

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

Identification of immuno-modulatory compounds from several type of rice brans and vegetables by metabolomics approach

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Plant metabolite has provided as an incomparable source for bioactive compounds. However, the wide range of the polarity of metabolites present in plants has been a big obstacle for its full use of the chemical diversity. Identification of active compounds needs tedious extraction and separation steps. To solve the problem, a new extraction method coupled to NMR- and fluorescence spectroscopy-based metabolomics was applied to identify bioactive natural products. A comprehensive extraction method consisting of a continuous flow of solvent mixtures through plant material was developed to provide extracts with a wider chemical variety than those yielded with a single solvent extraction, which is suitable for metabolomics-based work.

In the first part of our research, several type of rice brans and vegetables extracts were tested for histamine release inhibitory activity using RBL 2H3 cells line. Vegetable x showed the highest inhibitory activity. Comprehensive extraction in combination with NMR based metabolomics for the vegetable x showed that compound having chemical shift of 3.14 ppm are probably responsible for the activity. Based on ¹H NMR and 2D NMR data, this signal came from arginine. The OPLS data obtained from 3-D Fluorescence Spectra Measurement (Fluorescence Fingerprint) supported this conclusion. Our data was also supported by previous report that compound with strong histamine release inhibitor from vegetable x was a type of peptide/protein.

In the second part of our research, we screened our samples for several cytokines modulatory activity (IL-2, IL-4, IL-5, IL-10, IL-12p40 and IFN- γ). The screening results showed that Green Torbangun suppressed IFN- γ but it showed no effect in IL-2 secretion, thus we chose to undergo NMR based OPLS analysis for IFN- γ inhibitory activity of Green Torbangun fractions obtained from comprehensive extraction. The results indicated that triterpene acids and flavonoids were the active compounds. The most active signals were probably came from pomolic acid and methyl 3-epimaslinate which were reported to be present in Green Torbangun. However these compounds were not commercially available. We tested the other 4 available triterpenes (ursolic acid, oleanolic acid, maslinic acid and tormentic acid) and a flavonoid (eriodictyol) for IFN- γ and IL-2 inhibitory activities. The tested concentrations were 5, 10, and 20 μ M. Oleanolic acid and eriodictyol also showed IFN- γ inhibitory activity with eriodictyol had the stronger activity than oleanolic acid. IL-2 secretion and cells viability was not affected by the two compounds. On the other hand, ursolic acid inhibited secretion of not only IFN- γ but also IL-2. The data of Fluorescence Fingerprint based OPLS analysis also supported this finding. However, the compounds with the strongest active markers remained unidentified. As previously mentioned, OPLS of NMR data predicted that the compounds probably pomolic acid or methyl 3-maslinate but this prediction needs to be confirmed by isolating them from Green Torbangun and testing their IFN- γ inhibitory activity. In this research, comprehensive extraction coupled to NMR- and Fluorescence Fingerprint -based metabolomics were shown to be an effective tool to screen biological active compounds from natural sources.

Lipid-lowering ability of *Lactobacillus plantarum* IBT16 isolated from Vietnamese fermented shrimp in diet-induced obese mice

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Lactic acid bacteria (LAB) is widely distributed in nature. These bacteria have been used in traditional fermented food long time. Accumulated recent evidence indicates that, LAB, representing a major part of the microbial flora in the human gastrointestinal tract, participate to nutrient metabolism, generate essential amino acids, synthesize vitamins, and inhibit pathogenic bacteria to help maintain the balance of the host's health. In our research, by using chemical and molecular biology methods, we isolated and identified successfully three *Lactobacillus plantarum* strains comprising L.p-IBT5, L.p-IBT16, and L.p-IBT27 from Vietnamese traditional fermented products i.e. shrimp paste, sour shrimp and duachua (pickles) respectively. Analysis of 16S rDNA gene sequence indicated that the L.p-IBT5 and L.p-IBT16 showed 99% homology with *L. plantarum* ST-III, *L. plantarum* K-46, respectively while the L.p-IBT27 showed 100% homology to *L. plantarum* P8. Cholesterol assimilation of these bacteria was examined. Results showed that all of them could assimilate cholesterol from cultured media almost on the same level. Although the effect of bile salt on viability of these strains was unremarkable but the L.p-IBT16 had a highest tolerance to low pH. The L.p-IBT16 was therefore selected to feed diet-induced obese C57BL/6 mice with a daily dosage of 10^7 CFU. After 9 weeks, L.p-IBT16 have been found in the caecum of obese mice. Furthermore, some bacteria species which were reduced in obese mice have been recovered with the presence of L.p-IBT16. Although the L.p-IBT16 did not reduce serum cholesterol level significantly but this strain of bacteria decreased significantly serum triglyceride level in obese mice. The collecting results suggest that the L.p-IBT16 hold potential features as probiotics for obese people.

Development of Taxon-Specific Sequence(s) of Eggplant(s) for LAMP Detection Method

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Regulation for labeling of food that includes genetically modified organism (GMO) has been developed and came into effect by a number of countries to enforce consumer's right to know. Many PCR based methods have already been introduced for routine detection or are currently under investigation for their performance in nucleic acid detection. However, for the development of DNA-based detection methods, taxon specific endogenous gene is absolutely imperative for quantification of GMOs as a reference gene and for qualitative detection as a positive control. In addition, to obtain reliable results, the detection must be compared with the corresponding taxon specific reference gene. In this study, the development of taxon specific eggplant β -fructosidase gene primers for Loop Mediated Isothermal Amplification (LAMP), were described. Intra specific homogeneity and inter specific variability of β -fructosidase gene of eggplant with potato and tomato obtained from public database were also analyzed, the limit of detection has obtained as 50 copies of the genome sequence. The results depicted the high specificity and sensitivity of the primers. Therefore, these primers of β -fructosidase gene of eggplant can be used as a taxon specific endogenous reference gene for GMO analysis.

Key words: Genetically Modified Organism (GMO), Loop Mediated Isothermal Amplification (LAMP), β -fructosidase gene

Preparation and characterization of esterified xylo-oligosaccharides-stabilized oil-in-water emulsions using microchannel emulsification

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Amphiphilically esterified oligosaccharides are value-added and functionality-added products of hydrolyzed agricultural residues of annual plants such as wheat straw, grain hulls, corn cobs and corn stalks, thereby increasing the value and potential utilization of agricultural waste in the food industry. Esterified oligosaccharides have hydrophilic and hydrophobic subregions, therefore they can act like low-molecular-weight surfactants and they may present good ability for oil emulsification probably due to steric stabilization.

Esterified oligosaccharides were prepared by an enzyme-catalyzed reaction of maltodextrin/xylo-oligosaccharide and palmitic acid. Maltodextrin with dextrose equivalent (DE) of 16 palmitate (DE16_P) and 9 palmitate (DE9_P), as well as xylo-oligosaccharide palmitate (Xylo_P), were used as sole emulsifier to stabilize O/W emulsion prepared by high-speed homogenizer. The effect of the concentration (10-50% (w/w)) and type of esterified oligosaccharides on the Sauter mean diameter and droplet-size distribution, the rate of coalescence (K_c), and the creaming properties of O/W emulsions were investigated. Esterified oligosaccharides adsorbed to the surface of the oil droplets. Esterified oligosaccharides formed polydisperse O/W emulsions with particle sizes between 12 and 70 μm , depended on concentration of esterified oligosaccharides. The Sauter mean diameter, K_c , and the creaming index decreased markedly, with increasing concentration of esterified oligosaccharides. The type of ester minimally affected the Sauter mean diameter at each ester concentration. DE9_P inhibited coalescence and creaming more efficiently than other esterified oligosaccharides, mainly due to the higher viscosity of the continuous phase.

A series of an amphiphilically esterified xylo-oligosaccharides (xylo esters) with three fatty acids– decanoic acid (C-10), lauric acid (C-12) and palmitic acid (C-16) – were used as emulsifier to generate O/W emulsions by microchannel emulsification (MCE). Grooved and straight-through MCE was used to study the droplet generation and stability of emulsions, respectively. Xylo ester-stabilized oil droplets were generated smoothly from MCE, but xylo esters stabilized emulsions were less monodispersed due to low surface activity. The combination of xylo esters (2.5% (w/w)) and Tween series (0.1% (w/w)) can improve the monodispersity of oil droplet due to increased viscosity and/or sufficient stabilization layer at O/W interface. Combination of xylo laurate (2.5% (w/w)) and Tween 20 (0.1% (w/w)) inhibited coalescence and oiling off more efficiently than only Tween 20 stabilized emulsions during evaluated storage period. Xylo esters improved the efficiency of monodisperse emulsion generation by both grooved and straight-through MCE and also enhanced emulsion stability.

This study suggested that esterified-oligosaccharides may be attractive alternative ingredients to control over the stability of emulsions for various applications in food products.