also had sensitive in both combinations. Therefore, this study may be considered as a preliminary approach for combined effect of food preservative agent with nanoparticles against food spoiling organism, which may be used for the preparation of packaging materials to extend the shelf life of food in future.

Introduction

Nanotechnology offers higher hopes in food packaging by promising longer shelf life, safer packaging, better traceability of food products and healthier food (Suresh and Digvir, 2011). The term 'nanofood' describes food which has been cultivated, produced, processed or packaged using nanotechnology techniques or tools, or to which manufactured nanomaterials have been added (Joseph and Morrison, 2006). In general, about 200 companies all over the world are involved in an active research and development in the field of nanofood but at the same time, even though the progress is obvious, it is still more right to say that the potential of nanotechnologies in food industry is yet to be recognized (Popov *et al*., 2010).

Nanoparticles can act as antibacterial and antifungal agents, due to their ability to interact with microorganisms. Exerting their antibacterial properties, nanoparticles attach to the surface of the cell. This interaction causes structural changes and damage, markedly disturbing vital cell functions, such as permeability, causing pits and gaps, depressing the activity of respiratory chain enzymes and finally leading to cell death (Zawrah and Sherein, 2011). The demonstrated antibacterial activity of nanoparticles recommends its possible application in the food preservation field; otherwise it can be applied as a potent sanitizing agent for disinfecting and sterilizing food industry equipment and containers against the attack and contamination with foodborne pathogenic bacteria (Ahmed *et al*., 2010).

Over the recent decades, gold nanoparticles (NPs) have attracted significant interest as a novel platform for various applications such as nanobiotechnology and biomedicine because of convenient surface bioconjugation with molecular probes and remarkable plasmon-resonant optical properties (Burygin *et al*., 2009). There are few studies for combined effect on antibacterial effect of nisin-loaded chitoson/alginate nanoparticles as a novel antibacterial vehicle and result revealed the entrapment efficiency of nisin inside the nanoparticles was about 90-95% compared with free nisin (Maryam *et al*., 2010). Also, the antibacterial effects of the Bacillus amyloliquefaciens-produced bacteriocin subtilosin, both alone and in combination with curcumin, e-poly-L-lysine (poly-lysine), or zinc lactate, were examined against Listeria monocytogenes and result found more active with combination (Tahar *et al*., 2010). The antimicrobial effect of chitosan edible film incorporating garlic oil (GO) was compared with conventional food preservative potassium sorbate (PS) and bacteriocin nisin (N) at various concentrations against food pathogenic bacteria and result showed more efficacy on combination (Pranoto *et al*., 2005).

Conjugation of gold NPs with antibiotics

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and antibodies also has been used for selective photothermal killing of protozoa and bacteria (Zharov *et al.*, 2006; Williams *et al.*, 2006; Pissuwan *et al.*, 2007; Huang *et al.*, 2007). With respect to antibacterial activity, the study showed that gold NPs do not affect the bacterial growth or functional activity (Gu *et al.*, 2003); whereas conjugates of vancomycin to gold NPs decrease the number of growing bacterial cells (Zharov *et al.*, 2006). The synthesized stable gold NP's covered with vancomycin showed significant enhancement of antibacterial activity for this conjugate, in comparison with the activity of the free antibiotic (Williams *et al.*, 2006).

Therefore, in the present study we report the combined effect of bacteriocin produced by *Lactobacillus plantarum* ATM11 along with gold nanoparticles which was already synthesized, characterized by *Bacillus subtilis* (Thirumurugan *et al.*, 2012). The synthesized gold nanoparticles were incorporated with bacteriocin, alone and also combination study was conducted on nisin with gold nanoparticles and alone for comparison against food spoiling organism.

Materials and Methods

Bacterial growth and conditions

Bacteriocin producing *Lactobacillus plantarum* strain ATM11 was isolated from meat environment (slaughter house soil), routinely cultivated in MRS broth (HiMedia, Mumbai) at 30°C for 24 h. *Bacillus cereus* (MTCC 1272), *Escherchia coli* (MTCC 433), *Staphylococcus aureus* from GI disordered patients and *Micrococcus luteus* (MTCC 2987) were used as indicator microorganism and grown in nutrient agar medium at 37°C for 24 h.

Production of bacteriocin

The isolated strain was grown in MRS broth (Hi Media, India – pH 6.5) and maintained aerobically at 34°C for 24 h. After incubation, cells were removed from the growth medium by centrifugation (10,000 x g for 30 min, 4°C) and passed through 0.2 mm filter. The cell-free supernatant was adjusted to pH= 6.0 using 1N NaOH and used as crude bacteriocin.

Determination of bacteriocin activity

Bacteriocin activity was determined by the agar well-diffusion method using above mentioned organism as the indicator strain. The activity of cell-free supernatant was expressed in arbitrary units per ml (AU/ml). A unit activity of the bacteriocin was defined as arbitrary unit (AU); 1 AU is a unit area of inhibition zone per unit volume, in this case mm^2/ml (Sri and Tri, 2009). The bacteriocin activity was calculated using the following formula:

Bacteriocin activity (mm^2/ml) = Lz−Ls/V

Lz = clear zone area (mm^2), Ls = well area (mm^2)

V = volume of sample (ml)

Gold nanoparticles

Previously, we synthesized gold nanoparticles by Bacillus subtilis and evaluated increased antimicrobial activity against clinical isolates (Thirumurugan *et al.*, 2012). Gold nanoparticles thus obtained were used for the present study.

Combined antibacterial activity

The combined effects of antibacterial activity of nanoparticles along with bacteriocin and alone and nisin along with nanoparticles and alone were investigated by using an agar well diffusion method. The above mentioned bacteria were used as indicator organisms for testing the antibacterial activity. Sterilized Muller Hinton agar was poured in a Petri dish and then solidified for 1 h. Wells (diameter 6 mm) were made on each agar plate, and then the combined and alone (bacteriocin (20 µl), bacteriocin + nanoparticles (20 µl), nisin (Sigma aldrich) (20 µl), nisin + nanoparticles (20 µl) and nanoparticles as control (20 µl) solutions were dropped into the corresponding wells. The bacteriostatic activity showed a clear inhibition zone around the sample-loaded well after incubating at 37°C for 24 h. The inhibition zone, where no visible bacterial colonies formed, was measured by subtracting the well diameter from the total inhibition zone diameter (Pissuwan *et al.*, 2007).

Result and Discussions

This is one of the preliminary and first reports on combined effect of bacteriocin produced by *Lactobacillus plantarum* strain ATM11 along with gold nanoparticles synthesized by *Bacillus subtilis* (Thirumurugan *et al.*, 2012) and also nisin along with gold nanoparticles against food spoiling organisms such as *Bacillus cereus*, *Escherchia coli*, *Staphylococcus aureus* and *Micrococcus luteus* (Figure 1). Already some reports have been discussed on combined effect of both the peptides such as bacteriocin and nisin (Gu *et al.*, 2003; Pranoto *et al.*, 2005; Zharov *et al.*, 2006; Williams *et al.*, 2006; Pissuwan *et al.*, 2007; Huang *et al.*, 2007; Maryam *et al.*, 2010; Tahar *et al.*, 2010; A. Thirumurugan *et al.*, 2012) and its increased antimicrobial effect against target organism, also it includes nanoparticles combination (Lin Bi *et al.*, 2011).

According to Asharani *et al.*, (2011) the gold nanoparticles are non-toxic compared with other

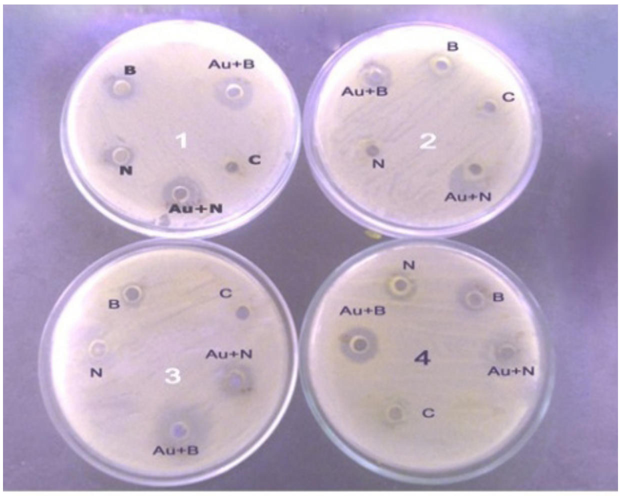
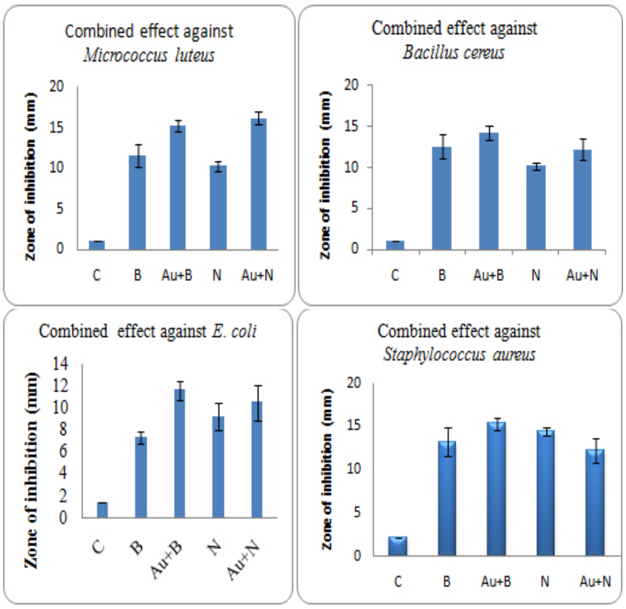


Figure 1. Shows the combined antimicrobial activity of peptides of bacteriocin and nisin with gold nanoparticles and alone against food spoiling microorganism. 1. *Micrococcus leuteus* 2. *Bacillus cereus* 3. *E.coli* 4. *Staphylococcus aureus*

metallic nanoparticles of silver and platinum. Also, the nanoparticles can act as antibacterial and antifungal agents due to their ability to interact with microorganisms. Exerting their antibacterial properties, nanoparticles attach to the surface of the cell. This interaction causes structural changes and damage, markedly disturbing vital cell functions, such as permeability, causing pits and gaps, depressing the activity of respiratory chain enzymes and finally leading to cell death (Gu *et al*., 2003).

When gold nanoparticles incorporated with drugs, the antimicrobial activity is increased because gold nanoparticles act as a good anchor carrying more amounts of drugs on the surface via electrostatic attraction between the amine groups of drugs and nanoparticles which gives a better activity (Zawrah and Sherein, 2011). Rodriguez *et al*. (2008) have already developed antifungal active paper packaging by incorporating cinnamon oil with solid wax paraffin using nanotechnology as an active coating and it was used as an effective packaging material for bakery products. Similar work has also been carried out with oregano oil and apple puree and created edible food films that are able to kill *E. coli* bacteria (Rojas-Grau *et al.*, 2006).

Previously, some of the authors studied with antimicrobial nanoparticles that have been synthesized and tested for applications in antimicrobial packaging and food storage boxes which include silver oxide nanoparticles, zinc oxide and magnesium oxide nanoparticles and also nisin particles produced from the fermentation of a bacterium (Suresh and Digvir, 2011). Similarly, here our produced bacteriocin by *Lactobacillus plantarum* ATM11 studied with gold nanoparticles. When



Graph.1-4. Shows comparison of antimicrobial activity of bacteriocin, nisin in presence and in absence of gold nanoparticles

bacteriocin was combined with gold nanoparticles, increased activity was observed but not much enhancement was observed with bacteriocin alone against food spoiling organism. Similarly when commercially available nisin (nisaplin) combined with gold nanoparticles an increased effect observed but not much enhancement was observed with nisin alone against food spoiling microorganism (Graph.1-4). Also the gold nanoparticles alone used as control, very minimal effect was observed because gold NP's they do not affect effectively bacterial growth or functional activity.

This study suggest, in future combination of gold nanoparticles with biopreservative agents may be further used for the preparation of antimicrobial packaging systems in order to extend the shelf life of food but there is need of further study on stability of biopreservative agent and nanoparticles.

Conclusion

We conclude that *Lactobacillus plantarum* strain ATM11 produced bacteriocin was studied; incorporating gold nanoparticles synthesized by *Bacillus subtilis* also nisin (nisaplin) along with gold nanoparticles was used for the comparison study. Here our findings reveal that the producer organism producing peptides (bacteriocin) have more antimicrobial activity against the above mentioned food spoiling organism when used as combination and alone than commercially available nisin with gold nanoparticles. We also conclude that there is a scope in future for combination of gold nanoparticles with food preservative agents to prepare the food packaging material to protect from food spoiling microorganism.

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