A review of next generation sequencing technologies used in the evaluation of the skin microbiome: what a time to be alive

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Abstract

The role of the microbiome in healthy and disease states of the human body is progressively being found to extend beyond the gastrointestinal tract and into other organ systems such as the skin. Researching the microbiome thus has become paramount to understanding additional physiological and pathophysiological mechanisms that may be at play between microbes and their hosts. Cell cultures have traditionally been used to study the microbiome, but in our current day and age, advanced metagenomic techniques — such as 16S rRNA amplicon sequencing and whole metagenomic shotgun sequencing — are better able to classify the microorganisms making up the microbiome. Utilizing metagenomics alone, however, does not allow for the study of the more complex effects of the microbiome, such as changes in gene expression and metabolic byproducts. Thus, incorporation of other modalities such as metatranscriptomics, metaproteomics, and metabolomics are needed to further elucidate the extensive intricacies of the skin microbiome.

Keywords: metagenomics, amplicon sequencing, metatranscriptomics, metaproteomics, metabolomics, skin microbiome

Introduction

It is estimated that 10^{14} microorganisms make up the human microbiome [1]. There is increasing evidence to support the significant role in human health of this diverse ecosystem made up of bacteria, fungi, viruses, and eukaryotes [2, 3]. Although much of the research done on the human microbiome has been related to the gastrointestinal tract, it has been shown that the microorganisms that live on humans can also affect the skin. The delicate balance of these rich communities of microorganisms is believed to contribute to skin barrier function, immunity, and lipid formation [4, 5]. Furthermore, a disruption of the skin microbiome has been associated with different diseases states such as atopic dermatitis, acne, and psoriasis [6-8]. Therefore, studying these microbial communities is vital to gaining insight into the various physiological and pathophysiological states of the skin.

In his essay "The Law of Accelerating Returns," author Ray Kurzweil argues that technology is advancing at an exponential rate. He argues that despite humans holding an "intuitive linear" perspective for technology growth, if one examines our ancestors or even biological evolution as a whole, it becomes strikingly clear that progress is being made in an exponential fashion. As it relates to genomic sequencing and bioinformatics technology, there has recently been a dramatic shift in how the skin microbiome is studied. Instead of examining skin microbiota communities through culture-based protocols, scientists have transitioned to applying sequencing techniques such as amplicon or whole

metagenomic shotgun sequencing — collectively known as metagenomics [9]. Although growing microbes in vitro has been an effective method in the past, culturing microorganisms in the laboratory setting erroneously underestimates the phylogenetic diversity of the skin microbiome [9, 10]. This underestimation is a byproduct of the bias that comes with culturing bacteria in a laboratory setting. For example, some species flourish more readily in artificial growth settings than others, effectively skewing data [9, 11]. Recognition of this limitation, combined with technological advances and cost reductions, are the motivating factors in researchers' decisions to utilize sequencing techniques in skin microbiome studies.

The progression to culture-independent profiling has ushered in a new era for microbiome research and revolutionized our understanding of the skin microbiome [12]. Yet, despite the development of higher resolution techniques, most skin microbiome researchers only utilize metagenomic sequencing for taxonomic classifications. With our knowledge of the skin microbiome expanding rapidly, it has become apparent that the pathogenesis of various skin diseases arises from more than just changes in microbial composition. Changes in gene expression, production of metabolites, and microbial interactions with the host are all factors that play a pivotal role in deepening our collective knowledge and elucidating the influence of the skin microbiome in disease. In this regard, it has become apparent that utilizing metagenomics alone without the other 'omics' (metatranscriptomics, metaproteomics, and/or metabolomics) paints a very limited picture of the skin microbiome. **Table 1** summarizes the modalities used in the analysis of the skin microbiome. Herein, we review the methodology behind amplicon and whole metagenomic sequencing and discuss the need to incorporate the other "omics" in skin microbiome research moving forward.

Metagenomics

At present, the two most common next generation sequencing techniques are 16S rRNA amplicon sequencing (16S) and whole metagenomic shotgun sequencing (WMS). Metagenomics collectively refers to the genomic analysis of a group of microorganisms using DNA extraction and replication techniques [13].

Amplicon sequencing

16S rRNA sequencing, also known as metataxonomics, involves selecting primers that bind to a specific portion of the highly conserved bacterial hypervariable loop region followed by PCR amplification of this region [14]. Recent studies have indicated that 16S tag primers for the V1-V3 region have yielded the most accurate and encompassing results in terms of bacterial abundances and genus classification [14]. The actual steps in performing 16S amplicon sequencing include: collection of cutaneous samples, bacterial DNA isolation and annealing of selected hypervariable loop primer, and amplification of the selected bacterial region through PCR sequencing [14].

Once the samples are sequenced and the results are collected, researchers are tasked with analyzing the metadata, which is typically done using programs such as Mothur or QIIME. The analysis pipeline can be broken down into three steps: preprocessing, clustering operational taxonomic units (OTUs), and annotating OTU tables [14]. Preprocessing can be likened to a quality assurance checkpoint, in which low quality reads and PCR generated chimeric sequences are filtered out. Clustering OTUs is essentially the grouping of similar sequences together. The last step of the analysis, annotation of OTU tables, is the process of comparing clustered OTUs to known reference databases (Silva132, GreenGenes, RDP) in order to assign the appropriate taxonomic classification to the clustered sequences [14].

Although what was described above discusses the methodology for targeting and sequencing bacterial genomes, amplicon sequencing is not limited to prokaryotic organisms. By targeting conserved ribosomal RNA genes specific to fungi, it becomes possible to amplify and sequence eukaryotic genomes as well [15]. Typically, the three most conserved and targeted regions used in fungal metagenomics are internal transcribed spacer (ITS1–ITS2), 18S ribosomal small subunit RNA gene, or D1/D2 domain of the 26S ribosomal large subunit

Table 1. *Various Methods Used to Study the Microbiome*

Technique	Description	Pros	Cons
Culture	Growing microbes on a culture media in the confides of a laboratory setting	Cost effective and relatively simple to perform Can grow populations from a single cell Able to isolate and selectively grow a particular microorganism	Bias toward bacteria that grow in artificial settings Significantly less sensitive in bacterial detection as compared to DNA methods Dramatically underestimates bacterial diversity in collected samples
Metagenomics-Amplicon	Targeting specific conserved genomic regions found in microbes in order to determine taxonomical classifications	Comprehensive reference databases Capable of analyzing large number of samples Plethora of usable primers	Selected variable region primers (V1-V3 and V4) can lead to skewed estimates of relative abundance Samples are subject to PCR amplification bias (i.e. chimera generation) Taxonomical classifications are typically limited to phyla and genera levels
Metagenomics-WMS	Parallel sequencing of thousands of different organisms that may otherwise be unculturable, in order to determine taxonomic classifications	Confidently assigns classifications for many sequences at the species level and in some instances at strain level Allows for simultaneous study of prokaryotes, archaea, viruses, virophages, and eukaryotes Can be complemented with metatranscriptomics, proteomics, metabolomics and metadata to derive mechanistic models that explain the structure and function of the microbiome	Library preparations/sequencing costs 5x more compared to 16S methods Susceptible to contamination of human DNA when there is low biomass available More difficult to analyze data because of significantly larger orders of magnitude of data that is generated requiring filtering from human sequences.
Metatranscriptomics	All RNA present in the microbial community is sequenced in order to determine level of expressional of the genes present and gain insight into the active metabolic pathways	Capacity to elucidate functional expression in collected samples Potential to reconstruct active metabolic pathways in microbial community Possible to determine a specific protein's rate of production	Cost Intensive Difficulty in purifying mRNA from rRNA Based on how samples are collected, high potential of contamination by host RNA Relatively small and limited number of reference databases
Metaproteomics	The functional characterization of the microbial community via measurement of protein abundances and determination of protein species	Can characterize all gene translation products Provides insights on post translational modification Offers information on protein stability and localization	Cannot provide information on protein abundance Subject to inefficient chemical labeling leading to compromised biological coverage
Metabolomics	The detection of metabolites and by-products of microbial activity, providing insight into the subtleties of how microbes may be communicating together	Analyses of hundreds of metabolites in a given sample High predictive capacity for phenotype Resolution of microbial metabolic products/ signaling molecules	Cost Intensive Rather nascent - currently not possible to translate all the data produced into a meaningful biological context Known data bases may contain low quality reference MS spectra

RNA gene [16]. Like amplicon sequencing in prokaryotes, DNA is isolated from the samples of interest and the selected primers targeting a conserved region are introduced to the sample and undergo amplification via PCR. The generated reads are filtered and analyzed by programs, such as QIIME, until data satisfactory to the researchers' standards is produced. The filtered data is compared to online databases (UNITE) for sequence homology and taxonomic classification. Although ITS1–ITS2 tends to be the region most targeted for fungal amplicon sequencing, it has been shown that the variability in length of this region between different organisms generates erroneous estimations of their relative abundances. Thus, it is suggested that researchers use primers targeting 18S or 26S regions in order to generate more accurate and reliable data [16].

In the past, amplicon sequencing was the gold standard for microbiome research and in some instances still is today. The utility of amplicon sequencing relates to the multiple comprehensive genomic reference databases that exist (Silva132, GreenGenes, RDP, UNITE) as well as the affordability and relative ease of performing this technique [17]. Although this form of metagenomics has been a powerful tool in skin microbiome research, it comes with inherent disadvantages.

The major drawbacks that come with amplicon sequencing are related to over exploitation of the conserved sequences within a genome. For example, preferential selection of primers for a region of interest generates a bias in the collected sample and can lead to various organisms being over or underrepresented. Furthermore, PCR amplification has the propensity to generate chimeric sequences within the collected samples being sequenced. Therefore, skewing or lowering the quality of the reads and possible misrepresentation of the taxonomic diversity can occur. In a parallel light, taxonomic classification of samples sequenced through amplicon methods tend be constrained to the phyla, and to a lesser extent, genera levels. With these limitations becoming more apparent over time, newer technologies have been invented in hopes to overcome these constraints [12, 17, 18].

Whole metagenomic shotgun sequencing

Whole metagenomic shotgun sequencing (WMS) is a newer next generation sequencing technology with the capacity to identify approximately twice as many species than amplicon sequencing at certain read depths. It also provides information on the functional potential of the sampled genes [19]. Similar to the methodology for amplicon sequencing, whole metagenomic shotgun sequencing requires collection of cutaneous samples, but instead of using primers to isolate and amplify the gene of interest, all collected DNA samples are sheared into small fragments and sequenced independently [14]. The small amplified sequences produced, known as contigs, are arranged to recreate their respective genomes and are compared against online reference databases to determine the organisms from which the DNA originated. WMS confidently assigns classifications to many sequences at the species level and in some instances at the strain level. This is all done while simultaneously enabling the study of prokaryotes, archaea, viruses, virophages, and eukaryotes along with functional potential classification of the associated gene sequences, ultimately driving the discovery of new microbial genes and genomes. Even though WMS provides insights into the functional potential of collected samples, it cannot resolve the level of expression of any of the present genes [19]. Therefore, the true value of using WMS is a result of its capacity to be coupled with the other 'omics' (metatranscriptomics/proteomics/metabolomics) to generate metadata that can explain the structure and function of the microbiome with greater precision and accuracy.

The major drawbacks of whole metagenomic shotgun sequencing can be narrowed down into two factors - cost and intensity to perform. To amplify the small sheared DNA fragments and analyze the resulting data is significantly more expensive and intense than amplicon sequencing. WMS is also constrained to adequate read depths in order to obtain accurate results, which can be difficult to achieve in skin microbiome research, although significant advances have been made to ameliorate the problem. Researchers encounter challenges in analyzing shotgun metagenomic data occur because of the extensive filtering that is required as a result of sequencing all sampled DNA

(host + microbial), leading to data sets of significantly larger orders of magnitude. Thus, analyses of WMS data take longer to perform and require far more computational power [20]. When utilizing WMS for skin samples, the overabundance of host DNA relative to microbial organisms inhabiting the skin, requires researchers to use more targeted DNA isolation methods and filtration. Otherwise, the contigs produced from these reads could err on the side of inaccuracy and misrepresent the microbes present in the collected samples.

Of the two technologies, WMS is being implemented increasingly in cutaneous microbiome research. This is partially related to the expanding size of the WMS reference databases and the more comprehensive and accurate nature of the technique, but it is mainly because of the reduction in read depth requirements and cost to perform [18].

Using a "Multi-Omic" approach

As researchers shift to utilizing WMS in their projects, they should remain mindful of the benefits that exist when using a "multi-omic" approach. Metagenomics has given researchers a wealth of new information, but even then, classification of sampled representative sequences are limited to the organisms that have been cataloged in known reference databases [10]. In attempts to overcome these limitations and produce accurate data that is more representative of the skin ecosystem, researchers have begun coupling metagenomics with metatranscriptomics, metaproteomics, and/or metabolomics.

Metatranscriptomics

Metatranscriptomics can be defined as profiling of a metatranscriptome, or community wide gene expression. The strength of metatranscriptomics is the ability to elucidate functional expression of microbial genes of microorganisms, despite their ability to be grown in vitro [20]. When complemented with WMS, it becomes possible to gain accurate insights into expression levels of the sequences annotated by metagenomics. With access to such functional data, researchers can determine the metabolic pathways active in microbial communities in different environments and generate profound information pertinent to biomedical advances [20].

The typical procedure for metatranscriptomics begins by collecting samples and purifying the mRNA. Once isolated, the mRNA is converted to cDNA and sequenced concurrently with metagenomic samples [21]. The sequences produced are referred to as RNA-Seq and are compared to online databases, such as HUMAnN, for analyses [20].

Metatranscriptomics is seldom used in skin microbiome research despite the fact that it can be coupled with whole shotgun metagenomic sequencing. This can be attributed to the high costof performing metatranscriptomic sequencing as well as the difficulty that comes with isolating mRNA from the samples. Even once mRNA samples are collected and isolated, mRNA has a short half-life and is degraded quickly, making it difficult to detect at times. Researchers are also faced with the possibility of contamination by host RNA depending on how the samples are collected (i.e. biopsies). The small, yet growing size of the databases to which the RNA-Seqs are compared is another point of contention when considering utilization of metatranscriptomics [20]. However, despite these obstacles and constraints, when coupled with metagenomics, metatranscriptomics proves to be an immensely powerful tool for cutaneous microbiome researchers because it is capable of providing a more holistic view of the skin microbiome [20].

A recent study examined the effects of Vitamin B12 in the pathogenesis of acne as modulated by microbiota. Using metatranscriptomics in order examine expression of the Vitamin B12 biosynthesis pathway in skin microbes, researchers discovered that supplementation of Vitamin B12 to the host repressed Vitamin B12 biosynthesis in the commensal skin species *Cutibacterium acnes* (*C. acnes*), one of the bacteria believed to have a role in the pathogenesis of acne. This increase in host vitamin B12 concentration also increased the concentration of porphyrins, chemicals shown to promote inflammation in acne, produced by *C. acnes* in the follicles of acne patients [22]. These discoveries suggest possibilities for the development of new

treatment options for acne. Evidently, as science marches forward, the combined use of multiple different 'omic' technologies will be necessary in order to yield data that truly represents the complexities of the skin microbiome in different environments.

Metaproteomics

Metaproteomics is defined as, "the large-scale characterization of the entire protein complement of environmental microbiota at a given point in time" [23]. By knowing exactly what proteins are present and in what concentration, it allows for the resolution of the components in a microbial ecosystem that perpetuate its survival. Typically, the identification and quantification of protein species are measured via a shotgun-like methodology, in which peptides are sheared and undergo liquid chromatography–mass spectrometry [23, 24]. The resulting data reveal amino acid sequence, abundance, and the presence of any post translational modifications (i.e. phosphorylation of residues). Finally, the sequence homology is compared to online reference databases (i.e. Pipasic, MetaProteomeAnalyzer, Unipept) to determine the exact protein species present. Experts have suggested to compare the resulting data against multiple databases in order to yield the most accurate results [25]. Like WMS, metaproteomics can be used to analyze the expression of active functional pathways, but with more precision. In metatranscriptomics, measuring the amount of transcript present indicates the rate of production of its protein product. However, knowing the rate of protein production does not accurately relate to the concentration of the protein present nor does it speak about the protein's stability. The appeal of metaproteomics is that it provides researchers more relevant and accurate data in terms of proteins [26]. To date, a very limited number of skin microbiome researchers have incorporated metaproteomics into their research designs. This is in stark contrast to what is seen with gut microbiome research in which various researchers are utilizing metaproteomics alongside metagenomic sequencing [25-27]. This disconnect between gut and skin microbiome research may exist for a number of reasons, such as limited read depths and/or cost restrictions. Nevertheless, as biotechnology continues to advance, the possibility for metaproteomics in cutaneous research becomes increasingly more viable. Metaproteomic techniques allow researchers to track functional genes and metabolic pathways, identify protein expression in various situations (such as microbes under stressors), and aid in alluding to new possible functional genes. All of these capabilities are instrumentally important in elucidating the role of microbes in skin disease pathogenesis.

Metabolomics

The final category of the four "omics" discussed here is known as metabolomics, which involves the identification and quantification of all metabolites present in a sample. Similar to metaproteomics, metabolites are identified and quantified through liquid/gas chromatography as well as mass spectrometry and nuclear magnetic resonance; the typically accurate results produced are contingent upon the purity of the collected samples. The spectra generated are subject to analyses and through it researchers are able to uncover the identity and abundances of metabolites present in the sample. Similar to comparing genomic sample sequences to known taxonomical databases, the generated metabolomic data is compared to known spectral databases, such as the Madison-Qingdao Metabolomics Consortium Database, in order to identify the sample metabolites [10]. As with metaproteomics, the majority of metabolomics research being conducted pertains to the gut microbiome rather than the skin microbiome. There have been a few pivotal skin metabolomic studies described in the literature, such as the work done by Kuehne et al. that demonstrated that there are only minor metabolomic and transcriptional changes as our skin ages [10, 28]. The field of lipidomics is a prime example of the role of metabolomics in cutaneous research, specifically regarding psoriasis. According to research by Zeng et al, lipid metabolites such as LDL-C, HDL-C, AND APOA-I have been found to be abnormal in serum lipids of psoriatic patients [29]. The researchers found that lysophosphatidic acid, lysophosphatidylcholine, and

phosphatidic acid were elevated in patients suffering from psoriasis, indicating a novel route of pathogenesis not previously examined in skin microbiome research.

Despite cost barriers, utilization of metabolomics allows cutaneous researchers to examine the subtle nuances of microbial communities, such as the signaling processes used between bacteria in communication [10]. Coupled with WMS, metabolomics paves the way to reconstruct the intricacies of whole networks of microbial communities.

Discussion

As it stands, a majority of skin microbiome research conducted today still heavily relies on amplicon sequencing, both because it is the least laborious and least cost intensive. However, as biotechnology continues to advance, the utility of next generation sequencing methods, such as WMS, are proving to be more useful than its amplicon sequencing counterpart. By its ability to distinguish variations in organisms at the strain level and allowing predictive measures of the functional capacity of genes, WMS is starting to take hold in skin microbiome research. However, despite this shift towards WMS, a majority of skin microbiome researchers have yet to incorporate other 'omic' methods in their research deigns. Coupling metagenomic research with metatranscriptomics, metaproteomics, and/or metabolomics paints a much more comprehensive and detailed picture of what is occurring between the host and skin microbes in healthy and diseased states. As skin microbiome research progresses it will become imperative to look beyond just "what microbes are present?" and focus in on, "what are the microbes present doing?" The logical next step in furthering the knowledge of pathological conditions of the skin involves examining what microbes are producing, how are they producing it, and what the downstream effects of these products are. As the requirements for read depths and labor intensity decrease, it is inevitable that the other "omics" will make their way into skin microbiome research; it is just a matter of how quickly researchers are willing to adopt these practices [10].

Conclusion

Today, researchers investigating the skin microbiome are placed into the unique position of being at the forefront of elucidating new information that was once considered out of reach. Gone are the limitations that encumbered scientists when growing microbes in vitro. With access to next generation sequencing technologies, investigators can now examine the nuanced interplays between microbes within the microbiome and between the microbes and host as well. With this wealth of new information being generated, we stand at the threshold of skin microbiome research. As we enter this new era ushered in by "omics" technology, one cannot help but think — what a time to be alive.

Potential conflicts of interest

RKS serves a medical editor for LearnHealth and as a consultant for Burts' Bees, Dermala, and Tomorrow's Leaf. The remaining authors declare no conflicts of interests.

References

1. Turnbaugh PJ, Ley RE, Hamady M, et al. The human microbiome project. *Nature*. 2007;449:804-10. [PMID: 17943116].
2. Ursell LK, Metcalf JL, Parfrey LW, Knight R. Defining the human microbiome. *Nutr Rev*. 2012;70 Suppl 1:S38-44. [PMID: 22861806].
3. Human Microbiome Project C. Structure, function and diversity of the healthy human microbiome. *Nature*. 2012;486:207-14. [PMID: 22699609].
4. Cogen AL, Nizet V, Gallo RL. Skin microbiota: a source of disease or defence? *Br J Dermatol*. 2008;158:442-55. [PMID: 18275522].
5. Rocha MA, Bagatin E. Skin barrier and microbiome in acne. *Arch Dermatol Res*. 2018;310:181-5. [PMID: 29147769].
6. Dessinioti C, Katsambas AD. The role of Propionibacterium acnes in acne pathogenesis: facts and controversies. *Clin Dermatol*. 2010;28:2-7. [PMID: 20082942].
7. Grice EA, Segre JA. The skin microbiome. *Nat Rev Microbiol*. 2011;9:244-53. [PMID: 21407241].
8. Owen CM, Chalmers RJ, O'Sullivan T, Griffiths CE. A systematic review of antistreptococcal interventions for guttate and chronic plaque psoriasis. *Br J Dermatol*. 2001;145:886-90. [PMID: 11899140].
9. Byrd AL, Belkaid Y, Segre JA. The human skin microbiome. *Nat Rev Microbiol*. 2018;16:143-55. [PMID: 29332945].
10. Aguiar-Pulido V, Huang W, Suarez-Ulloa V, et al. Metagenomics, Metatranscriptomics, and Metabolomics Approaches for Microbiome Analysis. *Evol Bioinform Online*. 2016;12:5-16. [PMID: 27199545].

11. Kong HH, Andersson B, Clavel T, et al. Performing Skin Microbiome Research: A Method to the Madness. *J Invest Dermatol*. 2017;137:561-8. [PMID: 28063650].
12. Ferretti P, Farina S, Cristofolini M, et al. Experimental metagenomics and ribosomal profiling of the human skin microbiome. *Exp Dermatol*. 2017;26:211-9. [PMID: 27623553].
13. Handelsman J. Metagenomics: application of genomics to uncultured microorganisms. *Microbiol Mol Biol Rev*. 2004;68:669-85. [PMID: 15590779].
14. Meisel JS, Hannigan GD, Tyldsley AS, et al. Skin Microbiome Surveys Are Strongly Influenced by Experimental Design. *J Invest Dermatol*. 2016;136:947-56. [PMID: 26829039].
15. Kong HH, Segre JA. The Molecular Revolution in Cutaneous Biology: Investigating the Skin Microbiome. *J Invest Dermatol*. 2017;137:e119-e22. [PMID: 28411842].
16. De Filippis F, Laiola M, Blaiotta G, Ercolini D. Different Amplicon Targets for Sequencing-Based Studies of Fungal Diversity. *Appl Environ Microbiol*. 2017;83:00905-17. [PMID: 28625991].
17. Jo JH, Kennedy EA, Kong HH. Research Techniques Made Simple: Bacterial 16S Ribosomal RNA Gene Sequencing in Cutaneous Research. *J Invest Dermatol*. 2016;136:e23-7. [PMID: 26902128].
18. Jovel J, Patterson J, Wang W, et al. Characterization of the Gut Microbiome Using 16S or Shotgun Metagenomics. *Front Microbiol*. 2016;7:459. [PMID: 27148170].
19. Ranjan R, Rani A, Metwally A, et al. Analysis of the microbiome: Advantages of whole genome shotgun versus 16S amplicon sequencing. *Biochem Biophys Res Commun*. 2016;469:967-77. [PMID: 26718401].
20. Bashiardes S, Zilberman-Schapira G, Elinav E. Use of Metatranscriptomics in Microbiome Research. *Bioinform Biol Insights*. 2016;10:19-25. [PMID: 27127406].
21. Franzosa EA, Hsu T, Sirota-Madi A, et al. Sequencing and beyond: integrating molecular 'omics' for microbial community profiling. *Nat Rev Microbiol*. 2015;13:360-72. [PMID: 25915636].
22. Kang D, Shi B, Erfe MC, et al. Vitamin B12 modulates the transcriptome of the skin microbiota in acne pathogenesis. *Sci Transl Med*. 2015;7:293ra103. [PMID: 26109103].
23. Wilmes P, Heintz-Buschart A, Bond PL. A decade of metaproteomics: where we stand and what the future holds. *Proteomics*. 2015;15:3409-17. [PMID: 26315987].
24. Zhang X, Chen W, Ning Z, et al. Deep Metaproteomics Approach for the Study of Human Microbiomes. *Anal Chem*. 2017;89:9407-15. [PMID: 28749657].
25. Heyer R, Schallert K, Zoun R, et al. Challenges and perspectives of metaproteomic data analysis. *J Biotechnol*. 2017;261:24-36. [PMID: 28663049].
26. Xiong W, Abraham PE, Li Z, et al. Microbial metaproteomics for characterizing the range of metabolic functions and activities of human gut microbiota. *Proteomics*. 2015;15:3424-38. [PMID: 25914197].
27. Xiao M, Yang J, Feng Y, et al. Metaproteomic strategies and applications for gut microbial research. *Appl Microbiol Biotechnol*. 2017;101:3077-88. [PMID: 28293710].
28. Kuehne A, Hildebrand J, Soehle J, et al. An integrative metabolomics and transcriptomics study to identify metabolic alterations in aged skin of humans in vivo. *BMC Genomics*. 2017;18:169. [PMID: 28201987].
29. Zeng C, Wen B, Hou G, et al. Lipidomics profiling reveals the role of glycerophospholipid metabolism in psoriasis. *Gigascience*. 2017;6:1-11. [PMID: 29046044].