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Isolation, Characterization and Bio-control Activities of *Bacillus subtilis* from in Fermented Soybean in Cambodia

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Fermentation is one of the popular techniques that has been used to process and store food. Fermented foods are commonly produced in Cambodia and in other South-East Asian countries since they play a very important role for nutrient supply and keeping health. Soybean has been the fourth main agricultural commodity which is actively generated yearly income for rural farmers in Cambodia. Soybean is used for producing fermented food (commonly called *SIENG* in Cambodian). *SIENG* is a similar food as Japanese *Natto*; the fermentation is occurred by naturally contaminated bacteria and the products sometime spoilage or cause food poisoning.

Some *Bacillus subtilis* strain isolated from fermented foods has been reported to produce strong antimicrobial compounds effective for Gram-positive pathogens or spoilage bacteria. So we tried to isolate such bacteriocin producing *B. subtilis* strains from *SIENG* to apply for starter cultures of Cambodian fermented soybean production.

One hundred and twenty *SIENG* sample were randomly purchased from 9 different local open markets in Phnom Penh, 1 local open market and 2 local producers in Kandal province in Cambodia. Twelve of 120 collected samples were contaminated with *Bacillus cereus*. Enterotoxin producing *B. cereus* strains was isolated from 2 of these 12 samples. This result suggested that *SIENG* has a risk to cause *B. cereus* food poisoning.

One hundred and nineteen *Bacillus subtilis* strains were isolated from same 120 collected samples. Fifteen of these isolated strains exhibited antimicrobial activity to *Lactobacillus plantarum* by agar well diffusion assay and one of them (*CeM6-7* strain) exhibited strongest antimicrobial activity. The filtered supernatant of this strain also suppressed the growth of several Gram-positive pathogens (such as *Staphylococcus aureus*) or lactic acid bacteria in broth. The filtrate could also suppress the growth of *L. plantarum* inoculated into soymilk.

Antimicrobial *B. subtilis* strain *CeM6-7* are thought to be applicable for making a starter culture to produce much safe fermented soybean foods by suppressing the growth of naturally contaminated *B. cereus* or *S. aureus* during production. In addition, the use of this starter culture may contribute to prevent spoilage or quality loss of soybean products caused by the over growth lactic acid bacteria or other Gram-positive bacteria.

Keywords: Fermented soybean, Bacteriocin, Food poisoning, Spoilage