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Cocoa Stimulates the Production Bioactive Components of Lactobacillus Casei and Competitively Excludes Foodborne Pathogens

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Abstract : Lactobacillus casei found in the human intestine and mouth is commonly applied for dairy production. Recently, it was found that some byproducts produced by Lactobacillus exhibited antimicrobial activities against multiple bacteria. Meanwhile, introduction of prebiotic-like foods (e.g. cocoa) or probiotics or both of them as food supplements in human diets as well as in farm animal feeds is believed to be an effective ways in control/reduce the colonization of foodborne bacterial pathogens infection in the gut environment. We hypothesized that cocoa may stimulate the production antimicrobial components of Lactobacillus casei and may potentially inhibit/reduce the colonization and infection of foodborne bacterial pathogens in the gut. Mixed culture of L. casei (LC) with enterohemorrhagic E. coli EDL933 (EHEC), Salmonella Typhimurium LT2 (ST), or Listeria monocytogenes LM2 (LM) showed that LC could competitively exclude (100%) them within 72 h. Further, investigation of cell-free culture supernatant (CFCS) revealed that the antimicrobial effects of LC came from CFCS. CFCS of LC eliminated (100%) EHEC, ST, and LM within 72 h, and 2 h CFCS treatment increased the hydrophobicity of EHEC (5.10 folds), ST (8.48 folds), and LM (2.03 folds). In addition, LC cells exhibited more inhibitive effects than CFCS on cell adhesive and invasive activities of EHEC (52.14% & 90.45%), ST (66.89% & 93.83%), and LM (61.10% & 83.40%). Two clusters of poly-peptides in CFCS were identified by SDS-PAGE, the molecular weights of which are ≈5 KD and 40-45 KD. LC CFCS with overnight growth in the presence of 3% strengthened all of the antimicrobial activities (growth inhibition, outer membrane disruption, and cell infective ability reduction). Liquid chromatography/Mass spectrometry analysis detected 5 unique components in class of flavonoids in LC CFCS with overnight 3% cocoa supplement. Furthermore, qPCR results showed that CFCSs up-regulated the expression level of genes responsible for flagellin synthesis and motility, but down-regulated genes for specific binding and invasion-associated proteins synthesis. The stimulatory effects of cocoa in producing bioactive components of probiotics may aid prevention of foodborne illness caused by major foodborne enteric bacterial pathogens.

Keywords : foodborne pathogens, probiotics, prebiotics, pathogen exclusion
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