

ANIMAL

Enhanced pig production: potential use of insect gut microbiota for pig production

Jiwon Shin^{1,†}, Bo-Ra Kim^{1,†}, Robin B. Guevarra¹, Jun Hyung Lee¹, Sun Hee Lee¹, Young Hwa Kim², Suphot Wattanaphansak³, Bit Na Kang⁴, Hyeun Bum Kim^{1*}

¹ Department of Animal Resources Science, Dankook University, Cheonan 31116, Korea

² National Institute of Animal Science, Rural Development Administration, Wanju 55365, Korea

³ Department of Veterinary Medicine, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand

⁴ Abbvie Bioresearch Center, Abbvie, Worcester 01581, MA USA

*Corresponding author: hbkim@dankook.ac.kr

†These authors equally contributed to this study as first author.

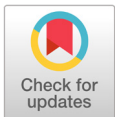
Abstract

The insect gut microbiome is known to have important roles in host growth, development, digestion, and resistance against pathogens. In addition, the genetic diversity of the insect gut microbiota has recently been recognized as potential genetic resources for industrial bioprocessing. However, there is limited information regarding the insect gut microbiota to better help us understand their potential benefits for enhanced pig production. With the development of next-generation sequencing methods, whole genome sequence analysis has become possible beyond traditional culture-independent methods. This improvement makes it possible to identify and characterize bacteria that are not cultured and located in various environments including the gastrointestinal tract. Insect intestinal microorganisms are known to have an important role in host growth, digestion, and immunity. These gut microbiota have recently been recognized as potential genetic resources for livestock farming which is using the functions of living organisms to integrate them into animal science. The purpose of this literature review is to emphasize the necessity of research on insect gut microbiota and their applicability to pig production or bioindustry. In conclusion, bacterial metabolism of feed in the gut is often significant for the nutrition intake of animals, and the insect gut microbiome has potential to be used as feed additives for enhanced pig performance. The exploration of the structure and function of the insect gut microbiota needs further investigation for their potential use in the swine industry particularly for the improvement of growth performance and overall health status of pigs.

Keywords: gut microbiome, insect, metagenomics, next-generation sequencing

Introduction

As the world population is increasing exponentially, the amount of food consumption and demand increases concurrently. In order to meet the requirement, the concept of edible insects



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as a sustainable alternative food can cope up with the emerging global food crisis (Van Huis, 2013). In Asian countries including South Korea, insects such as grasshopper and silkworm have been consumed as an alternative food protein source (Kim et al., 2016). Furthermore, insects play significant roles in contribution to food security because they are an environmentally friendly alternative to meat, healthy, and can offer livelihood strategies (Van Huis et al., 2013).

In addition, the insect gut microbiome has extensively been studied because of their beneficial functions. The genetic diversity of the insect gut microbiota has recently been recognized as potential genetic resources for industrial bioprocessing. Therefore, there is a need to better understand potential benefits of the insect gut microbiota for the enhanced pig production.

The basic structure of digestive tract of phytophagous insect is divided into three primary regions: foregut, midgut, and hindgut (Engel and Moran, 2013). Digestion of major plant components primarily occurs in the midgut where enzymes are produced by intestinal epithelial cells for utilization of nutrients. However, plant cell wall decomposition and utilization occurs in the hindgut of insects where enzymatic reactions are produced mainly by symbiotic microorganisms present in the specialized hindgut (Walters, 1990). Previously, these cellulolytic bacteria from the gut of many herbivorous insect species were studied and isolated (Shelomi et al., 2014; Shil et al., 2014; Sheng et al., 2015)

In order to understand the diversity of insect gut microbiota, the analysis of the taxonomical and functional properties of insect gut microorganisms is essential. Here, we introduce concepts to emphasize the necessity of the study on the insect gut microbiota and their applicability to pig production or bioindustry.

Insect gut microbiota

Previous studies using conventional microbiological methods have identified microorganisms based on phenotypic characteristics of the bacterial isolates. This established a fundamental basis for the studies on intestinal microorganisms, however, conventional methods are not able to identify many unculturable bacteria (Riesenfeld et al., 2004). With the advent of next-generation sequencing (NGS) technologies, development of sequence-based metagenomics has been accelerated (Thomas et al., 2012). Metagenomics is defined as a study of analyzing genome from environmental samples (DeLong, 2005; Thomas et al., 2012). These new approaches allowed not only the exploration of unculturable bacteria but also the investigation of microbial diversities from extreme environment such as hot springs, salt ponds, and gastrointestinal tracts of humans and animals (Singh et al., 2008).

The insect abdomen contains most of the digestive, respiratory, excretory, and reproductive organs. The gut contains 10 times more microorganisms than the whole cells of the host, and it is estimated that the microorganism genes are 100 times more diverse than the animal genes (Rajagopal, 2009). Insect intestinal microbial communities have been studied in many fields. Many factors influence the insect gut microbial communities including diet, pH, host specificity, host habitat, and life cycle (Colman et al., 2012).

Fundamentally, the gut microorganisms make a colony at the intestine through food intake and have a key role in the host's growth, digestion, and resistance to pathogens (Dillon et al., 2010; Engel and Moran, 2013). Depending on what they are fed, the metabolic function and structure of the microorganism community may change (Santo Domingo et al., 1998; Colman et al., 2012). In fact, it confirmed through experimentation that differences in diets influence biochemical activities such as rate of evolution of volatile fatty acids, ratio of butyrate production to acetate and

propionate production (Santo Domingo et al., 1998). Insect mainly ingests plants, therefore harboring a gut microbiota related to cellulose degradation is essential for their survival (Engel and Moran, 2013). Cellulose is a good carbon source, but it is not easy to access from the host point of view because of the crystalline or amorphous microfibrils (Sukumaran et al., 2005; Engel and Moran, 2013). In order to utilize the carbon source from the plant, hosts need to get help from bacteria which are involved in breakdown of cellulose into simpler sugars from (Engel and Moran, 2013). It has also been studied that species-rich communities of the host gut microbiota have much stronger resistance to invasion than species-poor case and more bacterial species increase the survival rate of the host (Dillon et al., 2005).

The cellulase from the insect gut microbiota

Many studies reported functions of the insect microbiota, such as cellulose and xylan hydrolysis, vitamin production, nitrogen fixation, phenolics metabolism, and antibiotic resistance through metagenomic analysis of the bacterial community residing in the insect gut (Engel and Moran, 2013; Krishnan et al., 2014). Among them, the most widely applied for biotechnology industry is the cellulase that breaks down the cell wall of the plant.

The plant cell wall is the principal energy source in the biosphere and an indispensable food source for herbivorous insects. Plant cell wall materials are made mostly of polysaccharides including cellulose, hemicellulose, xylan, and pectin. Cellulose contains plenty of D-glucose residues with β -1, 4-glycosidic linkages. Xylan is a group of hemicellulose that is found in plant cell walls. It is derived from units of xylose, a pentose sugar, with α -1, 3-glycosidic bonds. In plant biology, pectin is an important naturally occurring polymer which is usually found on the middle lamella and the primary cell wall of plants (Salisbury and Ross, 2001). With the catalyzing of cellulose degrading system, cellulose can be turned to glucose which has numerous utilization (Gupta et al., 2011).

Cellulase is the enzyme used to decompose cellulose and some related polysaccharides into monosaccharides and simple sugars, such as β -glucose or shorter polysaccharides and oligo saccharides. Utilization of cellulose is mediated by cellulase enzyme including three classes β -glucosidase, β -1,4-endoglucanase and β -1,4-exoglucanase (Gilbert, 2010). Additionally, hemicellulose decomposition enzymes are most represented by xylanase and xylooligosaccharidase (Calderon-Cortes et al., 2012). The enzymes which are responsible for breakdown of the pectin, pectinases, include pectinesterase, pectin/pectate lyase and poly galacturonase (Fig. 1) (Gilbert, 2010).

Cellulase is widely applicable to various industries. Cellulase is routinely used in the finishing phase of the textile industry to remove cellulose fibers, creating a soft, shiny appearance and brightening the color. In recent years, the addition of enzyme cocktails containing cellulase, lipase, and protease in the detergent industry has emerged as a new trend, which helps remove stains from the fabric. Cellulase also gives the fabric brightness and softness, even after repeated washing. In the food processing industry, cellulase, along with xylanase and pectinase are used for the extraction and purification of fruit and vegetable juices, helping to increase yield by improving the cloud stability and the aromatics of pulp. In the animal feed industry, the addition of enzymes during feed processing helps the growth of animals by helping digest the partially degraded feed resulting in the transfer of more nutrients to the animals (Sukumaran et al., 2005; Juturu and Wu, 2012, 2014)

Both fungi and bacteria have been used in bioindustry as a producer of cellulase and hemicellulase. The most attractive benefit of using fungi is their ability to produce plentiful amount of cellulase and the enzymes that can be

extracted and purified easily on the media. However, bacteria have a higher growth rate than fungi and it can reside in a variety of environments, even including extreme conditions. Besides, bacterial cellulolytic enzymes are more complex and are expressed as a multienzyme complex that increased functions of combined enzymes (Maki et al., 2009). For these reasons, isolation of cellulolytic enzymes from the bacteria is becoming widely exploited. These cellulolytic bacteria from the gut of many herbivorous insect species have been studied and isolated continuously (Schafer et al., 1996; Shil et al., 2014; Sheng et al., 2015).

According to cited research, the glycosyl hydrolase (GH) families including GH2, GH7, GH10, GH11 and GH36 were found in the *Holotrichia parallela* larvae hindgut (Sheng et al., 2015). Moreover, it was revealed that tens of gut microflora including *Bacillus*, *Streptomyces*, *Klebsiella*, *Pseudomonas*, and *Acinetobacter* were involved in cellulose and hemicellulose degradation (Schafer et al., 1996; Anand et al., 2010; Shil et al., 2014). Through the biochemical experiment on respective agar plates which contained carboxymethylcellulose (CMC), oat spelt xylan, citrus pectin, and starch, the taxonomical identification and characterization of functions related to the utilization of the plant cell wall materials of cultivatable intestinal bacteria in entire digestive tract of silkworm were identified by cited research (Anand et al., 2010).

The influence of insecticides on the gut microbiota of insects

Approximately 10 thousand of 1 million insects feed on crops, of which about 700 species that cause insect damage to crops in farm around the world (Ware and Whitacre, 2004). Insecticides are drugs that have the effect of killing insects that harm human or crops. Insecticides are classified into five categories: inorganic insecticides, organophosphates, carbamates, organochlorines, and natural insecticides (botanicals) (Abdollahi et al., 2004). Among them, organophosphates are mainly composed of an organic phosphorus compounds, and it occupies the largest number of insecticide currently used in practical use. It is almost ester-like and relatively low in stability, and is easily hydrolyzed, so that there are minimal residual problems. Characteristically, it is a neurotoxic agent works by infringing the nervous system of insects.

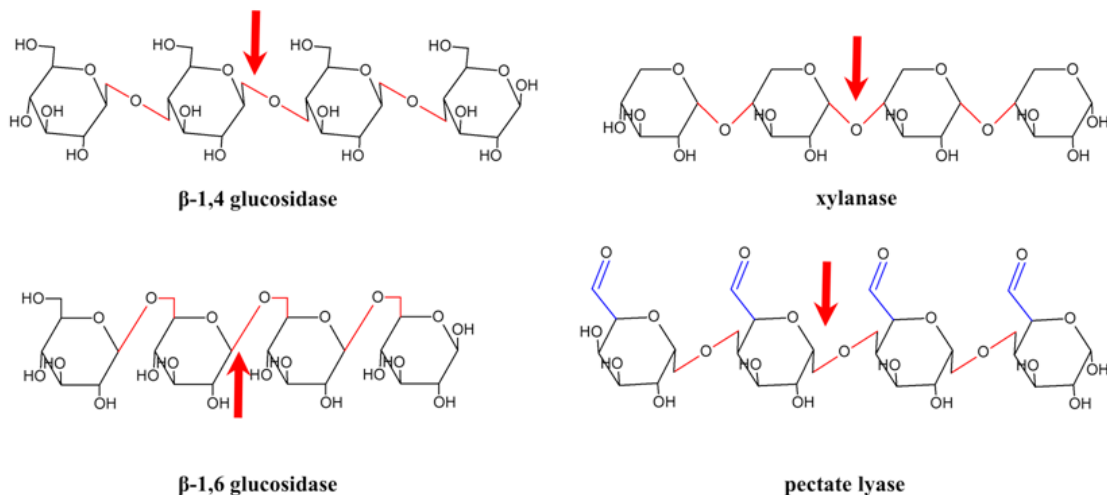


Fig. 1. The enzymes that are involved in the degradation of cellulose, hemicellulose and pectin. Red arrows indicate the active site of degradation.

The organochlorines are characterized by containing a large amount of chlorine. It is excellent in efficiency, but is currently prohibited from being used for reasons of residual toxicity. The oldest and most representative type is Dichloro-Diphenyl-Trichloroethane (DDT) (Ware and Whitacre, 2004). The mechanism of toxicity in most pesticides is the stimulation of free radical generation, induction of lipid peroxidation and the deterioration of antioxidant function (Abdollahi et al., 2004; Jang et al., 2018).

The intestinal microbiome in insects have another significant function that is insecticide resistance. The insecticide resistance symbolizes a remarkable instance of natural selection. Some of the enzymes in insect that are involved in the tolerance to insecticides, such as the glutathione S-transferases (GST) and cytochrome P450s, that regulate discharging or decomposing xenobiotics including DDT (Feyereisen, 1999; Daborn et al., 2002; Enayati et al., 2005; Li et al., 2007). These enzymes are a protective line against a number of chemicals and enable insects to avoiding toxic plants, microbial contamination of their food, insecticides, and organic pollutants existing in their living environment. In addition, they help the insects adapt easily to their host plants (Feyereisen, 1999; Gui et al., 2009). Both enzymes have important antioxidant properties in most organisms. They are capable of preventing damage to significant cellular components caused by reactive oxygen species (ROS) such as free radicals, peroxides and heavy metals. ROS play roles in controlling the homeostasis of cells as signaling messengers. When the production of ROS is more than innate anti-oxidant defenses, oxidative stress has arisen (Burton and Jauniaux, 2011). Exposure to insecticides is one of the factors that produce oxidative stress.

Oxidative stress is simply defined as the imbalance between antioxidant protection in the body and free radical generation which can lead to peroxidation of the lipid layer of the cell (Abdollahi et al., 2004). Oxidative stress adjusts crucial cellular functions, especially in astrocytes and microglia, including apoptosis program activation and ion transport and it can cause excitotoxicity that is a main reason of neuronal death (Emerit et al., 2004).

Excitotoxicity is a pathological process in which neurons are damaged by glutamate and its analogues, which may lead to cell death. The most abundant amino acids in insect central nervous system (CNS) are L-glutamate which act on excitatory and inhibitory neurotransmitter (Gilbert and Gill, 2010). Glutamine-glutamate circulation is managed by astrocytes and neurons. In this cycle, the glutamine synthetase can be subjected to damage by oxidative stress easily. Thus, it leads to the accumulation of intracellular glutamate and its release to outside of cell (Emerit et al., 2004). Glutaminergic neurotransmission is controlled to stabilize extra cellular glutamate concentration at 0.6 mmol in healthy conditions. However, when the concentration increases to be 3 - 8 times over, it can damage cortical or hippocampal neurons (Emerit et al., 2004).

The concept of edible insects as a sustainable alternative food

Insects are the largest group of invertebrates and the number of species reported to be over the world is over one million. It accounts for 73% of the total group of invertebrates (Chapman, 2009). Moreover, insects are the most diverse group in the animal kingdom with a high survivability rate.

As the global population increases, the amount of food needed increases accordingly. In 2050, the culmination of obtainable world food production is about 3300 Mt, 60% above at 1999 (Table 1) (Gilland, 2002, 2006). In order to reach the demand, the concept of edible insect has been risen as solution against a food crisis (Van Huis, 2013). Insects such as grasshopper (*Oxya chinensis sinuosa*) (Kim et al., 2016) and silkworm (*Bombyx mori*) (Zhou and

Han, 2006) have been known and utilized as a nutritional food source in Southeast Asian countries including South Korea. There are three reasons for entomophagy: health, environmental, and livelihoods. Tens of insects have rich protein and healthy fats and are high in essential minerals such as calcium, iron and zinc. Additionally, insect rearing requires minimal technology and low-funded industrial conditions that offer entry even in poor societies (Van Huis et al., 2013). Besides, the insects serve humans not only as food, but also as valuable producers of products such as honey, silk, wax and natural medicines.

In addition, using insects as a feed for livestock has nutritional and economic benefits. As the demand for meat grows, the need for grains and proteins in feed is also growing. The cost of producing meat, fish, and soybean flour for animal feed accounts for 70% of the livestock production costs (Van Huis, 2013). Depending on the animal's rating and the type of production used, the amount of feed required to gain 1 kg of weight varies greatly. When fed a diet with metabolizable energy of 3,200 kcal/kg, cumulative feed/gain ratios of chicken, pork, and beef are 2.5, 5.0, and 10, respectively (Smil, 2002). However, cumulative feed/gain ratios of insects, such as crickets, is 1.7 (Collavo et al., 2005). An insect is a cold-blooded animal, theoretically because it does not need feed to maintain body temperature (Van Huis et al., 2013). If this figure is adjusted to the weight of the food, the benefits of eating insects are much greater (Van Huis, 2013). Moreover, insects have relatively high amounts of nutrients, less environmental impact, and require less space to raise. As such, when the insects were incorporated into broiler feed, the result has shown some cases of increasing growth rates of chick (Rumpold and Schlüter, 2013).

Conclusion

The microbiome is known to play an important role in host nutrition, digestion, and immunity, thus its importance has become undeniable. With the help of high throughput sequencing through the NGS, sequence information of the various bacteria has been generated. In summary, bacterial metabolism of feed in the gut is often significant for the animals' nutrition intake, and insect gut microbial community has a potential to be used as feed additives for the enhanced pig performance. The exploration of insect gut microbial community structure and function needs further investigation for their potential use in the swine industry particularly for the improvement of growth performance and overall health status of the pigs.

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Table 1. Cereals and world population in 1999 and 2050. Adopted from (Gilland, 2002).

Items	Year	
	1999	2050
World population (Million)	6003	9104
Cereal area (Mha)	671	725
Cereal production (Mt)	2077	3300
Cereal yield (kg/ha)	3094	4550

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