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Antibacterial activity of protein hydrolysates from Thai rice grain

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Abstract:

Rice is a well-known cereal grain in most parts of the world. The world's human population consumes rice as a major food staple, especially in Asia. Rice has considerably benefit as shown in previous works. This research was designed to study bioactive peptides in Thai rice grain, including Khao Dawk Mali 105, Niaw Kiaw-Ngoo, Khao Tah Haeng 17, Sangyod Phatthalung and Hawm Surin. Rice were cooked, digested with pepsin and tested for their antibacterial property. The results showed that protein hydrolysate from Sangyod Phatthalung was able to inhibit the growth of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*.

Introduction

Rice is a staple food for more than half of the world's population, mainly those living in Asia. For Thai people, rice is a history of living, rice-farmer is a main occupation. There are plenty of rice cultivars planted throughout Thailand. Each cultivar has individual quality in morphology such as grain color, shape and nutrition.

In the past decade, many researches have focused on rice as a natural source for human health such as high vitamins, antioxidant property and antibacterial activity. Kondo and coworkers (2011) showed antimicrobial activity of bran extract on bacteria causing diarrheal disease by disk diffusion and broth dilution methods[1]. The rice bran extracts showed the most effective antibacterial activity against *Vibrio cholera*. Strong inhibitory activities on *Escherichia coli* and *Pseudomonas aeruginosa* were observed after exposure to rice bran oil[2]. Pumirat and Luplertlop reported that colored-rice crude extracts had antibacterial effects against *Staphylococcus aureus* associated with skin and soft-tissue infection[3].

Antimicrobial peptides (AMPs) are oligopeptides that interact with microbes by peptide penetration of microbial lipid membranes[4]. AMPs are commonly found in prokaryote and eukaryote. There are many classes of targets including viruses, bacteria, fungi, and parasites[5].

The digestive system of human consists of gastrointestinal tract such as esophagus, stomach, small and large intestine. Foods take about six to eight hours to pass through stomach and intestine. While opportunistic pathogens cause gastrointestinal diseases such as *E. coli*, *S. aureus*, and *P. aeruginosa* usually live in digestive tract. Hence, this work was aimed to study the possible antibacterial activity of protein hydrolysates from rice against the opportunistic pathogens in gastrointestinal tract.

Methodology

Plant Materials

Five cultivars of Thai rice were collected from Chachoengsao Agricultural Extension. These were Khao Dawk Mali 105, Niaw Kiaw-Ngoo, Khao Tah Haeng 17, Sangyod Phatthalung and Hawm Surin as shown in Figure 1.

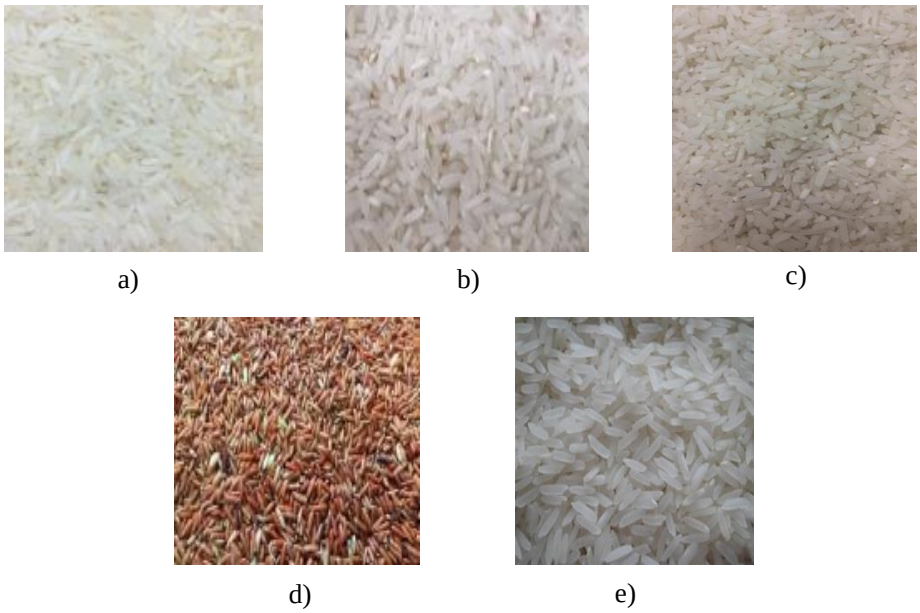

Figure 1.
Rice Sample; a) Khao Dawk Mali 105, b) Niaw Kiaw-Ngoo, c) Khao Tah Haeng 17, d) Sangyod Phatthalung and e) Hawm Surin

Preparation of protein hydrolysate

Total protein from 50 g of rice grain were extracted using 200 mM sodium acetate, pH 3.7, with heat at 121°C followed by hydrolysis with pepsin at 37 ºC for 16 h. The crude hydrolysate was filtrated with cheesecloth. The total protein concentration of the supernatant was measured by the method of Lowry using bovine serum albumin as standard.

Antibacterial activity determination

The antibacterial activity was determined against *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923) and *P. aeruginosa* (ATCC 27853) using broth dilution method in triplicate. While the starting OD at 600 nm of all bacterial was 0.05. The sterile water and tetracycline was used as negative control and positive control, respectively. The final concentration of protein hydrolysate and and tetracycline was 100 µg/ml.

Results and Discussion

Antibacterial activity of protein hydrolysate from rice samples were tested by broth dilution method, while sterile water was positive control and tetracycline was negative control. We found that peptides from Sangyod Phatthalung were able to inhibit the growth of *E. coli* and *S. aureus* within 25 h and inhibit growth of *P. aeruginosa* within 5 h after exposure, while other samples gave negative results as shown in Figure 2.

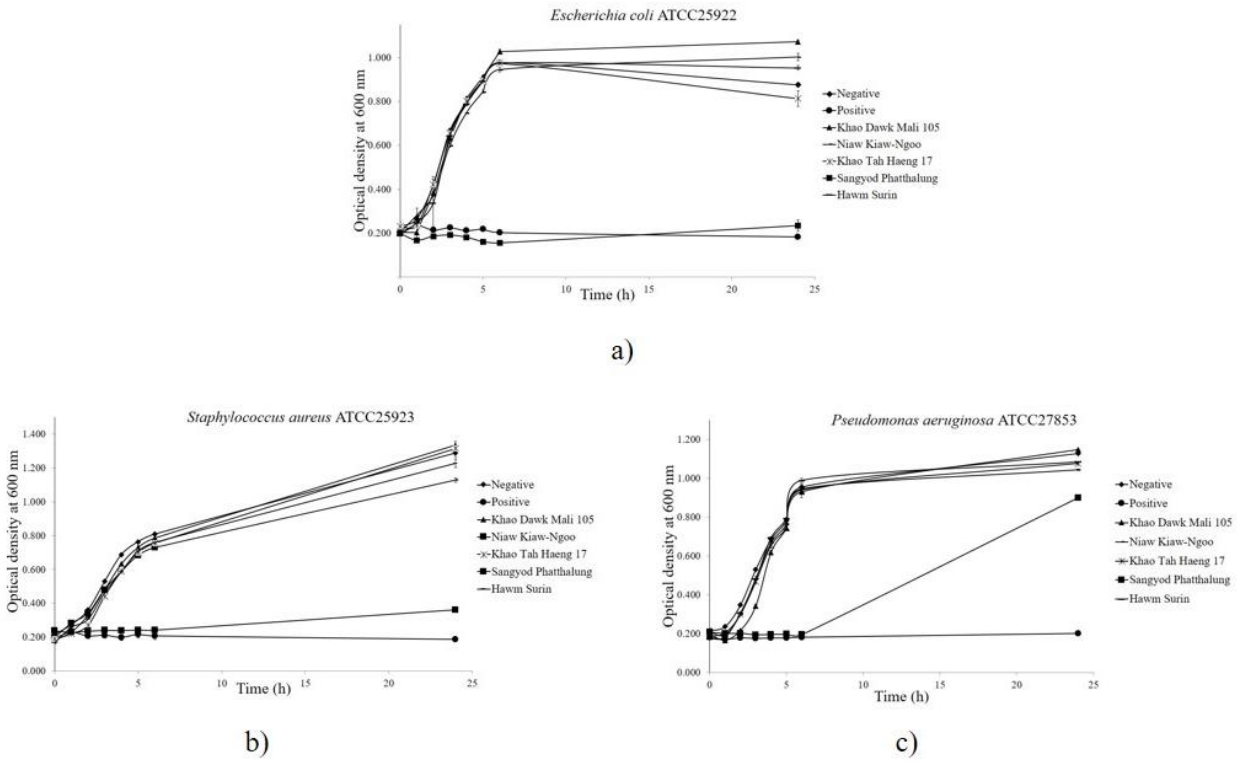

Figure 2. Antibacterial activity of protein hydrolysates from 5 Thai rice against bacteria a) *E. coli* ATCC25922 b) *S. aureus* ATCC25923 c) *P. aeruginosa* ATCC27853

In human stomach, high concentration of hydrochloric acid (HCl) and protein-digesting enzyme called pepsin carry out protein digestion. Pepsin works best in a strongly acidic environment (pH of gastric juice is about 2 and pepsin working pH range is 1-4). By breaking peptide bonds, it cleaves proteins into smaller polypeptides. Further digestion to individual amino acids occurs in the small intestine and the contents of the stomach typically pass into the small intestine within 2-6 h after meal. After digestion with pepsin, the protein hydrolysates from Sangyod Phatthalung were able to inhibit growth of all those three opportunistic pathogens, *E. coli*, *S. aureus* and *P. aeruginosa*. Diarrheal illness, urinary tract infection (UTI) and food poisoning, that usually occur in Thailand for long time, were caused by infection of these pathogens. Thus, the risk from infection of these pathogen will be decreased after eating Sangyod Phatthalung rice.

From Figure 1, Sangyod Phatthalung is a colored rice cultivar and its protein hydrolysate was able to inhibit growth of bacteria as shown in figure 2. These results were similar to Pumirat and Luplertop[3] reporting colored-rice crude extracts had antibacterial effects against *S. aureus*. In addition, anthocyanin in Sangyod Phatthalung may be a factor affecting growth of bacteria. *Bacillus cereus* was the most sensitive to anthocyanin extracted from HomLanna rice, HomNil rice, Black glutinous LeumPua rice and Riceberry followed by *E. coli*, *S. aureus*, *Salmonella enteritis*, *Enterobacter aerogenes* and *P. aeruginosa*, respectively[6]. It's probably that antibacterial property worked from protein hydrolysates related to anthocyanin production.

Conclusion

The protein hydrolysates from Sangyod Phatthalung were able to inhibit the growth of *E. coli, S. aureus* and *P. aeruginosa*. Daily intake of Sangyod Phatthalung rice might prevent bacterial pathogenesis.

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