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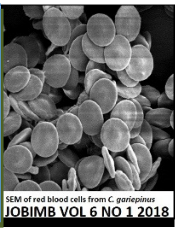

Metagenomic Studies of the Gut Microbiota: The Snake Gut Microbiota as a Model Organism

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ABSTRACT

Microbes play a very important role in each individual. The microbial communities and its genetic blueprint greatly influence in many human diseases. Most of the microbe populations are grow in an individual's gut. Therefore, metagenomics studies on gut microbes are essential to understand the microbial diversity in gut and the knowledge on microbial composition associates with terrestrial animals will be very important for further understand nutrition, diseases and physiological state. Besides, the availability of next generation sequencing technologies gives a better understanding on gut microbiotas communities compare to the first generation sequencing. This paper, we suggested snakes as a model to study microbial metagenomics due to its various compounds can help to cure various illnesses, even kill off unwanted germs from body. Therefore, this paper mainly review on snake gut microbes, secondary metabolites produce by microbes and the benefits of molecular technologies used in metagenomics which can be useful in medical industries and treatment of infectious diseases.

INTRODUCTION

Microbes play crucial role in health and well-being of all life on earth. It produces many primary metabolites and secondary metabolites. The primary metabolites include vitamins, amino acid and nucleotides which function in growth, development, reproduction and physiological functions in the body. Whereas, secondary metabolites which are derivatives of primary metabolites include antibiotics, pigments and drugs play role in ecological function. Pharmaceutical industries are using microbial secondary metabolite in producing antibiotics and drugs to control infectious diseases. It is due to the presence of anti-infective properties and biological active agent of the secondary metabolites. For an example, active agent produced by Penicillin notatum called penicillin kills Staphylococcus aureus. Besides, some microbial metabolites have shown antitumor, antiproliferative properties and some industries use enzyme inhibitors which are isolated from microbes. The compound derived from microbes plays a role as immunosuppressive agent which helpful in rejecting organ transplantation. Thus, microbes play an important role in medical field and pharmaceutical industry [1].

Generally, bacteria produce natural antibiotic resistance mechanisms to survive and many antibiotics have been found ineffective due to the multidrug resistance formed by the microbes. Therefore, searching for new antibiotics and new batches of anti-infective agents against those resistant bacteria and to tackle new diseases is necessary. Previous studies have shown that large number of bacteria and fungi is yet to be cultivated in the laboratories. Therefore, many researchers are sourcing microbial DNA extraction from environment such as soil and marine habitats, cloning large fragments into artificial chromosomes of bacteria, expressing in host bacterium and screening the library for new antibiotics.

The effort of this metagenomics analysis will introduce many untapped reservoir of genetic and metabolic diversity [1]. Studies indicated that many researchers focus on gut microbiota because of the total microbial communities present in gut which is approximately 3 to 10 times greater than the host cells present in an individual's body. Gut microbes do beneficial to host body and alteration in those microbes causes digestive tract diseases, inflammatory bowel diseases and so on. To further understand the mechanism of gut microbes in human system, scientist needed more animal models which are have almost similar physiology, biochemical, metabolic and genetic similarities as compare to humans. Therefore, most of the researches and pharmaceutical industries are using pig or swine to manufactured medicine and study the metagenomics of microbes [2]. Whereas, based on Judaism and the Islamic religion Harris M., [3] stated that pig or swine is prohibited and it's the dirtiest animal compared to other domesticated animal. It also prohibited eating the pork or even touching the swine's carcasses.

Snake is useful in the pharmaceutical industries although it is still prohibited. Snake's venom can be used in pharmaceutical companies and biomedical studies to produce pain killer drugs and uses as power enzyme to breakdown complex protein. So, based on Islamic believes, it can be said as permissibility of prohibited things in the case of necessity [4]. Besides, in Malaysia, local snakes are commonly found in many zoos and it is easier to collect gut samples with the permission of Department of Wildlife and National Parks Peninsular Malaysia. Furthermore, the availability of advanced technologies such as next generation sequencing (NGS) will ease the reconstruct of the genome presents in a community. NGS is efficient in the study of microbes with high throughput sequencing compared to the traditional method such as Sanger sequencing [5]. In this article, the review focus on the snake's gut microbes, secondary metabolites produce by microbes and the benefits of molecular technologies used in metagenomics which can be useful in medical industries and treatment of infectious diseases.

Gut microbiota in human health and diseases

Generally, gut microbiotas includes bacteria and other microorganisms develop in gut from an individual born includes bacteria, viruses, eukarya and archaea. In a new born baby, the number of microbial species is low especially when in mother womb and it will increases after birth due to environment factor like method of delivery (from the mother to baby), nutritional factor like method of feeding (breastfeeding) and epigenetic factors. These factors influence the health of gut and symbiosis of microbes.

The microbe's population starts to grow rapidly at the unweaning stage and changes occur in microbial diversity as the individual grow from child to adult. Studies shown that at old age, some of the specific microbes such as Bacteroidetes species will be decrease whereas the Firmicutes species will be increase. The function of the gut microbiotas varies from an individual to another based on the diet, lifestyle and host genome [6]. Gut microbiota mainly beneficial in regulating immune system of an individual and they are important in providing essential nutrients, digest indigestible compounds, protect against opportunistic pathogens and involves in intestinal architecture development [7].

The interaction between microbes and the host's immune system which link with the chemical composition act upon many organs modulates metabolic reactions and produce metabolites such as bile acids, choline and short-chain fatty acids. These metabolites play an important role in human health. The gut microbes play crucial role in preventing presence of pathogenic microbes [6].

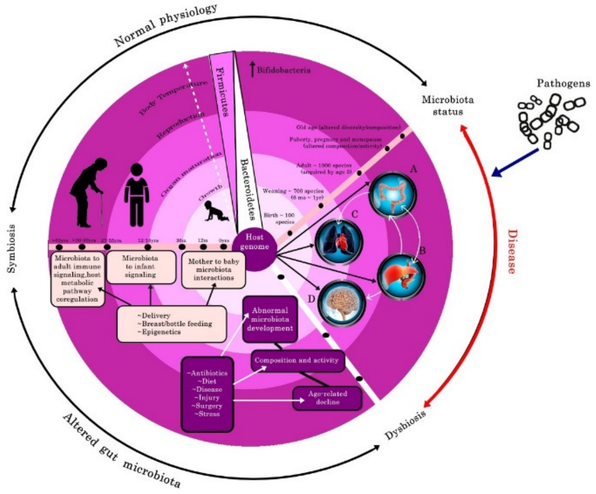

Fig. 1. The role of gut microbiota in an individual's growth and disease.

Based on **Fig. 1**, the alteration in gut microbiotas which is called dysbiosis due to environmental stresses and diet will leads to health risk and diseases. As shown, abnormalities in gut microbes cause (A) colon diseases like colon cancer and inflammatory bowel diseases; (B) affect liver and cause gastric ulcers, (C) lung affection and leads asthma and hypertension; (D) hormone signaling affected and changes happen in behavior and mood [6]. Therefore, National Institute of Health has introduced Human Microbiome Project to understand the characteristics of microbial population in human and those microbes contribution in causing diseases [8].

Gut bacteria and it's metabolites

Individuals are maintaining body regulation by the interaction between microbes with metabolites, microbes with immune system and metabolites with immune system. The gut microbes are different among each individual which influenced by gender, age, body mass index and others. Whereas some specialize metabolites like secondary metabolites, natural products and small molecules virulence faster almost present in most of microbes. Those metabolites are playing a part in human health to control the normal body regulation. They used in clinical as antibiotics, immune system regulator and cholesterol lowering drug (**Table 1**). Most of metabolites found in gut mainly in intestinal wall where it interact with immunological processes and function systematically on host [9].

Various models for gut microbial metagenomics studies

Some researchers have conducted microbial metagenomics analysis on human and mice, indicated that microbes associated with the alteration in adipose tissue and supply nutrients to host during it is in fasting or lack of food consumption due to the alteration in gastrointestinal microbiota. Gut microbiota also shown to alter the size of internal organ in which the increase of adipose tissue and heart mass during the transplantation of gut microbiota to a germ-free animal [10].

Table 1. Bacteria and metabolites related with the function of metabolites.

Metabolites	Bacteria involved	Role in human body	References
Short-chain fatty acid: acetate, Butyrate, propionate	A subset of bacteria, including several *Bacteroides* spp. and some *Firmicutes*	Decrease in colonic pH, as an energy source, anti-inflammatory agents and promote apoptosis of cancer cells	[10,11]
Choline metabolites: methylamines, dimethylamine, trimethylamine, and trimethylamine-*N*-oxide	*Bacteroides thetaiotaomicron, Lactobacillus* spp.	Develop insulin resistance, regulates lipid metabolism, involves in nonalcoholic fatty liver disease, high fat diet/ obesity	[12]
Bile acids: cholate, hyocholate, deoxyxholate, chenodeoxycholate, taurocholate	*Lactobacillus, Bifidobacteria, Eubacterium, Bacteroides, Fusobacterium*	Absorb dietary fats and lipid soluble vitamins, maintain cholesterol homeostasis, maintaining glucose and energy homeostasis	[1,13]
Other Acids: lactate, succinate and formate	*Eubacterium hallii* and *Anaerostipes* spp	important role in maintaining homeostasis, reduce pH, formation of lactate and its low pKa	[14]

In the Human microbiome project, researchers found some difficulties in certain aspect especially studies that cannot perform on real life human [15]. Therefore, many studies of gut microbial establishment have been performed mostly in adult animals or newborn offspring, such as human neonates [16], insects [17] zebra fish [14], chickens [18], snakes [19,20], alligators [12,21], and lizards [22].

Snakes as a model for gut's microbial metagenomics studies. Snakes are vertebrate animal which common found everywhere in Asian countries. There are many different species of snakes available and it can be good model to study diversity of microbial communities. Nowadays, upon the merging of global economic wild snakes are harvested and traded internationally instead of used locally as food or for the production of traditional medicine, leather, and other products. Snakes also have been used for food and medicine for centuries, and ancient Chinese texts list snake among the recognized traditional medicinal ingredients [23,24].Annually, there are three million kilogram of snakes has been consumed by the Chinese population and they believes it has many health benefits [25]. Besides, snakes are widely used in Chinese Traditional Medicine (CTM) [25].

A lot of ancient medical books stated the therapeutic effects of snakes or their organs, including gall bladder and liver [26–28]. Study conducted by Alves and his colleagues on snake usage as a remedy for diseases by interviewing local community in Northern Brazil reported that some snakes species have been used to treat 15 various diseases. For instance, rattlesnakes used in treating asthma [29]. Therefore, snakes can be a good model to study especially the microbial diversity of the intestinal to understand its function for the possible health benefits using molecular approaches such as real time PCR, next generation sequencing and some other molecular tools [20]. The potential microbes from snakes that has been be identified and characterized may possess many possible health benefits. It can be use as alternative to replace the traditional ways of consuming snakes as medicine, in which consumption of snakes will lead to extinction of certain snakes [25]. Even though snake is non halal to consume in the Islamic point of view because of their poisonous nature as they can be harmful to human, the finding from metagenomics greatly contribute to the understanding of beneficial microbes to mankind. In addition, this will also eventually contribute novel and additional information to the microbial biodiversity data presently available.

Molecular approaches in metagenomics

In 1985, there is a paradigm shift where there is a huge changes in visualize and describe the world of un-culturable microbes. Pace and his colleagues developed few methods to analyze phylogenetic and identify species of naturally occurring microbial communities. They developed 5S and 16S ribosomal RNA (rRNA) gene sequences to analyses the diversity of microbes [30]. A term called metagenomics has been introduced which using modern genomic methods to study diversity of microbes in natural sources without losing its geniture by rRNA sequencing [31,32]. Initially, first generation sequencing has been developed for genome analysis which known as automated Sanger sequencing plays a dominant role in most of the industrial areas [13]. Whereas, the application of this sequencing has some limitation such as only analyses small sample size, high chances of sample contamination and sequencing error mainly at amplification step [33].

In the evolution of modern technologies, there is a new sequencing method has been developed which is called "Next Generation Sequencing" (NGS). NGS is more advance compare to Sanger sequencing with its high throughput, cheap, less error and run high sample size. It has various useful features such as resequencing target regions or whole genome [13]. Some commercially available next generation sequencers are Illumina, Pyrosequencing (QIAGEN), Ion Torrent (Analisa Resources) and PacBio. NGS technology has attracted high demands in many fields and widely used everywhere especially in diagnose diseases by elucidate the role of non-coding RNA to characterizing evolutionary genome. It can be also use to obtain quality sequence data of a genome isolated from single cell that is associated in cancer genomics [13]. Besides, NGS method is highly recommended in metagenomics profiling due to the ability to reconstruct all the genome within an environment [5].

Paired end sequencing in NGS is one of the most commonly approach among the other available technologies due to its data accuracy, wide application in many field and simple procedures. Some NGS platform provides more sequences per run with in-depth coverage of data compare to others. Therefore, selection of appropriate analyzing technology tools is extremely important to understand the total diversity of microbes in an environment [34].

CONCLUSION

Microbial studies or metagenomics of microbes become important topics to be investigated because activities of microbes are very beneficial in human health. The metabolite contributed by the specific microbes' species has become very important in maintaining normal body regulation and alternative medicine. Each specific species has a unique microbiota which involves in physiological function such as digest indigestible foods, production of vitamins and crucial role in immune system. Besides, consumption of animal for the purpose of treatment can lead to extinction of certain. To overcome the extinction of animals, understanding the mechanism of microbes and harnessing their beneficial properties is essential to combat various diseases and health benefits. In order to study the microbial diversity, molecular approaches are mostly recommended tools such as next generation sequencing, real-time PCR and others due to their reliability and accurate data obtained. Genome sequencing has been evolved with technologies to improves the outcome of biology, medicine, agricultural sciences in many ways and most helpful in studying diversity of microorganisms in their natural habitats.

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