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Antibacterial activity of selenium-enriched lactic acid bacteria against common food-borne pathogens in vitro

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ABSTRACT

Selenium (Se) is an essential trace element for human health and animal nutrition. The aim of this study was to evaluate the inhibitory activities of Se-enriched lactic acid bacteria (LAB), *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*, against pathogenic *Salmonella typhimurium*, *Escherichia coli*, *Staphylococcus aureus*, and *Listeria monocytogenes* in vitro. The results indicated that the accumulation amount of Se by *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* reached 12.05 ± 0.43 μg/mL and 11.56 ± 0.25 μg/mL, respectively, accompanied by the relative maximum living cells when sodium selenite was 80 μg/mL. Oxford cup double plate assay showed that bacterial culture solution and cell-free culture supernatant (CFCS) from Se-enriched LAB exerted stronger antibacterial activity than those from the non-Se strains. The growth of pathogenic bacterial culture with CFCS at any growth stages was worse than that without CFCS; moreover, the inhibiting effect of CFCS of Se-enriched LAB was more significant than that of non-Se strains. Results from a scanning electron microscope equipped with energy dispersion X-ray spectrometry showed that elemental Se nanoparticles, which characteristically energy peak around 1.42 keV, were deposited on the cell surfaces of *Lactobacillus delbrueckii* ssp. *bulgaricus*. In addition, CFCS of Se-enriched LAB induced more serious cell structure damage of pathogenic bacteria than did non-Se LAB.

Key words: selenium, *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Streptococcus thermophilus*, antibacterial activity

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INTRODUCTION

Diseases triggered by food-borne pathogen bacteria such as *Salmonella typhimurium*, *Escherichia coli*, *Staphylococcus aureus*, and *Listeria monocytogenes* are becoming serious public health problems in both developed and developing countries (Olsen et al., 2000; Tayel and El-Tras, 2010). Various approaches have been taken to address the issue, including the application of antibiotics, which is recognized as an effective way to prevent or treat pathogenic bacteria. However, resistance of pathogenic bacteria to current antibiotics is an increasing public health concern. The development of antibiotic-resistant super bacteria has resulted in currently used antibiotics failing to cure many bacterial infections, and overuse of antibiotics has caused precipitate kidney complications (Mølbak et al., 2002; Cock, 2007). Therefore, alternative strategies that could control food-borne pathogen bacteria contamination effectively and harmlessly have drawn extensive attention.

Selenium (Se), an essential trace element with a recommended dietary allowance for adults of 55 μg/d (tolerable upper level is 400 μg/d), plays a crucial role in human health (Institute of Medicine, 2000). Previous studies have demonstrated that Se possesses potent immunoregulative, fertility-promoting, anticarcinogenic, antiaging, antioxidant, detoxification, and antibacterial activity (Navarro-Alarcón and Lopez-Martinez, 2000; Li et al., 2002; Mater et al., 2005), which indicated its potential benefit as a dietary supplement in food.

Lactic acid bacteria (**LAB**) play a significant role in food fermentation, where they contribute not only to the development of the desired sensory properties in the final product but also to the inhibition of harmful microbe contamination (Smaoui et al., 2010). It is difficult to avoid food-borne pathogenic bacteria in food during the processing, preservation, and transportation of products (Amer et al., 2010; Kim et al., 2016). A large part of food-borne diseases are caused by ingestion of contaminated food products, including fermented dairy products such as yogurt (Tajik et al., 2007). Lee et al. (2014) reported on a serious food-borne illness that broke out after people ingested yogurts contaminated with mold; more than 200 individuals suffered from vomiting, nausea, and diarrhea. Esena and Owusu (2013) reported on an epidemic outbreak caused by food-borne *E. coli* O157:H7 in yogurt, fermented sausages, cheeses, and unpasteurized fruit juices. Considering that this problem occurs at a high frequency, much more attention should be paid to the prevention of food-borne pathogens in products.

The antibacterial effect of LAB is mainly related to the metabolites such as organic acid (mainly lactic, acetic, propionic, sorbic, and benzoic acids), hydrogen peroxide, diacetyl, ethanol, phenols, and proteinaceous compounds that are produced during the growth of the bacteria (Bajpai et al., 2016). Moreover, some strains of LAB are able to synthesize bacteriocins, which possess significant antibacterial activity (Dalie et al., 2010). Previous reports have shed light on the combination of LAB and Se that achieved a synergistic effect, exceeding the antibacterial effect of either Se or *Lactobacillus acidophilus* alone, both in vivo and in vitro (Yang et al., 2009). This may imply that other Se-enriched LAB might also have great potential as a novel antibacterial formula in fermented food. Plenty of studies have indicated that Se-enriched dairy products such as Se-enriched yogurt might be effective functional foods that meet the demands of Se utilization and resolve the problem of Se deficiency in some areas (Alzate et al., 2007, 2008, 2010; Palomo et al., 2014). It was reported that absorbable and high-nutritive organic Se could be easily generated by microbes such as *Lactobacillus brevis* (Deng et al., 2015), *Lactobacillus bulgaricus* (Xia et al., 2007), *Enterococcus faecium* (Mego et al., 2005a), *Enterococcus durans* (Pieniz et al., 2013), *Lactococcus lactis* ssp. *lactis* (Guo et al., 2013), *Lactobacillus rhamnosus*, and *Lactobacillus fermentum* (Andreoni et al., 2000b) via microbial biotransformation during the process of fermentation.

However, comprehensive study of the activity of Se-enriched LAB against food-borne pathogen bacteria is still lacking. Thus, it might be worthwhile to explore whether Se-enriched LAB have stronger inhibition against common gram-negative and gram-positive food-borne pathogenic bacteria. *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*, 2 common probiotic bacteria, have been most widely used in fermented dairy foods such as yogurt. Both can improve the flavor of dairy products as well as increase the nutritional value of fermented products, including antioxidant and antibacterial activity. In this study, the antibacterial activities of *Lb. delbrueckii* ssp. *bulgaricus* and *Strep. thermophilus*, which were isolated from local yogurt and further enriched with Se, against *E. coli*, *S. typhimurium*, *Staph. aureus*, and *L. monocytogenes* were evaluated to prove that Se-enriched *Lb. delbrueckii* ssp. *bulgaricus* and *Strep. thermophilus* could be used as healthy starter cultures with Se supplementation to prevent the growth of food-borne pathogenic bacteria in fermented foods and improve the safety and nutritional function of dairy products.

MATERIALS AND METHODS

Strains and Reagents

Lactobacillus delbrueckii ssp. *bulgaricus* (**XN-L**) and *Strep. thermophilus* (**XN-S**), which were previously isolated from local yogurt and identified by 16S rDNA analysis, were cultured in de Man, Rogosa and Sharpe (**MRS**) broth (Zhou et al., 2012). *Escherichia coli* ATCC 25922 (**XN-EC**), *S. typhimurium* LT2 ATCC 19585 (**XN-LT**), *Staph. aureus* ATCC 29213 (**XN-SA**), and *L. monocytogenes* ATCC 54004 (**XN-LM**) were cultured in Luria-Bertani (**LB**) broth (Sezonov et al., 2007). All strains (stored at −80°C) were obtained from the College of Food Science and Engineering in Northwest A&F University (Yangling, China). Sodium selenite (Na_2SeO_3) was purchased from Tianjin Zhiyuan Chemical Engineering Factory (Tianjin, China), and 3,3′-diaminobenzidine was purchased from Tokyo Chemical Industry (Tokyo, Japan). All chemicals were analytical grade.

Choice of Suitable Sodium Selenite Concentrations for 2 LAB

To select suitable selenite concentrations for XN-L and XN-S to grow, the viabilities of 2 cultures in different selenite concentrations were determined by counting colony-forming units per milliliter (Deng et al., 2015). Simple colonies of XN-L and XN-S were cultivated to the post-log phase in MRS broth at 37°C, and then 2% (vol/vol) of their cultures was transferred to 40 mL of MRS broth with 0, 20, 40, 60, 80, 100, 120, 140, or 160 μg/mL of sodium selenite in 100-mL Erlenmeyer flasks. When reaching post-log phase, 1 mL of aqueous solution of fermentation broth from each flask was sampled to prepare for the gradient dilutions, which then were plated on MRS agar and incubated at 37°C until the colonies appeared. The selenite concentrations in which XN-L and XN-S had the highest viabilities (relative maximum) were used in the next experiments.

Preparation of Se-Enriched LAB and the Determination of Se Content in Cell Pellets

The quantities of selenium accumulated by XN-L and XN-S were determined through the mixed acid diges-