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Activities of amylase, proteinase, and lipase enzymes from *Lactococcus chungangensis* and its application in dairy products

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ABSTRACT

Several enzymes are involved in the process of converting milk to lactic acid and coagulated milk to curd and, therefore, are important in dairy fermented products. Amylase, proteinase, and lipase are enzymes that play an important role in degrading milk into monomeric molecules such as oligosaccharides, amino acids, and fatty acids, which are the main molecules responsible for flavors in cheese. In the current study, we determined the amylase, proteinase, and lipase activities of *Lactococcus chungangensis* CAU 28^T, a bacterial strain of nondairy origin, and compared them with those of the reference strain, *Lactococcus lactis* ssp. *lactis* KCTC 3769^T, which is commonly used in the dairy industry. *Lactococcus chungangensis* CAU 28^T and *L. lactis* ssp. *lactis* KCTC 3769^T were both found to have amylase, proteinase, and lipase activities in broth culture, cream cheese, and yogurt. Notably, the proteinase and lipase activities of *L. chungangensis* CAU 28^T were higher than those of *L. lactis* ssp. *lactis* KCTC 3769^T, with proteinase activity of 10.50 U/mL in tryptic soy broth and 8.64 U/mL in cream cheese, and lipase activity of 100 U/mL of tryptic soy broth, and 100 U/mL of cream cheese. In contrast, the amylase activity was low, with 5.28 U/mL in tryptic soy broth and 8.86 U/mL in cream cheese. These enzyme activities in *L. chungangensis* CAU 28^T suggest that this strain has potential to be used for manufacturing dairy fermented products, even though the strain is of nondairy origin.

Key words: *Lactococcus chungangensis*, amylase, proteinase, lipase, dairy product

INTRODUCTION

Lactic acid bacteria (**LAB**) have been widely used in the dairy industry to produce cheese, yogurt, butter, and fermented milk. The LAB often play an important role in the starter cultures of commercial dairy products, and ensure consistency of the process and product quality. *Lactococcus* is a genus of LAB that serves as a starter for several types of cheese, such as hard cheese without eyes or with small eyes (Leroy and De Vuyst, 2004), Cheddar (Cretenet et al., 2011), Moroccan soft white cheese (Ouadghiri et al., 2005), Domiati cheese (El-Baradei et al., 2007), and cream cheese (Konkit et al., 2015).

Lactococcus lactis ssp. *lactis*, *Lactococcus lactis* ssp. *cremoris*, and *Lactococcus raffinolactis* are well-known strains used as starters in cheese and fermented milk (Leroy and De Vuyst, 2004; Ouadghiri et al., 2005). For many fermented dairy products, the most important properties and functions of LAB are (1) fermentation, including the depletion of milk sugar and the production of acids in milk; (2) reduction of the redox potential; (3) citrate fermentation; and (4) casein degradation (Olson, 1990). The primary role of LAB, however, is to utilize milk as a substrate and convert it into monomeric molecules for use as nutrients for their metabolism and growth (Vihinen and Mantsiila, 1989). These changes during the fermentation process, including the secretion of nutritional and chemical substances, are associated with bacterial enzymes (Gurr, 1987).

Microbial enzymes are more valuable in manufacturing than animal and plant enzymes because of their variety of catalytic activities and the high yields possible (Seitz, 1990; Kunji et al., 1995). Furthermore, their rapid growth on inexpensive media and the stability of their enzyme products make bacteria the preferred enzyme source in the food industry (McSweeney and Sousa, 2000). Among the technical enzymes, amylase, proteinases, and lipases are the principal enzymes used in food and animal feed production. Amylase catalyzes the hydrolysis of starch, resulting in products such as glucose, maltose, and maltotriose units (Gupta et al., 2003; Kandra, 2003; Rajagopalan and Krishnan, 2008). Amylase was the one of the first indigenous enzymes to be identified in milk, with α-amylase being the principal enzyme and β-amylase the lesser (Sato, 1920). Milk starch is broken down by amylase and converted into primarily dextrin and then into maltose, to a lesser extent (Guzmán-Maldonado et al., 1995).

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The proteolytic systems of LAB are essential for their growth in milk and contribute significantly to the development of flavor in fermented milk products (Kunji et al., 1995, 1996). Proteinase is the one of the enzymes that converts milk casein to free AA and peptides necessary for growth and acid production (Law and Haandrikman, 1997). Some *Lactococcus* strains serve as starters for dairy fermentation and have proteolytic activity. Proteolysis is the first biochemical step in the process that determines the flavor and texture of dairy products (Fox et al., 1996; McSweeney and Fox, 1997). Specifically, lactococci possess a proteolytic system that, together with other protein-hydrolyzing enzymes, is responsible for the conversion of casein into peptides and AA.

Lipolytic enzymes are involved in the breakdown and mobilization of lipids within the cells of an individual organism as well as the transfer of lipids from one organism to another (Beisson et al., 2000). Milk fat is essential for the development of a desirable flavor in fermented dairy products. Lipase is the enzyme that hydrolyzes triglycerides to fatty acids and glycerol, and mono- or diglycerides that are the key to flavor development (McSweeney and Sousa, 2000). *Bacillus* species have been found to possess amylase, proteinase, and lipase enzymes that can be used in the food and household industries (Hasan et al., 2006). Among those, *Bacillus subtilis* is a well-known enzyme-producing species that plays an important role in the production of natto by solid-state fermentation of soybeans (Hara and Ueda, 1982). Their proteinase enzymes have been used in the production of household and heavy detergents (Schallmey et al., 2004). Moreover, *Bacillus* species have been used as probiotics (Ziaei-Nejad et al., 2006).

Lactococcus chungangensis CAU 28^T is a strain of nondairy origin, which was isolated from activated sludge in our laboratory (Cho et al., 2008). Transcriptomic analysis showed that the strain possessed genes such as cystathionine β-lyase (*MetC*) and O-acetylserine sulfhydrylase (*CysK*), which are important factors in the processing of cheese (Konkit et al., 2014). Moreover, the strain has been found to play a role in functional activities, such as alcohol dehydrogenase and aldehyde dehydrogenase, which can moderate the level of alcohol and aldehyde in vivo (Konkit et al., 2015, 2016). The objective of this study was to evaluate the potential amylase, proteinase, and lipase activities of *L. chungangensis* CAU 28^T in broth culture, yogurt, and cream cheese. A comparison was made with another culture, *L. lactis* ssp. *lactis* KCTC 3769^T, which is routinely used in the dairy industry.

MATERIALS AND METHODS

Bacteria Strains

Lactococcus chungangensis CAU 28^T and *L. lactis* ssp. *lactis* KCTC 3769^T were cultured in tryptic soy broth (Becton, Dickinson and Co., Sparks, MD) at 30°C for 24 h. *Lactococcus lactis* ssp. *lactis* KCTC 3769^T was obtained from the Korean Collection for Type Cultures (KCTC; Taejon, Korea).

Broth Culture with Each Enzyme Substrate

One percent (wt/vol) each of starch, casein, and olive oil was added to basal medium, as described by Zhang et al. (1983), which contained (g/L) $(NH_4)_2SO_4$ 1.0, K_2HPO_4 6.0, KH_2PO_4 3.0, $MgSO_4 \cdot 7H_2O$ 0.01, $CaCl_2 \cdot 2H_2O$ 0.05, $MnSO_4 \cdot 2H_2O$ 0.01, $FeSO_4 \cdot 7H_2O$ 0.001, and $ZnSO_4 \cdot 7H_2O$ 0.001. An inoculum of each strain was added (1.0%, vol/vol) to each broth culture and then incubated at 30°C for 54 h.

Cream Cheese Making

According to a published method used for making cream cheese (Konkit et al., 2015), pasteurized milk (Pasteur Milk Co. Ltd., Seoul, Korea) was heated at 68°C for 30 min and cooled down, 5% (vol/vol) of a starter *Lactococcus* strain was added, and the mixture was incubated at 30°C for 48 h. During this period, the milk was acidified. It was then stirred and heated at 70°C for 5 min, and subsequently the whey was separated through a cloth bag. The curd was set and whey drained by adding 0.5% salt. Finally, each cream cheese sample was freeze-dried and stored in the dark at 4°C until further tests.

Yogurt Making

Pasteurized milk was heated to about 93°C and stirred gently to prevent it from boiling over and then cooled to around 44 to 46°C. The milk was stirred occasionally to prevent skin formation; then, a cup of warm milk was added to the inoculum strain (1% vol/vol) and allowed to set overnight.

Amylase Activity

Maltose (0.5 *M*) dilutions ranging from 0.3 to 0.5 μmol/mL, including a blank tube, were prepared. One milliliter of each dilution of maltose was pipetted into a series of corresponding numbered tubes, and 1 mL