

Distribution of Bacteriocin Producing *Bacillus subtilis* Strains effective for Controlling Pathogenic/Spoilage Gram-positive Bacteria in Asian Countries

Yasuhiro Inatsu^{*1}, Yukie Hosotani¹, Chiraporn Ananchaipattana¹, Md Mahfuzul Hoque² and Kong Thong³

1. Food Hygiene Laboratory, National Food Research Institute, NARO 2-1-12, Kannondai, Tsukuba, Ibaraki 305-8642, Japan

2. Department of Microbiology, University of Dhaka, Bangladesh,

3. Faculty of Agro-Industry, Royal University of Agriculture, Cambodia

*Corresponding author's e-mail: inatu@affrc.go.jp

Abstract: A variety of fermented soybean foods produced in Southeast and East Asia are used for common seasoning. Some of them are fermented without salt and sun-dried for long-term preservation and other are eaten in raw. Unlike Japanese fermented soybeans of the non-salted type (natto), which are fermented using a pure starter strain of *Bacillus* (*B.*) *subtilis*, naturally occurring microorganisms or seeds (a portion of the product) are used to produce fermented soybeans in other Asian countries. Some *B. subtilis* strains are known to produce bacitracin or other peptide antibiotic such as bacilysin, sublancin 168 and bacilysocin. *B. subtilis* also produce lysozyme-like-enzyme or phenolic derivatives (such as dipicolinic acid and amicoumacin) which are thought to have antibiotic or bacteriostatic activities. Comparing with the bacteriocins produced by lactic acid bacteria, the antibacterial compounds produced by *Bacillus* however, have not been studied from the perspective of the application for food hygiene. Authors collected fermented soybeans from 10 South-East to East Asian countries and isolated *Bacillus subtilis* strains that produced typical sticky material. One hundred and 34 fermented soybean foods were obtained from 27 cities in these countries. Of those, 118 samples consisted of non-salted types of food that included Japanese “Natto”, Thai or Laos “Tua Nao”, Cambodian “Sieng”, Myanmar or Chinese “Doucha” and Nepalese “Kinema”. About one tenth of total *B. subtilis* isolates exhibited bactericidal activities to broad range of gram-positive strains including *Listeria monocytogenes* and *Lactic acid bacteria*. These strains isolated from different sources exhibited different RAPD-PCR patterns. The active compound produced at late log to early stationary growth phase was heat tolerant and protease sensitive small peptide. Even they could also produce potent antibacterial compounds, dipicolic acid and lysozyme, their levels were similar to that of the type strain of *Bacillus subtilis* or commercial strains used for natto production in Japan, which have no antimicrobial activity. The strong antimicrobial compound from isolated *B. subtilis* strains will be able to use for bio-preservation of food such as lightly fermented vegetables.

Key words: Bacteriocin, *Bacillus subtilis*, pathogens, spoilage microorganisms, Asian countries.