

การทำงานวิจัยร่วมกับภาคเอกชนอย่างมืออาชีพ

วรรณพ วิเศษสงวน

**ศูนย์พันธุวิศวกรรมและเทคโนโลยีชีวภาพแห่งชาติ
สำนักงานพัฒนาวิทยาศาสตร์และเทคโนโลยีแห่งชาติ**



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องค์ความรู้ด้านเคมีอาหารกับการยกระดับอาหารหมักไทย



เน้นศึกษาเกี่ยวกับการเปลี่ยนแปลงองค์ประกอบทางเคมีและสมบัติเชิงหน้าที่ขององค์ประกอบหลักที่เป็นส่วนผสมในการหมัก เพื่อปรับปรุงคุณภาพและกระบวนการผลิตอาหารหมักไทย นำไปประยุกต์ใช้ในด้านต่าง ๆ ดังนี้

ตัวบ่งชี้คุณภาพและแนวทางการสูญเสียผลิตภัณฑ์
โดยศึกษาความสัมพันธ์ระหว่างปัจจัยในการผลิตกับคุณสมบัติทางเคมี-กายภาพ และคุณสมบัติทางประสาทสัมผัสของผลิตภัณฑ์ เพื่อใช้เป็นตัวบ่งชี้ชี้คุณภาพและการยอมรับของผู้บริโภค

เครื่องมือและเทคโนโลยีเพื่อติดตามจุลินทรีย์ต้นเชื้อในกระบวนการหมัก
โดยการสร้างและใช้จุลินทรีย์ต้นเชื้อที่มีโปรตีนเรืองแสงสีเข้ร่วมกับเทคนิคทางชีววิทยาโมเลกุลใหม่ๆ ในการติดตามการเปลี่ยนแปลงกลุ่มประชากรเชื้อจุลินทรีย์ในระบบการหมัก

แบบจำลองทางคณิตศาสตร์สำหรับการคาดการณ์อัตราการหมักหมมที่ใช้น้ำเชื่อมธัญพืช
โดยนำค่าการอบต่างๆ (parameter) ในกระบวนการหมักหมมมาสร้างเป็นแบบจำลองทางคณิตศาสตร์สำหรับการคาดการณ์อัตราการหมักหมมที่ใช้น้ำเชื่อมธัญพืช จากการเปรียบเทียบผลการคาดการณ์ของแบบจำลองกับผลการหมักจริง พบว่ามีความถูกต้องประมาณร้อยละ 75-85



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การผลิตและใช้ประโยชน์เซลลูลินทรีย์และเอนไซม์

กระบวนการหมักน้ำปลาโดยใช้เอนไซม์ พืชน้ำสีเขียวที่สกัดจากผักกาดหอม จากปกติ 18 เดือน ให้เหลือ 11 เดือน ที่น้ำปลาที่ได้มีปริมาณไนโตรเจนร้อยละ 27 มีปริมาณกรดอะมิโนอิสระที่ให้รสชาติมากกว่าน้ำปลาที่หมักด้วยวิธีปกติ

การผลิตและใช้ประโยชน์เซลลูลินทรีย์จากข้าว พัฒนาการหมักและใช้ประโยชน์จากข้าวเพื่อผลิตอาหารสัตว์ที่มีคุณค่าทางโภชนาการสูงสำหรับสัตว์ปีกและสัตว์เคี้ยวเอื้อง โดยนำเอาเอนไซม์ที่ผลิตจากข้าวไปใช้ร่วมกับวัตถุดิบอื่น ๆ เพื่อเพิ่มประสิทธิภาพการย่อยอาหารของสัตว์

ต้นเชื้อแบคทีเรียกรดแลคติกสำหรับหมักผักกาดดองเปรี้ยว ช่วยให้ได้ผักกาดดองที่มีคุณภาพที่ดีขึ้น มีความเหมาะสมในการหมัก การผลิต และการขนส่ง และลดระยะเวลาในการหมักจาก 12 วัน เหลือเพียง 8 วัน

เชื้อจุลินทรีย์จากปลาเพื่อเป็นอาหารหมักชีวภาพสำหรับสัตว์ สร้างเอนไซม์ที่มีความสำคัญต่อการย่อยวัตถุดิบอาหารสัตว์ สร้างสารต้านการเจริญเติบโตของจุลินทรีย์ก่อโรค สารต้านการดูดน้ำ/สารต้านการดูดน้ำ การเจริญเติบโตของสัตว์ได้ดีกว่าหรือเท่ากับจุลินทรีย์ที่เติมเข้าจากภายนอก

การพัฒนาเทคโนโลยีการผลิตเอนไซม์เสริมสำหรับสัตว์ พัฒนาการหมักและใช้ประโยชน์จากเอนไซม์เสริมสำหรับสัตว์ที่สกัดจากปลา ช่วยเพิ่มประสิทธิภาพการหมักและใช้ประโยชน์เป็นสารเสริมอาหารสัตว์และวัตถุดิบอาหารสัตว์ที่ผ่านการหมักทางชีวภาพ

น้ำส้มสายชูหมักจากผลไม้ในขั้นตอนเดียวและสูตรจุลินทรีย์สำหรับหมัก สามารถลดระยะเวลาการผลิตน้ำส้มหมักของหมักจากเดิม 1-2 ปี เหลือเพียง 2-4 เดือน กระบวนการหมักมีต้นทุนต่ำหมักได้โดยไม่ต้องทำให้ออกซิเจน ใช้กรดน้ำส้มปริมาณสูง น้ำส้มหมักที่ได้มีคุณภาพและมีความปลอดภัยต่อสุขภาพและมาตรฐาน



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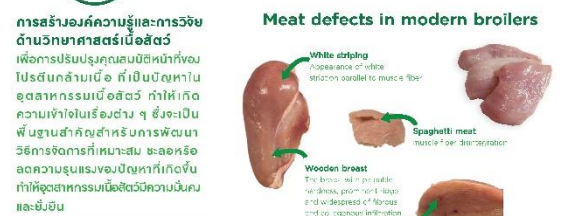
การใช้ประโยชน์จากไข่เนื้อสัตว์และวัสดุเหลือจากกระบวนการผลิตอาหาร

การใช้ประโยชน์จากไข่กระดูกพรุน เริ่มตั้งแต่การปรับปรุงคุณภาพการปรับปรุงกระบวนการผลิตไข่กระดูกพรุน (ไข่ขาว ไข่แดง และไข่รวม) พัฒนาการหมักและใช้ประโยชน์จากไข่และวัสดุเหลือจากกระบวนการผลิตอาหารเพื่อผลิตอาหารสัตว์ที่มีคุณค่าทางโภชนาการสูง

การใช้ประโยชน์จากไข่เนื้อสัตว์และวัสดุเหลือจากกระบวนการผลิตอาหาร เพื่อผลิตอาหารสัตว์ที่มีคุณค่าทางโภชนาการสูง

การพัฒนาเทคโนโลยีการผลิตเอนไซม์เสริมสำหรับสัตว์ พัฒนาการหมักและใช้ประโยชน์จากเอนไซม์เสริมสำหรับสัตว์ที่สกัดจากปลา ช่วยเพิ่มประสิทธิภาพการหมักและใช้ประโยชน์เป็นสารเสริมอาหารสัตว์และวัตถุดิบอาหารสัตว์ที่ผ่านการหมักทางชีวภาพ

การสร้างองค์ความรู้และการวิจัยด้านวิทยาศาสตร์เนื้อสัตว์ เพื่อการปรับปรุงคุณสมบัติของโปรตีนกล้ามเนื้อ ที่เป็นปัญหาในอุตสาหกรรมเนื้อสัตว์ ทำให้เกิดความเข้าใจในเนื้อสัตว์มากขึ้น ซึ่งจะเป็นพื้นฐานสำคัญสำหรับการพัฒนาวิธีการจัดการที่เหมาะสม ของหรือลดความรุนแรงของปัญหาที่เกิดขึ้น ทำให้อุตสาหกรรมเนื้อสัตว์มีความมั่นคงและยั่งยืน

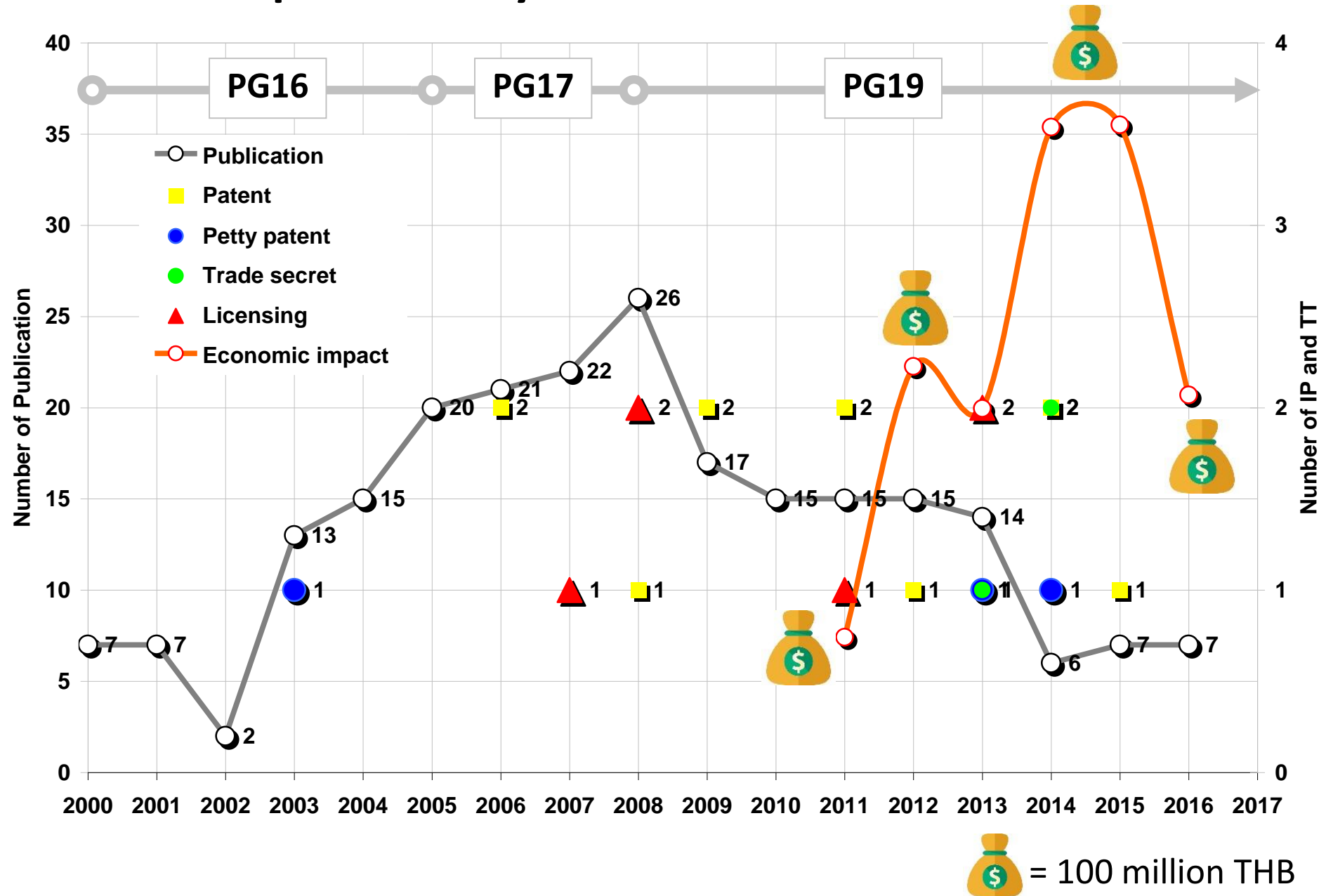


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มูลค่าผลกระทบบางทางเศรษฐกิจสะสม 1,800 ล้านบาท



Research productivity 2000-2017



ประเภทโครงการ		0340	ปี 2563
		โครงการ	โครงการ
วิจัย	การพัฒนากระบวนการเร่งหมักน้ำปลาโดยใช้เอนไซม์		67
	สูตรเชื้อจุลินทรีย์ในการผลิตต้นเชื้อเหวมและการใช้ต้นเชื้อจุลินทรีย์ ในการหมักเหวม		Ongoing
	การหมักผักกาดเขียวปลีด้วยต้นเชื้อ <i>L. plantarum</i>		.,50
	การศึกษาสมบัติทางกายภาพและสมบัติทางเคมีของไข่	014.	Ongoing
	การผลิตน้ำส้มสายชูหมักจากมันาคุด		Ongoing
	การพัฒนากระบวนการผลิตชีวมวลของเชื้อจุลินทรีย์โพรไบโอติก <i>Lactobacillus paracasei</i> SD1 ให้ได้ปริมาณสูง	.,7	Ongoing
วิจัยขั้น	เทคโนโลยีกระบวนการผลิตเอนไซม์เพนโตซานเนสจากเชื้อราสายพันธุ์ <i>Aspergillus</i> sp.BCC7178 เพื่อใช้ในอุตสาหกรรมอาหารสัตว์	03,21	370
	การอนุญาตให้สิทธิการใช้เชื้อแบคทีเรีย และการถ่ายทอดเทคโนโลยีการผลิตต้นเชื้ออาหารหมักสัตว์	/16,54	1,250
	สารทดแทนไขมันต่อการย่อยของอาหารสัตว์และการดูดซึมกลูโคส	201	Ongoing



Economic Impact



Social Impact



Environmental Impact

Recipe of success

- Technological capability and readiness
- Mindset & Responsibility
- Strategic thinking
- Trust
- Networking
- Timing
(*Speed ** quality and ***Ability to Deliver)
- Effective communication
- Teamwork



COVID-19



←-----→
MINIMUM 2M (6FT)

←-----→
MINIMUM 2M (6FT)



- ตอบสนองต่อการเปลี่ยนแปลง ที่ไม่แน่นอน ได้อย่างรวดเร็ว
- สร้างความเข้มแข็งจากภายใน บนรากฐานของทรัพยากรที่มี อย่าง

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Changes in composition and functional properties of proteins and their contributions to Nham characteristics

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Abstract

Changes in composition and functional properties of proteins during fermentation of Nham, a Thai-fermented sausage, were studied. An alkaline-soluble fraction constituted a major protein component of Nham. The amount of each protein fraction in Nham varied, depending on the fermentation time. As fermentation proceeded, the progressive decrease in sarcoplasmic and myofibrillar protein fractions was accompanied by an increase in the alkaline-soluble fraction and non-protein constituents ($P < 0.05$). Slow pH lowering to pH 4.6 during fermentation as a result of bacterial growth and accumulation of lactic acid affected the molecular conformation of the muscle proteins and resulted in changes in protein functional properties. The acid produced resulted in changes in solubility, water-binding capacity, textural properties, and color characteristics. Proteolysis of Nham proteins occurred during fermentation, resulting in increases in TCA-soluble peptides and free α -amino acids, which may contribute to the taste and aroma of Nham.

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Keywords: Fermented pork sausage; Acid-induced gelation; Functional property

1. Introduction

Fermentation is one of the oldest techniques in food preservation as it not only extends the shelf-life but also enhances the flavor and nutritional quality of the product. Nham is a Thai-style fermented pork sausage, which has gained popularity with an estimated production value of 20 million USD annually. Nham is normally made of minced pork, shredded cooked pork rind, 2–3% NaCl, cooked rice, garlic and 100–125 ppm of sodium nitrite, mixed well and wrapped tightly in banana leaves or plastic bags. Fermentation of Nham generally takes 3–5 days at room temperature (~30 °C) without further ripening. Nham usually has a pH of 4.4–4.8 with titratable acidity values of 0.77–1.60% (Phithakpol, Varanyanon, Reummaneeapatton, & Wood, 1995).

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Influence of crude xylanase from *Aspergillus niger* FAS128 on the *in vitro* digestibility and production performance of piglets

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Abstract

The influence of crude xylanase produced from *Aspergillus niger* FAS128 or FAS128 on the *in vitro* digestibility of pig diets and production performance of piglets was investigated in comparison with two commercial crude enzyme products, IE1 and IE2. The addition of FAS128 in all pig diets resulted in higher *in vitro* digestibility of dry matter (DM), crude fibre (CF), ether extract (EE), and ash than those supplemented with IE1 and IE2 ($P < 0.05$). In 6-week feeding trials, weaned piglets fed with diets supplemented with FAS128 had a significantly higher average daily gain (ADG) and lower feed conversion ratio (FCR) than those given diets with IE1 and IE2 ($P < 0.05$). The incidence of diarrhoea and concentration of blood urea nitrogen (BUN) of piglets at all growth period was also lowered. Based on the improved *in vitro* digestibility and production performance, crude xylanase produced from *A. niger* FAS128 has the potential for use as feed enzyme supplement.
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Keywords: Xylanase; *Aspergillus niger*; Digestibility; Production performance; Pig

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Degradation of histamine by extremely halophilic archaea isolated from high salt-fermented fishery products

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ABSTRACT

The presence of high level of histamine is detrimental to the quality and safety of fish sauce. Therefore, this study aimed to study the ability of extremely halophilic archaea to reduce histamine under high salt condition and to examine the enzyme activity potentially involved. Of 156 extremely halophilic archaea isolated from various salt-fermented fishery products, HD53-1 from an anchovy fish sauce sample fermented for 3 months, exhibited the highest histamine degradation activity when cultured in halophilic medium containing 5 mM histamine (free-base), followed by HD51-1, HRC1-2, and HD40-3, respectively. HD53-1 was classified as *Natrialba* gari based on 16S rRNA gene sequence similarities and did not exhibit decarboxylase activity toward all tested amino acids. Based on *in vitro* cytotoxicity assay, the treatment with whole cell extract of HD53-1 to all cell lines tested resulted in dose-dependent inhibitions of the cell growth with the IC_{50} values higher than 250 μ g/ml⁻¹. Histamine-degrading activity of HD53-1 was located in the intracellular fraction and required 1-methoxy-5-methylphenanzinium methylsulfate (PMS) as an electron carrier. The optimal pH, salt concentration, and temperature for histamine degradation were pH 6.5–8, 3.5–5 M NaCl, and 40–55 °C, respectively. The activity was fully retained at pH 6.5–9, in the presence of NaCl above 2.5 M, and at temperature lower than 50 °C. The results suggested that histamine-degrading activity of HD53-1 was most likely associated with salt-tolerant and thermo-neutrophilic histamine dehydrogenase.

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1. Introduction

The presence of high levels of histamine is detrimental to the quality and safety of foods, particularly fish sauce and other fermented fishery products from scombroid species. Histamine in foods is mainly induced by histamine decarboxylase activity from several kinds of bacteria. The presence of histamine in fermented foods does not usually represent any health hazard to individuals unless large amounts are ingested. Typical symptoms may be observed in certain individuals, and include nausea, sweating, headache, and hyper- or hypotension [1]. The Food and Drug Administration (FDA) established an advisory level of 500 ppm to be toxic to human health [2]. Histamine is heat stable and is not detectable through organoleptic analysis by even trained panelists. Except for the gamma irradiation, no other food processing

methods are available for histamine degradation [3]. Therefore histamine, if present, is difficult to destroy and poses a risk of food intoxication.

The presence of histamine-degrading enzymes either histamine oxidases or histamine dehydrogenases has been reported in various higher organisms [4–6] as well as in microorganisms [7–10]. Therefore, the application of starter strains possessing histamine-degrading activity might be a way for decreasing the amount of histamine produced *in situ* [5,7]. Nevertheless, the applications of these microorganisms and enzymes have been restricted by unfavorable physiological conditions for growth and enzyme activity such as low oxygen concentration, low pH values, undesirable temperature, and especially in the high salinity. The extremely halophilic archaea, in particular, are well adapted to saturated NaCl concentrations (grow optimally above 3.4–5.1 mol l⁻¹ or 20–30% NaCl). They have a number of novel molecular characteristics, especially for their enzymes that function in high salt concentration (3–4 M NaCl), such as lipase [11], protease [12], and glucose dehydrogenase [13]. Therefore, in this present study, extremely

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2015

Food Control 55 (2015) 176–184



Two putatively novel bacteriocins active against Gram-negative food borne pathogens produced by *Weissella hellenica* BCC 7293

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ABSTRACT

Weissella hellenica BCC 7293, isolated from Thai fermented pork sausage called Nham, produced two putatively novel bacteriocins, 7293A and 7293B. Both bacteriocins had broad antimicrobial spectra and exceptionally inhibited several important Gram-negative food-borne pathogens (*Pseudomonas aeruginosa*, *Aeromonas hydrophila*, *Salmonella Typhimurium* and *Escherichia coli*). The highest amount of bacteriocin was produced in MRS and APT media at 30 °C without agitation. Bacteriocin 7293A showed relatively higher antimicrobial activity than bacteriocin 7293B. However, pH and thermal stability of bacteriocin 7293A was lower. These bacteriocins were of proteinaceous nature, in which the complete inactivation of their antimicrobial activity after treatment by proteolytic enzymes, including trypsin, α-chymotrypsin, pepsin and protease K was observed, whilst lipase and α-amylase showed no effect. Antimicrobial activity of both peptides was also not inactivated by organic solvents (ethanol, isopropanol, acetone, acetonitrile) and surfactants (Tween 20, Tween 80 and Triton X 100). Bacteriocins 7293A and B exhibited bactericidal effect against both Gram-positive and Gram-negative indicators without cell-lysis. According to ESI/MS analysis, the molecular masses of bacteriocin 7293A and B were determined to be 6249.302 and 6489.716 Da, respectively. Because their molecular masses were not similar to those of other known bacteriocins, both bacteriocins 7293A and B could be novel bacteriocins. Thus, both novel bacteriocins hold promise for applications in the prevention or treatment of pathogenic infections as food and feed additives to replace antibiotics for enhancing the productivity and sustainability of food animals.

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1. Introduction

One of the most important problems always found in food industry is the contamination of pathogenic bacteria (Marie et al., 2012). Among the techniques used to control the microbial contamination in food, the application of natural antimicrobial agents has received wide attention. The demand for natural, chemical preservative-free, minimally processed, and healthy products with microbial safety is increasing (Deegan, Cotter, Hill, & Ross, 2006; Papagjanni & Anastasiadou, 2009). Bacteriocins or antimicrobial peptides produced by lactic acid bacteria (LAB) are

members of natural antimicrobial agents which have received great attention (Cleveland, Montville, Nes, & Chikindas, 2001; Cotter, Hill, & Ross, 2005; Zacharof & Lovitt, 2012). Although many bacteriocins from LAB, such as nisin and pediocin, have been approved and widely used in food products (Zacharof & Lovitt, 2012), the inability to inhibit Gram-negative pathogens limits their applications (Cleveland et al., 2001; Deegan et al., 2006; Gillet, Etzion, & Riley, 2008). Some bacteriocins from LAB could exhibit antimicrobial activity against Gram-negative bacteria when unpurified form was used (Benjeddo, Fons, Stocker, & Sadoun, 2012; De Kwaadsteniet, Todoro, Knoetze, & Dicks, 2005; Gong, Meng, & Wang, 2010; Jena, Trivedi, Chaudhary, Sahoo, & Senhadri, 2013; Lin, Tsai, Lin, Tien, & Tsai, 2008; Marie et al., 2012; Ravi, Prabhu, & Subramanyam, 2011) or they were used together with chelating agent such as EDTA (Cutter & Siragusa, 1995; Lappe,

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2016

Meat Science 120 (2016) 118–132



Bacteriocins from lactic acid bacteria and their applications in meat and meat products

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ABSTRACT

Meat and meat products have always been an important part of human diet, and contain valuable nutrients for growth and health. Nevertheless, they are perishable and susceptible to microbial contamination, leading to an increased health risk for consumers as well as to the economic loss in meat industry. The utilization of bacteriocins produced by lactic acid bacteria (LAB) as a natural preservative agent has received a considerable attention. Incorporation of bacteriocin-producing LAB as starter or protective cultures is suitable for fermented meats, whilst the direct addition of bacteriocins as food additive is more preferable when live cells of LAB could not produce bacteriocin in the real meat system. The incorporation of bacteriocins in packaging is another way to improve meat safety to avoid direct addition of bacteriocin to meat. Utilization of bacteriocins can effectively contribute to food safety, especially when integrated into hurdle concepts. In this review, LAB bacteriocins and their applications in meat and meat products are revisited. The molecular structure and characteristics of bacteriocins recently discovered, as well as exemplary properties are also discussed.

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1. Introduction: the need for natural antimicrobials in meat application

Microbial contamination causes serious safety and quality problems in meat industry. Meat and meat products, particularly fresh meat, contain adequate amount of water and abundance of proteins and essential nutrients with favorable pH for supporting microbial growth. The microorganisms present on meat and its products are in broad spectrum, ranging from bacteria to yeasts, molds and viruses, depending on type of the products. By far, microbial issues in meat industry have arisen mostly due to bacteria (Hui, 2012). As reviewed by Jayasena and Jo (2013), the main spoilage bacteria in meat include *Pseudomonas*, *Acetivibrio*, *Brochothrix thermosphacta*, *Moraxella*, *Enterobacter*, *Lactobacillus*, *Leuconostoc*, and *Proteus*. Upon a substantial growth of those spoilage organisms, proteins and lipids of meat and meat products undergo degradation, adversely changing appearance, texture and flavor of the products (Borch, Kant-Muermans, & Blact, 1996). Normally, spoilage microbes do not harmfully affect the health but they can stimulate gastrointestinal disturbances when consumed in high concentrations (Jayasena & Jo, 2013).

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2016

Differences in textural properties of cooked caponized and broiler chicken breast meat

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ABSTRACT This study was aimed at evaluating textural properties of cooked chicken breast meats obtained from 3 production systems (conventional raising, food modification, and caponization) and determining the relationship between instrumental parameters and sensory attributes associated with the texture of capon meat. Texture of cooked broast meats was determined using 3 instrumental methods: Warner-Bratzler Shear (WBS), texture profile analysis (TPA), and uniaxial compression (UC), and sensory analysis by trained panelists. The results indicated that cooked caponized meat showed the lowest values of WBS force, shear energy, hardness, Young's modulus of UC, and the 2 sensory attributes (firmness and number of chews) ($P < 0.05$). In contrast, springiness and juiciness were the highest in the caponized meat ($P < 0.05$), suggesting that capon meat was more tender and juicier than the others. Food-modified chicken samples showed intermediate textural characteristics between the samples of capon and conventionally raised broiler. Pearson's correlation revealed that WBS force, shear energy, Young's modulus of UC, gumminess, and springiness were strongly

correlated with 3 sensory attributes (firmness, number of chews, and juiciness). Partial least squares regression (PLSR) demonstrated that 72% of all sensory attributes for the first 2 PLSR components were explained by 36% of the instrumental parameters and the production systems. Loading and score plot illustrated that conventional raising contributed to a high degree of firmness and number of chews, and positively correlated with shear energy, WBS force, gumminess, hardness, and Young's modulus. Contrarily, caponization was negatively correlated with those sensory attributes. The univariate analysis indicated that firmness and number of chews were positively correlated with all instrumental parameters, except springiness. Juiciness was positively correlated with springiness but negatively correlated with the others. The study suggested that the cooked meat of capons could be differentiated from those of broilers raised conventionally and with food-modified diets based on textural properties. Based on the optimized simulating equation, texture of caponized broast could be explained by WBS force, shear energy, Young's modulus, and gumminess.

Key words: capon meat, instrumental, texture, sensory attributes, partial least squares regression
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INTRODUCTION

Broilers have been a predominant poultry consumed globally as they are an inexpensive, good source of high-quality protein. Nonetheless, a number of consumers also demand different varieties of poultry and their products. In this aspect, the caponized chicken or capon, a male chicken with testes artificially removed, has gained more popularity due to its unique textural

characteristics. Capon meat is not only tender and juicy, but it also provides stickiness and gumminess characteristics to some extent. Initially, caponization applies a surgical operation or implantation of a synthetic hormone (huxotestrol) to manifest sexual maturation of male chickens, resulting in tremendous changes in physical characteristics and fat accumulation in the birds (Tor et al., 2002; Migoul et al., 2008; Sirri et al., 2009). The increased fat, including abdominal, subcutaneous, and intramuscular fat, enhances flavor, juiciness, and tenderness of the meat (Maat et al., 1981; Chen et al., 2006; Sirri et al., 2009). However, testes removal via surgical operation has raised some ethical concern. Furthermore, hormone implantation has

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2018

2019

2020

RESEARCH ARTICLE

Absolute expressions of hypoxia-inducible factor-1 alpha (HIF1A) transcript and the associated genes in chicken skeletal muscle with white striping and wooden breast myopathies

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Full length article

Effects of Bacillus aryabhattai TBRC8450 on vibriosis resistance and immune enhancement in Pacific white shrimp, Litopenaeus vannamei

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Keywords: Bacillus aryabhattai, Probiotic, White spp, Muscle immunity, Antioxidant, Litopenaeus vannamei

ABSTRACT

The use of probiotics in aquaculture is a practical alternative to promote animal health and disease prevention. Meanwhile, this practice can also reduce the use of prophylactic antibiotics. The purpose of this study was to identify candidate probiotics that could control pathogen populations in larval gastrointestinal (GI) tract and stimulate host immunity in shrimp aquaculture. Bacillus aryabhattai TBRC8450, a bacterial strain isolated from the environment in a shrimp farm, has an antimicrobial activity against many pathogenic strains of Vibrio Harveyi and V. parahaemolyticus. Supplementation of B. aryabhattai to Pacific white shrimp (Litopenaeus vannamei) not only decreased the abundance of Vibrio populations, but also shifted the bacterial community in the shrimp GI tract. We found that supplementation of B. aryabhattai triggered shrimp innate immunity and antioxidant activities, and RNA expression of genes encoding microbial peptides and antioxidant enzymes, including C-type lectin, peroxidase-2, heat shock protein 60, thioredoxin, and ferritin, was significantly upregulated in the hepatopancreas of shrimp fed B. aryabhattai. Furthermore, phenoloxidase activity in the hemocytes and the total antioxidant activity in the plasma were increased, indicating enhanced immune and antioxidant response at the systemic level. In contrast, supplementation of B. aryabhattai had no effect on the total hemocyte count and superoxide dismutase activity in the plasma and hepatopancreas. Importantly, a pathogen challenge test using V. Harveyi 1562 showed a significant increase in survival rates of shrimp fed B. aryabhattai compared to the control group. Our findings suggest that B. aryabhattai TBRC8450 can likely be used as a probiotic to reduce the population of V. Harveyi in the shrimp GI tract and to enhance shrimp innate immunity and antioxidant capacity for vibriosis resistance in shrimp aquaculture.

1. Introduction

Shrimp aquaculture is a rapidly growing industry with a total production in million metric tons each year [1]. L. vannamei, known as Pacific white shrimp, is the most dominant species in shrimp world production [1]. Pacific white shrimp are predominantly produced from aquaculture in China and Southeast Asian countries [1]. L. vannamei was originally cultivated in the Pacific coast of the Central and South America was introduced into Asia due to its high growth performance and disease resistance [2]. However, after a few decades of massive cultivation, Pacific white shrimp became susceptible to many disease

outbreaks [3–5]. As the result, the production of Pacific white shrimp has decreased dramatically, leading to massive decline in economic incomes.

Infectious diseases resulting from Vibrio spp. have been a major burden in shrimp aquaculture worldwide [6–8]. Although Vibrio spp. are considered as commensal bacteria in marine animals. Some strains of V. Harveyi and V. parahaemolyticus have been known to cause serious shrimp outbreaks [9–12]. Infection of V. Harveyi causes luminous vibriosis, white tail disease, and bright-red syndrome, leading to massive mortality in shrimp hatcheries [13]. On the other hand, V. parahaemolyticus is a causative pathogen of acute hepatopancreatic necrosis

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Aquaculture

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Suppression of white feces syndrome in Pacific white shrimp, Litopenaeus vannamei, using hen egg white lysozyme

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ARTICLE INFO

Keywords: Hen egg white lysozyme, Immune response, Antioxidant activity, White feces, Pacific white shrimp, White feces syndrome

ABSTRACT

White feces syndrome (WFS) is one of the major constraints for shrimp cultivation. Although the pathogen causing WFS remains largely unknown, WFS has been associated with an increase in vibriosis abundance in shrimp hepatopancreas and intestine. We demonstrated that hen egg white lysozyme (HEWL) inhibited in vitro growth of Vibrio spp. including V. alginolyticus, V. Harveyi, and V. parahaemolyticus. To alleviate shrimp losses from WFS, HEWL was supplemented into feed at 0.005, 0.025, 0.125, and 0.625 μg diet. Supplementation at 0.125 (HEWL0.125) and 0.625 (HEWL0.625) μg diet retained the antimicrobial activity under the induction water. Both of the supplemented levels also showed a significant antimicrobial activity and reduction in vibriosis in shrimp gastrointestinal tract. We further analyzed the effect of HEWL supplementation on the expression of immune and antioxidant related genes in hepatopancreas. HEWL0.125 substantially up-regulated the expression of prophenoloxidase, serpin proteinase, superoxide dismutase, thioredoxin, and ferritin. Growth performance analysis of weight gain, average daily gain (ADG), feed conversion ratio, and survival rates was also determined, but there was no difference in those parameters. When shrimp were challenged with a pathogenic strain of V. Harveyi, we observed the greater survival rate in HEWL0.125 than that of the control group (66.67% vs. 16.67%). Importantly, the effect of HEWL0.125 on WFS was evaluated in a shrimp farm. The incidence of WFS was disappeared with our treatment in shrimp fed HEWL0.125. As a result, the survival and ADG were improved significantly. In conclusion, dietary supplementation of HEWL0.125 could inhibit vibriosis growth and stimulate immune- and antioxidant-related gene expression leading to resistance against WFS in shrimp.

1. Introduction

Pacific white shrimp, Litopenaeus vannamei, is one of the most cultivated penaeid in the world (The Fish Site, 2018). However, infectious diseases including luminous vibriosis, acute hepatopancreatic necrosis syndrome (AHPNS), and white feces syndrome (WFS) have deteriorated shrimp cultivation. WFS outbreaks have been frequently reported in Thailand which are responsible for a 1.0 to 15% loss in shrimp production (Somsriwong et al., 2014). The disease can be detected by the presence of white stringy feces (Srisartwong et al., 2014; Masten, 2015). Infected shrimp exhibit lower molting and pale hepatopancreas and intestine. As a consequence, the survival rate and growth

performance of shrimp were declined dramatically. Intersystemic Acroparasitosis (ISA), a metacarpid parasite in shrimp hepatopancreas (Tangpannapijit et al., 2013), has been proposed as the cause of WFS (Ito et al., 2013; Tang et al., 2016). WFS shrimp collected from the field largely showed ISA infection. Nevertheless, experimentally infected with ISA, shrimp failed to demonstrate WFS symptoms (Tangpannapijit et al., 2013). An elevation in vibriosis richness was also reported in WFS shrimp (Somsriwong et al., 2014). The number of vibrios including V. vulnificus, V. fluvialis, V. parahaemolyticus, V. alginolyticus, V. ordalii, V. cholerae and V. damsela in hepatopancreas and intestine of WFS shrimp were two-fold higher than that of healthy shrimp (Somsriwong et al., 2012). It was therefore speculated that vibriosis infection

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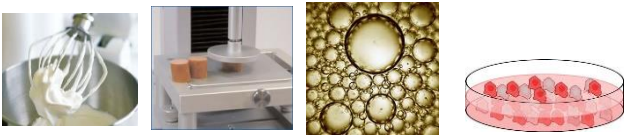
Core capability & Technology platform: Protein & Peptide Technology

Industrial interest

Core capability

Collaboration & network

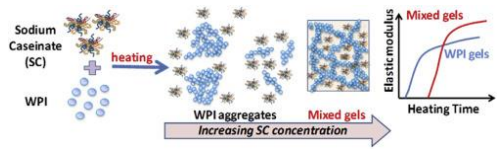
Protein/Peptide Functionality



Physical & Protein Functionality Analysis

- Texture analysis
- Viscosity
- Gelling, foaming & emulsifying properties
- Solubility
- Food structure

Food Matrix Interaction



Chemical Analysis

- Total protein
- Amino acid profile
- Enzyme activity
- Degree of hydrolysis
- Peptide analysis
- Enzymatic screening

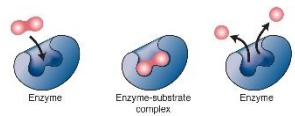
Alternative Protein Sources



Bioaccessibility & Bioavailability & Biological Activity

- Dynamic *in vitro* gastrointestinal model (TinyTIM®)
- Cell-based Assay

Enzyme-aided food processing



Food Processing & Protein Purification

- Protein purification
- Protein modification
- Thermal process



Core Capability & Technology Platform: Starter Technology

Industrial interest

Core capability

Collaboration & network

Fermented Foods and seasoning

- Process control & reliability
- Consistent quality
- Safety
- Value-added products

Screening technology of potential microbes

Lactic acid bacteria	Acetic acid bacteria	Bacillus
<i>L. plantarum</i>		<i>Bacillus spp.</i>

Fermented Feed

- Shelf-life extension
- high-quality feed



Culture optimization



Scale up

เอแอนด์พี ออร์ชาร์ด (A&P Orchard)



สหกรณ์โคนม



Platform Technology for Probiotics

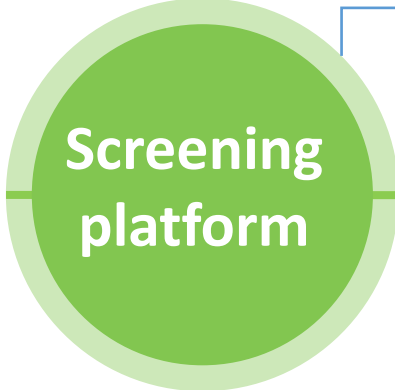


Collaboration network

- Food industry
- Feed industry
- Dairy industry

Industrial interest

- Probiotic specification



- In vitro*
- 10 strains for porcine ETEC
 - 3 strains for human ETEC



- Cell line model
- monocyte
 - macrophage

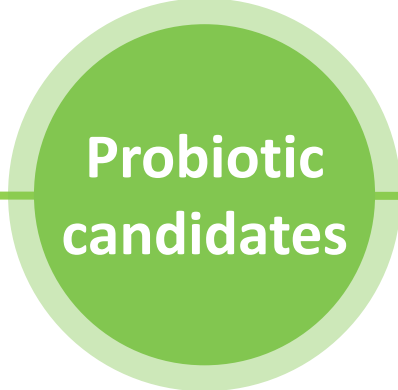
- 7 strains for porcine ETEC
- 3 strains for human ETEC



- *Bifidobacterium thermophilum* IFBT1159
- *Lactobacillus reuteri* IFBT1687

Limitation

- Animal trials



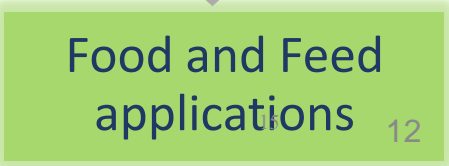
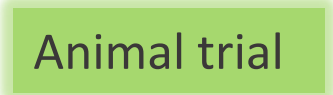
Safety evaluation

(WGS analysis)

- *Lactobacillus plantarum*
- *Bacillus velezensis*
- *Bifidobacterium animalis*



- Optimization
- Scale up



Core capability & Technology platform: Meat Science & Food Innovation

Industrial interest

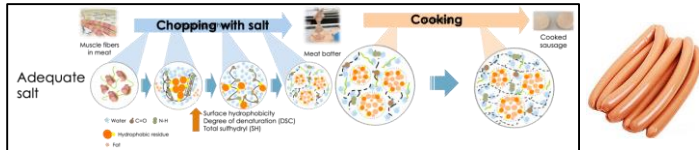
Core capability

Collaboration & Network

Meat Defects: White striping (WS) & wooden breast (WB) myopathies



Quality improvement of meat products



Value-added food products



Plant-based meat/Foods for special needs



Gene Expression Analysis

- Real-time PCR and digital droplet PCR techniques
- Gene expression analysis in muscle tissue

Physicochemical Analysis

- Meat texture and color analysis
- Chemical & biochemical analysis in muscle meat
- Muscle structure and composition
- Muscle & meat quality
- Meat defect characterization

Meat Product Processing and Formulation

- Conversion of muscle to meat
- Meat product processing (ea. tumbling, emulsifying, and retorting)
- Meat-food ingredient interactions (ea. salt and fat reduction)



Mindset and Strategy

- **Researcher ... Solution Provider**
- **Laboratory scale ... Commercial scale**
- **Customer ... Team and Strategic Partner**





1 Pentosanase production from a local microorganism

Economic and Social Impact in 2007-2020 = 370 million THB

- Profit to licensee
- Import substitution
- Investment
- Benefit to farm operators (better meat yield and healthier animals)



2 Starter culture and fermentation process for producing animal feed

Economic and Social Impact in 2010 - 2020 = 1,250 million baht

- Profit to licensee
- Import substitution
- Investment
- Benefit to farm operators (better meat yield and healthier animals)



3 Acceleration of fish sauce fermentation with enzymes

Economic and Social Impact in 2012 and 2014 = 67 million THB

- Profit to licensee
- Reduction from 18 months to 11 months
- The company produced new product in April 2009



Aspergillus niger BCC5639



SSF



Formulation



Field trial



Technology transfer

1



The technology, which includes the pentosanase-producing microorganism, *Aspergillus* sp., carefully screened from BIOTEC Culture Collection and the production process to achieve product in a powder form, was licensed to Asia Star Animal Health Co., Ltd. (ASAH) to manufacture and distribute pentosanase as feed additives.

Multi-enzyme preparation for swine and poultry

- Containing multi-enzyme activities such as amylase, cellulases, hemicellulases and acid protease that aids the nutrient utilisation in animal
- Improve energy utilization and protein digestibility in animal
- Increased average daily gain (ADG)
- Efficient nutrient utilization/ less pollution/ reduced production cost

Economic and Social Impact in 2007-2020 = 370 million THB

- Profit to licensee
- Import substitution
- Investment
- Benefit to farm operators (better meat yield and healthier animals)

2

DS-1 Feed Additive (*Bacillus* cells and enzymes)

Commencing in 2006, BIOTEC has collaborated with SPM Feed Co. Ltd. on a project to develop the fermentation process for *Bacillus* spp. and subsequently licensed this technology to Micro Innovate to produce Bacillus-fermented product, called DS-1 as a feed additive used in the SPM Group's pig farms. The use of DS-1 has increased the profit of the company, saving them from importing feed additives.



Economic and Social Impact in 2010 - 2020 = 1,250 million THB

- Profit to licensee
- Import substitution
- Investment
- Benefit to farm operators (better meat yield and healthier animals)

Research

BIOTEC has collaborated with Thai Fish sauce Factory (Squid Brand) Co., Ltd on a project to develop the fermentation process.

Technology transfer

The company produced new product in April 2009

3



To reduce from 18 months to 11 months.

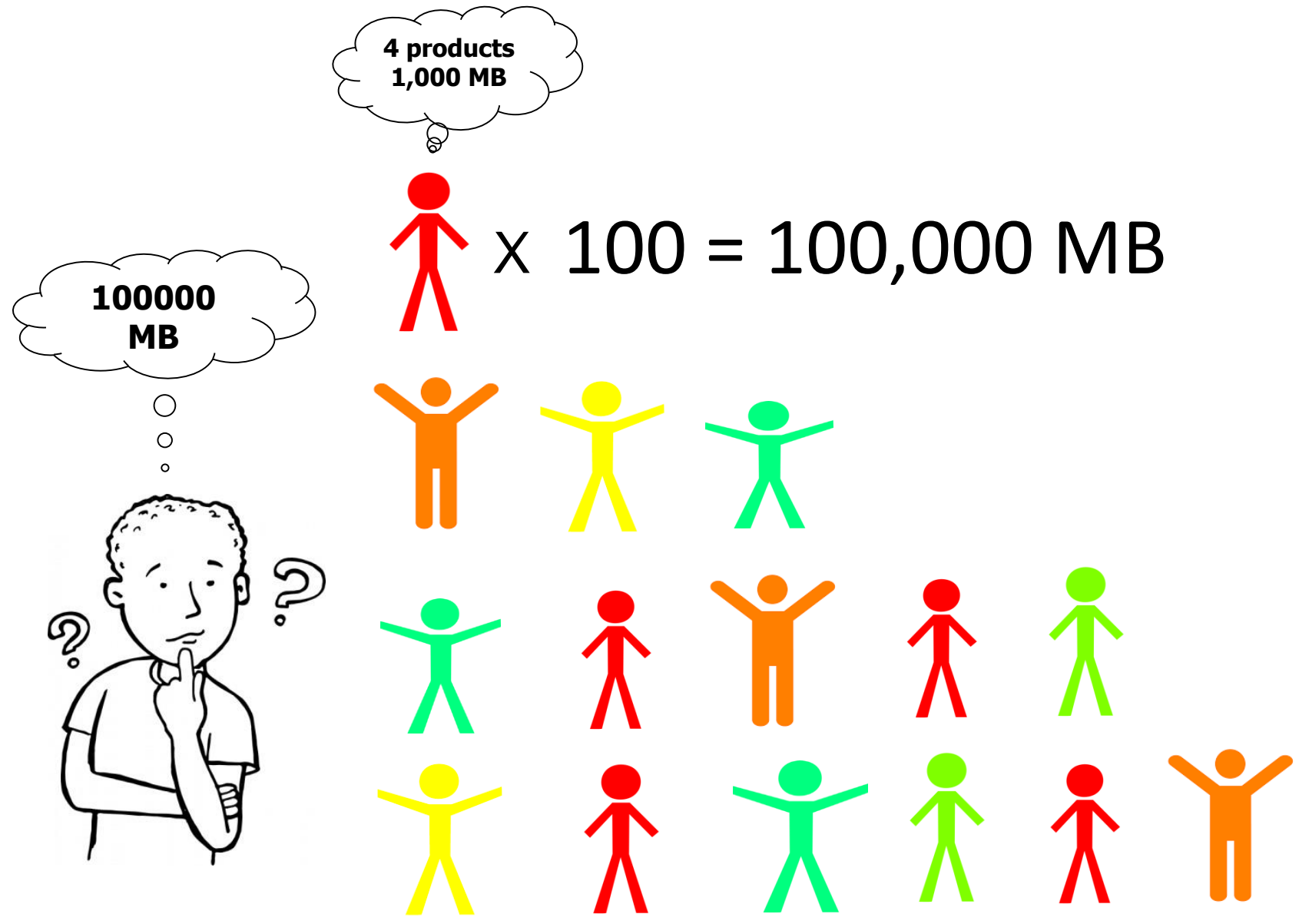


Economic and Social Impact in

2012 and 2014 = 67 million THB

- Profit to licensee
- Reduction from 18 months to 11 months
- The company produced new product in April 2009





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Thank you

A Driving Force for National
Science and Technology Capability



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