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Research Paper

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Synergistic Effect of Gold Nanoparticles and Bacteriocin Against Food Blemishing Microbes: A Novel Approach for Food Packaging Material Preparation

Ashok V. Gomashe Department of Microbiology, Shri Shivaji Science College, Congress Nagar, Nagpur-440012, MS, India. * Corresponding author

Preeti G. Dharmik Department of Microbiology, Shri Shivaji Science College, Congress Nagar, Nagpur-440012, MS, India

ABSTRACT *The present research has been made an attempt to investigate the bactericidal efficacy of gold nanoparticles (AuNPs) synergistically with the bacteriocin and commercially available peptide nisin on food blemishing microbes. The peptide of bacteriocin produced by Lactobacillus plantarum and Lactobacillus lactis incorporated with gold nanoparticles 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 as compared to nisin with nanoparticles. In addition to this Bacteriocin obtained from Lactobacillus plantarum showed most promising synergism as compared to Lactobacillus lactis peptide. 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.*

KEYWORDS : synergistic, Bacteriocin, Gold nanoparticles, food blemishing microbes

INTRODUCTION:

Unlike bulk materials, nanoparticles have been intensively studied over the last decade due to their characteristics: physical, chemical, electronic, electrical, mechanical, magnetic, thermal, dielectric, optical and biological properties (Schmid 1992; Daniel and Astruc 2004). Therefore, nanoparticles are considered as building blocks of the next generation of technology with applications in many industrial sectors. Of these, food industries utilised numerous nanoparticles for different purposes. 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 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).

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).

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).

The worldwide escalation of bacterial resistance to conventional medical practices is a serious threatens for human health. Microorganisms have been developing resistance to many antibiotics due to the indiscriminate use of antimicrobial drugs, increasing clinical problems in the treatment of infections (Hema *et al*., 2012). In view of the increasing incidences of infections with emerging multidrug resistance, there is very little choice left for the physicians to treat such infections (Kagithoju *et al*., 2012).

Therefore, there is an urgent need to develop new approaches to handle this problem. One of the promising approaches for overcoming bacterial resistance is the use of metallic nanoparticles (Chaloupka *et al*., 2010). Owing to their small size and higher surface-to-volume ratio, nanoparticles have an enlarged contact area with microorganisms.

This feature enhances biological and chemical activity of the nanoparticles with high antibacterial efficacy. Another important property of metallic nanoparticles is their ability to target different bacterial structures (Gordon *et al*., 2010). Among the various metallic nanoparticles, gold nanoparticles have wide range of applications in nano-scale devices and technologies due to its chemical inertness and resistance to surface oxidation (Sugunan *et al*., 2005). Gold nanoparticles also have potential activity against microbial pathogens and it mainly depends on the size and shape of the particles.

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 current investigation, we report the synergistic effect of bacteriocin produced by *Lactobacillus lactis and Lactobacillus plantarum* along with gold nanoparticles which was synthesized during the study period. The synthesized gold nanoparticles were incorporated with bacteriocin, alone and also combination study was conducted on nisin (commercially available in the market) with gold nanoparticles and alone for comparison against food blemishing organism.

MATERIALS AND METHODS:

a. Isolation of Lactobacillus species from the curd sample:

Bacteriocin producing *Lactobacillus lactis and Lactobacillus plantarum were* isolated from curd, routinely cultivated in MRS broth (HiMedia, Mumbai) at 30°C for 24 h. *Escherchia coli*, *Staphylococcus aureus, Bacillus cereus*, *Klebsiella pneumonia, Proteus mirabilis* were used as indicator microorganism and grown in nutrient agar medium at 37°C for 24 h.

b. Production of bacteriocin:

The isolated strains of *Lactobacillus* were grown in MRS broth (Hi Media, Mumbai) 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.

c. 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)

***d.* Preparation of aqueous dispersion of AuNPs (AuNPs)**
Gold nanoparticles were prepared by reducing HAuCl4.3H2O with trisodium citrate (Grace *et al*., 2007). In this method, HAuCl4.3H2O (0.5ml, 1mM) solution was heated to boiling. This solution, 0.5 ml of 0.01 M trisodium citrate was added. During this process, solution was mixed vigorously. After the addition of trisodium citrate, the previous yellow colored solution of HAuCl4.3H2O suddenly became transparent and colorless which is due to the formation of citric acid from trisodium citrate. Ultimately, the solution color changed to black and after then slowly to wine red indicates the formation of gold nanoparticles.

Mechanism of reaction could be expressed as follows:

2HAuCl4 + 3C6H8O7 (citric acid) →2Au + 3C5H6O5 (3-ketoglutaric acid) + 8HCl + 3CO2→

e. Synergistic activity:
The combined synergistic 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 Hi-sensitivity agar (Hi-Media, Mumbai) 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 (10 µl), bacteriocin + nanoparticles (10 µl), nisin (Sigma aldrich) (10 µl), nisin + nanoparticles (10 µl) and nanoparticles as control (10 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 DISCUSSION:
Appearance of wine red color colloids indicated the formation of gold nanoparticles (**Figure 1**). The formation of color in the reaction solution arises from excitation of surface plasmon vibration in the metal nanoparticles. Grace and Pandian (Grace et al., 2007) reported that the formation of wine red colloids is the characteristic of gold nanoparticles.

Fig. 1: Colloidal solution of gold nanoparticles

This is one of the first and preliminary reports on synergistic interaction of gold particles and bacteriocin produced by *Lactobacillus Species* and also nisin along with gold nanoparticles against food blemishing microbes such as *Escherchia coli*, *Staphylococcus aureus, Bacillus cereus*, *Klebsiella pneumonia, Proteus mirabilis*.

Some findings have been discussed on synergistic interaction of both the antimicrobial 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). Similar findings were also shown by Thirumurugan *et al*., 2012. They showed the combined effect of gold particles and bacteriocin.

According to Asharani *et al.*, (2011) the gold nanoparticles are non-toxic compared with other metallic nanoparticles of silver and platinum. Also, the nanoparticles can act as antibacterial and antifungal agents due to their ability to interact with microorganisms.

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).

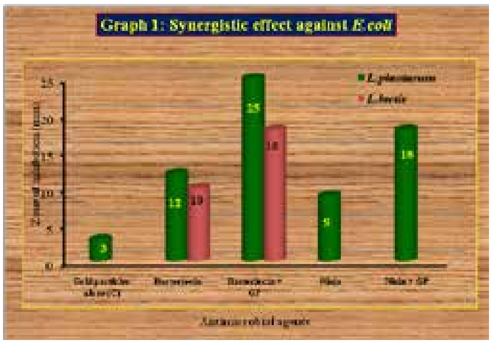
Rodriguez *et al*. (2008) have already developed antifungal active paper packaging by incorporating cinnamon oil with solid wax paraffin using nanotechnology as an active coating and it was used as an effective packaging material for bakery products. Similar work has also been carried out with oregano oil and apple puree and created edible food films that are able to kill *E. coli* bacteria (Rojas-Grau *et al.*, 2006).

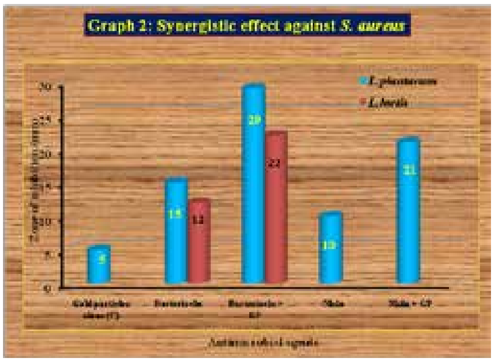
In the current study we have produced bacteriocin by *Lactobacillus plantarum* and Lactobacillus lactis studied with gold nanoparticles. When bacteriocin was combined with gold nanoparticles, increased activity was observed but not much enhancement was observed with bacteriocin alone against food spoiling organism.

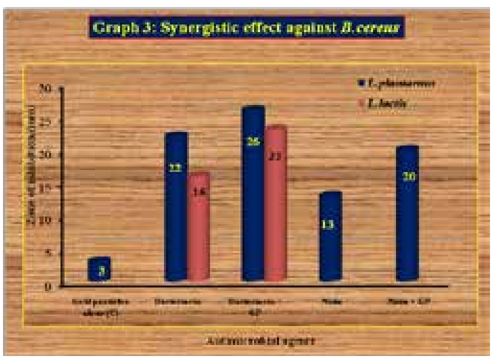
Similarly when commercially available nisin combined with our produced gold nanoparticles, an increased effect observed but not much enhancement was observed with nisin alone against food spoiling microorganism. Also the gold nanoparticles alone used as control, very minimal effect was observed because gold NP's they do not affect effectively bacterial growth or functional activity (Graph 1-5). Our findings correlate with the findings of Prema *et al.,* 2013 as well.

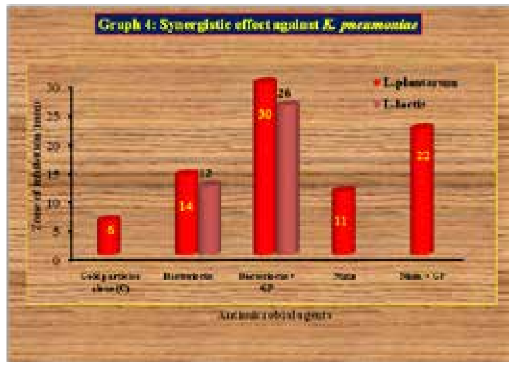
Current data thus show that gold nanoparticles together with antimicrobial peptide bacteriocin tested exhibit at least an additive effect. Genuine synergy was observed when gold nanoparticles were used together with bacteriocin as well as commercially available nisin. The combination of gold nanoparticles and Bacteriocin showed the most pronounced synergy than Gold nanoparticles and nisin. In addition to this Bacteriocin obtained from *Lactobacillus plantarum* showed most promising synergism as compared to *Lactobacillus lactis* peptide.

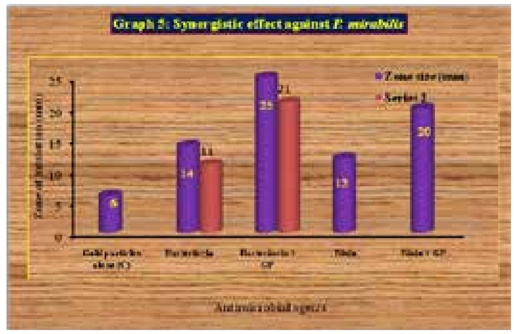
Here our results reveal that the peptides (bacteriocin) have more antimicrobial activity against the above mentioned food spoiling organism when used as combination and alone than commercially available nisin with gold nanoparticles. We also conclude that there is a scope in future for combination of gold nanoparticles with food preservative agents to prepare the food packaging material to protect from food blemishing microbes.











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