

## Hyper production of Acid protease by food grade fungi using food by-product

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Screening of food grade and industrial *Aspergillus oryzae* strains was performed and the potential strain *A. oryzae* RIB 40 (ATCC 42149) yielding 20.5 U/ml of acid protease on enrichment media was identified. However, productivity of the parent strain was improved by UV mutagenesis with a lethality of  $1.8 \times 10^{-4}$ . The mutant strain (F6) produced 115 U/ml, which is 5.6 times higher than the parent. Solid state fermentation of potato pulp powder and optimization of biovariables, namely moisture (50 %), temperature (30 °C) and fermentation time (120 h) for efficient protease production, accomplished using a central composite rotatable experimental design, led to an 11-fold increase in protease activity (31 U/g dry substrate). The protease from *A. oryzae* also released glycine ( $0.57 \pm 0.25$  U/g) and 40.65 nkat/g carboxy peptidases activity which can enhance food palatability. Further basic studies considering that *A. oryzae* is able to respond to light and as conserved light related genes in genome sequence, light regulation with respect to morphology, growth, sporulation and protease production of *A. oryzae* strains were explored. The colonies resulted with circular stripe pattern containing white and green rings illustrating poor and rich spores besides mycelia formation when exposed to white light than in the dark in few and vice versa. Thus, the results indicate that *A. oryzae* perceives and responds to light as a trigger of conidiation and influence protease production. Hence light as one of the variables for enzyme optimization needs to be considered. Also, molecular mechanisms of photoreaction of this fungus would provide physiological significance for basic and also for biotechnological processes

Key words: *A. oryzae*, Acid protease, process optimization, bio- variables, Light

## Genetic approach for bacteriophage resistance of *Bacillus subtilis* (natto)

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*Bacillus subtilis* strains are the essential bacteria responsible for soybean fermentation. Bacteriophage contamination is a problem, which may occur during the fermentation and spoils the product. At present the *yueB* is the only gene reported as a bacteriophage receptor gene in *B. subtilis* and responsible for irreversible binding of bacteriophage SPP1 to cell surface. It is very interesting to study relationship between bacteriophage sensitivity and variation of *yueB* in *B. subtilis* species. With this preliminary study we obtained *yueB* gene from total 42 strains of *B. subtilis* including Japanese natto fermenting strain and strains isolated from Thua-nao of Thailand by PCR. The nucleotide and translated amino acid sequence of *yueB* gene were analyzed to examine phylogenetic relationship with each other. Deduced amino acid sequences of YueB revealed large diversity in the middle part of it which was supposed to be topologically exposed to cell surface. Moreover, we constructed the knock out mutant of *yueB* by insertion of erythromycin resistance cassettes and determine phage sensitivity in the absence of *yueB*. The phage sensitivity assay confirmed that the *yueB* gene knockout mutant of the laboratory strain *B. subtilis* 168 is resistant to SPP1 phage, but the mutant of natto strain *B. subtilis* NAFM5 was still sensitive to several phages including phages isolated from spoiled natto. These results suggest co-evolution of *yueB* and bacteriophages and *B. subtilis* might have bacteriophage receptor gene(s) other than *yueB*. The study on bacteriophage resistance in *B. subtilis* could be extremely useful and applicable in fermented food industry.

Keywords: *Bacillus subtilis*, *yueB* gene, natto, bacteriophage

## Identification and Control of Microbiological Hazards in Fermented Vegetables and Fermented Fishes Produced in Cambodia

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Foodborne outbreaks are common in Cambodia, but only a few investigating documents have been conducted due to the poor public awareness and the lack of scientific report. The objectives of this study were (i) to identify and evaluate the contamination rate of microbial hazards in fermented vegetable and fish products in Cambodia, (ii) to determine histamine level in fermented fishes as well as histamine producing bacteria, and (iii) to define targets for control histamine producing bacteria through starter culture (LAB). Total 128 samples including fermented vegetables (60 samples) and fermented fishes (68 samples) were purchased randomly from 5 wet markets in Cambodia from March 2014 to July 2014. In this study, *E. coli*, *Cronobacter sakazakii*, *shigella* spp., *Enterobacter* spp., other Opportunistic *Enterobacteriaceae*, Opportunistic *Non-Enterobacteriaceae*, *Vibrio* spp., *Enterococcus* spp., *Aerococcus* spp., *Staphylococcus* spp., *Listeria* spp. and *Bacillus cereus* were found in both fermented foods. The contamination rates in both categories of fermented foods were different, especially for *Enterococcus* spp. Coliform bacteria, *Enterococcus* spp., *Staphylococcus* spp. and *Bacillus cereus* in fermented fishes were significantly higher than in those fermented vegetables. Twelve samples (20%) of fermented fishes exceeded the Food Standards Code (FSC) maximum permitted level (200 ppm) for histamine. *Enterobacter aerogenes* was identified as potential histamine producing bacteria in fermented fishes. Initial pH of fermentation and fast-growing lactic acid bacteria as starter culture play an important role for control histamine producing *Enterobacter aerogenes* as well as other *Enterobacteriaceae*. In this case, the combination of initial pH (6.0) with *Lactobacillus pentosus* or pH (5.0) with *Lactococcus lactis* ssp. *lactis* are able to suppress the growth of *Enterobacter aerogenes* and minimize the content of histamine at below FSC.

The results in this works could be information of bacterial contaminants in Cambodian fermented foods that should be prevented and reduced in the future. The quality of the raw material and good hygiene practices in food facility should be required to improve food safety in Cambodia. The strategy of target suitable starter cultures under optimal initial pH of fermentation indicated positive result to reduce poisoning histamine in final fermented foods.

Keywords: Fermented vegetables, fermented fishes, hazardous bacteria, histamine, histamine producing bacteria, Lactic acid bacteria, initial pH of fermentation, biological control, Cambodia.

## Functional Components of Colored Rice and Selected Vegetables

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Plants are known rich sources of biologically active compounds that provide physiological benefits, including antioxidative and anti-inflammatory effects, and reduction of risks of degenerative conditions. In this work, the different bioactive constituents in some plant foods, particularly pigmented rice (*Oryza sativa* L.) and some commonly consumed vegetables in Japan, were investigated.

Unpolished forms of three varieties of Philippine *indica* rice differing in pericarp color and a nonpigmented *japonica* cultivar (Koshihikari) were evaluated for their antioxidant potential using *in vitro* and *in vivo* techniques. Black rice (var. Ittum) contained the highest level of monomeric anthocyanins, 27.0 mg/100 g fresh weight (FW), but red rice (var. Saluyaw) had the highest antioxidant capacity among the samples, as measured by hydrophilic-oxygen radical absorbance capacity (H-ORAC) assay, 7,284  $\mu\text{mol}$  Trolox equivalent/100 g FW. This indicated the presence of other antioxidants in red rice. Feeding of Balb/c mice with diets containing 65.95% rice for 30 d did not induce changes in the antioxidant status in serum and liver, nor in the anti-inflammatory markers in adipose tissues.

In order to explore naturally occurring antioxidants in various foodstuffs, antioxidant capacities of spinach (*Spinacia oleracea*), komatsuna (*Brassica rapa*), and Japanese parsley or seri (*Oenanthe javanica*) were assessed. Seri exerted the strongest antioxidant capacity, 4,085  $\mu\text{mol}$  TE/100 g FW. Further study on the effect of location and seasonal variations in seri indicated that the mean total ORAC value of 22 seri samples cultivated in three regions (Kyushu, Kanto, Tohoku) was 3,287  $\mu\text{mol}$  TE/100 g FW, with 82% attributed to hydrophilic antioxidants. The major hydrophilic antioxidants in seri were chlorogenic acid (CGA) and quercetin derivatives, namely, isoquercitrin (quercetin-3-*O*-glucoside), rutin (quercetin-3-*O*-rutinoside), hyperoside (quercetin-3-*O*-galactoside), and quercetin-3-*O*-rhamnosyl-galactoside. CGA was the primary contributor to antioxidant capacity of seri. Seri samples cultivated in Kanto area contained higher levels of CGA, corresponding to higher H-ORAC values. Lipophilic ORAC values tended to increase in December to January, but this did not affect the total antioxidant capacity of seri. These results provided information on seri bioactive compounds and the conditions favorable for cultivation of seri possessing higher antioxidant capacity.

The potential effect of unpolished rice cultivars in modulating the intestinal bacterial population in mice was examined. Bacterial community in cecal contents analyzed by Polymerase Chain Reaction (PCR) amplification of DNA followed by Denaturing Gradient Gel Electrophoresis (DGGE) showed distinct DGGE profiles between the control (AIN-93G diet) and rice-fed groups. Banding patterns of samples from rice groups were similar, although intensities of the bands varied. Cluster analysis showed two separate major clusters for AIN and rice-fed groups. Results of quantitative PCR and DGGE showing various microbial populations further confirmed differences in microbial diversity between AIN and rice-fed groups. This work showed that diets containing rice resulted in shifts in the composition of intestinal microbiota of Balb/c mice.

## Screening and identification of enzyme inhibitors in local foods and analysis of lipase inhibition mode by anti-hyperlipidemic polyphenols

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### Background

Elevated blood levels of TG and Cholesterol are two very important factors for hyperlipidemia symptoms. Blocking cholesterol biosynthesis and TG hydrolysis are two most effective mechanisms to cope hyperlipidemia.

### Rationale

A number of plant polyphenols showed potential lipase and HMG CoA reductase inhibitory activity. Therefore, it is important to evaluate local dietary for their anti-hyperlipidemic activity.

### Objectives

Searching inhibitors from local dietary sources was the central focus of the present study by conducting: (1) Screening of selected local agricultural produces for lipase and HMG CoA reductase inhibitors; (2) Extraction and quantification of selected inhibitors by HPLC; (3) Screening standard flavonoid and non-flavonoid phenolics for lipase inhibition potency; and (4) Diagnosis of lipase inhibition modality of the resultant high potency inhibitors to evaluation the enzyme structure-activity relationship.

### Methodology

Selected foods were first extracted with different solvent systems followed by measuring their IC<sub>50</sub> value as an index of their inhibitor potency. Standard phenolics were screened for lipase inhibition and high potency inhibitors were further analyzed for their enzyme inhibition kinetics.

### Results

Nine bean extracts showed insignificant HMG CoA reductase inhibitions while, out of 16 bean, cereals, and fruits, only few showed significant lipase inhibition potency. HPLC assay identified presence of free gallic (GA) and ferulic acids (FA) in cereals as well as anthocyanins in black rice. A number of standard flavonoid and non-flavonoid polyphenols when screened for their lipase inhibition potential, only GA and gallol moiety containing catechins, namely, Epigallocatechin and Epigallocatechin gallate were found significantly inhibiting pancreatic lipase (IC<sub>50</sub> are 387.2, 237.3, and 391.2  $\mu$ M respectively). Analysis of lipase inhibition modality by these polyphenols identified a mode of inhibition that were best fit to competitive inhibitions as revealed by visual inspection of Lineweaver-Burk and Dixon plots.

### Conclusions

Structural similarity and a common pattern of competitive inhibitions exerted by the studied polyphenols pointed to a role of their galloyl moiety in enzyme binding and inhibiting the substrates competitively.