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Investigation into the antibacterial behavior of suspensions of magnesium oxide nanoparticles in combination with nisin and heat against *Escherichia coli* and *Staphylococcus aureus* in milk

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ABSTRACT

The antibacterial activities of magnesium oxide nanoparticles (MgO NP) alone or in combination with nisin against *Escherichia coli* and *Staphylococcus aureus* were investigated. Synergistic antibacterial effects existed and at lower levels of nisin when compared to when nisin was used alone. Also the antibacterial activities of MgO NP in combination with other antimicrobials (nisin and heat) against *E. coli* and *S. aureus* were investigated in milk. A synergistic effect of MgO NP in combination with nisin and heat was observed as well. Scanning electron microscopy was used to characterize the morphological changes of *E. coli* after antimicrobial treatments. It was revealed that MgO NP treatments in combination with nisin distort and damage the cell membrane, resulting in a leakage of intracellular contents and eventually the death of bacterial cells. This is the first report describing the antibacterial activity of MgO NPs and nisin in milk. It leads the way to development of treatment combinations which could result in a decrease in pasteurisation temperatures and the level of MgO NP required for pasteurising milk and maintaining pathogen control.

1. Introduction

Control of microorganisms in foods and beverages continues to be a worldwide problem (Krishnamoorthy, Moon, Hyun, Cho, & Kim, 2012). Outbreaks caused by foodborne pathogens continue to draw public attention to improve food safety. It is estimated that approximately 48 million cases of pathogenic diseases occur in the United States (Jin & He, 2011; Morris, 2011). Therefore, in order to solve this problem, it is necessary to develop effective antimicrobial agents to control the bacterial population (Kumar, Vemula, Ajayan, & John, 2008; Li, Leung, Yao, Song, & Newton, 2006). In recent years, the application of inorganic antimicrobial agents has attracted much attention for the control of pathogenic microorganisms (Okouchi, Murata, Sugita, Moriyoshi, & Maeda, 1995; Wilczynski, 2000). The specific advantages of inorganic antimicrobial compounds, as compared to their organic counterparts, are the improved safety and stability during high temperature treatments (Fu, Vary, & Lin, 2005; Hewitt, Bellara, Andreani, Nebe-von-Caron, & Mcfarlane, 2001; Makhluf et al., 2005; Wang et al., 1998).

Nanotechnology provides a method for develop new antibacterial agents to control multi-drug resistance bacteria (French, 2005). The toxicity of nanoparticles toward microorganism is due to either physical disruption or oxidative stress (Hu et al., 2010; Veerapandian & Yun, 2011). Nano structured materials have been used in textiles and in food industry to limit the growth of bacteria (Ugur, Sarnsik, Aktas, Ucar, & Erden, 2010).

Inorganic nano metal oxide such as ZnO, MgO and CaO have been investigated as antimicrobial agents (Roselli, Finamore, Garaguso, Britti, & Mengheri, 2003; Stoimenov, Klinger, Marchin, & Klabunde, 2002; Tang et al., 2012). MgO is an important inorganic oxide with typical wide band-gap (Al-Gaashani, Radiman, Al-Douri, Tabet, & Daud, 2012). It has been used in many applications such as catalysis, catalyst supports, toxic waste remediation, refractory materials and adsorbents, additive in heavy fuel oils, reflecting and anti-reflecting coatings, superconducting and ferroelectric thin films as the substrate, etc (Mirzaei & Davoodnia, 2012; Ouraipryvan, Sreethawong, & Chavadej, 2009).

MgO NPs have the advantage of non-toxicity, high thermal stability, biocompatible, low cost (Krishnamoorthy et al., 2012), and have considerable potential as an antibacterial agent (Jin & He,

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2011; Tang, & Lv, 2014). Mg plays several vital roles in human biology. Its deficiency has been implicated in regulation of blood pressure. Institute of Medicine of National Academy of Sciences has established Recommended Dietary Allowances which defines the average daily intake that is sufficient to meet the requirements is 420 mg (Mureinik & Guy, 2003). Mg is ingested as a dietary supplement in the form of MgO and MgOH. These are insoluble in water, but readily soluble in gastric juice, and the Mg ion becomes bioavailable upon dissolution in the stomach. MgO and MgOH serve as pH control agents in dairy products and in the manufacture of canned vegetables such as peas. They also serve as flow enhancer and anti-caking agents in dry breakfast cereal, salt, and powder concentrates such as soft-drink mixes (Krishnamoorthy et al., 2012).

In medicine, MgO is used for the relief of heartburn, upset stomach, and acid indigestion, as an antiacid, detoxifying agent, and for bone regeneration (Bertinetti et al., 2009; Boubeta et al., 2010; Krishnamoorthy et al., 2012). Recently, MgO nanoparticles have been applied in cancer therapy such as nano-cryosurgery and hyperthermia (Di, He, Sun, & Liu, 2012; Krishnamoorthy et al., 2012). Hence, the evaluation of antibacterial activity MgO NPs toward different bacterial strains is of potential interest.

Nisin is ribosomal synthesized peptides with antimicrobial activity. Also nisin is being presently widely used as a preservative in several food products such as processed cheese, dairy desserts, and canned foods. This antimicrobial protein exhibits inhibitory activity against Gram-positive bacteria including spore-forming bacteria and other spoilage and pathogenic bacteria (Schillinger, Chung, Keppler, & Holzapfel, 1998; Shin et al., 2015; Sobrino-Lopez & Martın-Belloso, 2008).

However, few studies have been reported on the use of nisin in combination with MgO NP.

The objective of this study was to investigate the antibacterial activities of MgO NP and its synergistic effect in combination with nisin and heat against foodborne pathogens (*Escherichia coli* and *Staphylococcus aureus*).

2. Materials and methods

2.1. Bacterial strains and culture conditions

The following bacterial strains were used in this study: *E. coli* PTCC1330, and *S. aureus* PTCC1112. These bacteria were obtained from the culture collection of the I.R. Department. Stock cultures were maintained at −80 °C. The strains were propagated on Tryptic Soy Agar (TSA; Merck, Darmstadt, Germany) at 37 °C and maintained at 0–2 °C before use. MgO nanoparticles with a diameter of 20–30 nm from US Nano Company with purity of 98–99% and nisin from Sigma Company USA were used.

2.2. Antibacterial activity of the combination of MgO NPs and nisin

The tryptic soy broth (TSB; Merck, Darmstadt, Germany) containing (0, 2, 4) mg/ml MgO NP, (0, 0.008, 0.01, 0.16, 0.2) mg/ml nisin and (2 + 0.008, 4 + 0.008, 2 + 0.01, 4 + 0.01, 2 + 0.16, 4 + 0.16, 2 + 0.2, 4 + 0.2) mg/ml MgO NP and nisin were prepared, respectively. Samples were then inoculated with the 10^6–10^7 CFU mL^{-1} of each strain. The samples were shaken at 50 rpm at 25 °C and examined at 3, 6, 8 and 24 h, respectively (Mirhosseini & Barzegari Firouzabadi, 2013; Peñuelas-Urquides et al., 2013; Premanathan, Karthikeyan, Jeyasubramanian, & Manivannan, 2011).

Aliquots (1 mL) of the treated TSB samples were dispersed in 9 mL of 0.2% (w/v) sterile peptone water and then serially diluted (10^{-1}–10^{-5}) in 0.1% sterile peptone water. Mannitol salt agar (MSA, Merck, Darmstadt, Germany) was used for isolation and enumeration of *S. aureus*. Eosin methylene blue (EMB, Merck, Darmstadt, Germany) agar was employed for isolation and identification of *E. coli*. Microscopic examination of isolates was performed by staining smears according to the Gram method. Identification and characterization of bacterial isolates up to species level was implemented using various biochemical and sugar fermentation tests (Brayner et al., 2006; Cowan, Steel, Barrow, & Feltham, 2003; Mirhosseini & Arjmand, 2014).

2.3. Antibacterial activity of the combination of MgO NPs and nisin in milks

2.3.1. Culture conditions

Each strain was cultured in TSB at 37 °C for 24 h, harvested by centrifugation at 4000 g for 20 min at 4 °C and washed three times with buffered peptone water. The final pellet was resuspended in buffered peptone water, corresponding to approximately 10^7–10^8 CFU mL^{-1} and mixed cocktails prepared by blending together equal volumes of each test strain.

2.3.2. Sample treatments

Unpasteurized milk cow bought from the supermarket in the city of Yazd, Iran. The milks containing (0, 2) mg/ml MgO NPs, (0, 0.008) mg/ml nisin and (2 + 0.008) mg/ml MgO NPs and nisin were prepared, respectively. The UV–visible spectra of MgO NPs suspended in milk were recorded with a spectrophotometer (Optizen 2120UV Plus. Mecasys, Daejeon, Korea) from 200 to 800 nm. As can be seen in the Fig. 1 absorption peak in the 246 nm area proves the existence of magnesium oxide nanoparticles (Nemade & Waghuley, 2014).

Samples were then inoculated with the prepared mixed culture cocktails (10^6–10^7 CFU mL^{-1} of each strain). The samples were shaken at 50 rpm at 25 °C and examined by colony count method at 3, 6, 8 and 24 h, respectively. Mannitol salt agar was used for isolation and enumeration of *S. aureus*. Eosin methylene blue agar was employed for isolation and identification of *E. coli*.

2.4. Antibacterial activity of the combination of MgO NPs, nisin and heat in milks

The milks containing (0, 0.5) mg/ml MgO NP, (0, 0.008) mg/ml nisin and (0.5 + 0.008) mg/ml MgO NP and nisin were prepared, respectively. Samples were then inoculated with the prepared

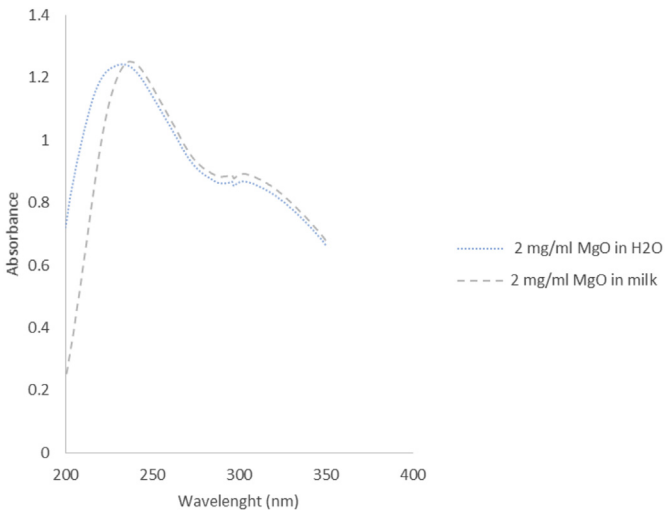

Fig. 1. UV–VIS of MgO nanoparticles.