



## Role of Next Generation Sequencing (NGS) in Searching for Promising Lactic Acid Strains Used as Animal Probiotic

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### Abstract

Next generation sequencing (NGS) has revolutionized the research related to the study of animal gut. Scientists point out, that little information has been investigated regarding the link between the animal health production and ruminal gut ecosystem. Hence, we review the advance progress in studying the host-microbial interactions. Scientists have developed/used new molecular approaches and computational techniques for metagenomic and whole genome sequencing analysis to search the promising probiotic strains which has the capability to improve milk yield. For the preparation of the unique probiotic product researcher should work on the isolation of microbial flora to conventional microbial molecular techniques merged with complex algorithms of computational efficiency for whole genome sequence analysis. Ruminal-gut microbiota is considered as a major source of unique probiotic bacterial strains. Probiotics are "live microorganisms which when administered in adequate amounts confer a health benefit on the host".

**Keywords:** Next Generation Sequencing (NGS); Lactic Acid Bacteria (LAB); Animal Probiotic

### Introduction

Lactic acid bacteria (LAB) and yeast strains are commonly used as probiotic. These strains has the ability to ferment foods, improve milk yield in dairy animals and meat quality in poultry and fish industry [1,2]. Literature showed that for getting the best results from these microbial species, the selection and source of the bacterial and yeast strains plays a major role. There is limited information available in the searching of promising probiotic and metabolic capabilities that associated with these fermentations and stunted ability to identify appropriate starter strains and/or modifications to the fermentation environment in pursuit of improved product [3]. Consequently, the dairy industry has been subject to the widest application of next-generation sequencing to the LAB involved (Table 1), yielding the benefit of well-developed and characterized probiotic that allows increased probiotic potential as a whole. Microbial culture approaches are primary methods for the study of lactic acid bacterial strains but the culturing are time taking and laborious method. While these methods have drawbacks, for microbial identification, classification and advanced level characterization, the livestock and dairy industries are reliant on advanced molecular techniques, such as NGS [4]. Literature told us that the phenotypic and biochemical characterization are

not sufficient to differ between the microbial strains since some bacterial strains of *Lactis* ssp. *cremoris* display the characteristics of *L. lactis* ssp. *lactis* [5,6].

Items	Methods	Used for
Genomics	Single organism DNA.	Study the genetic capacity.
Functional genomics	Specific genes of known function in single organisms and/or a mixed microbial community.	To understand the presence and distribution of functional genes or known genetic attributes within isolates or a microbial community.
Metagenomics	NGS sequencing of a mixed microbial community via sequencing a specific genetic marker (i.e. 16S rRNA amplicon) or the total DNA content of a community.	To understand the composition (what microbial species are present) and complexity of a microbial community. Provides information for potential metabolic capacity of the community.
Transcriptomics	NGS sequencing of mRNA transcripts of a single organism growing in a given condition.	To understand what genes are expressed and/or required for growth or survival in a given condition.

**Table 1:** Next-generation sequencing techniques.

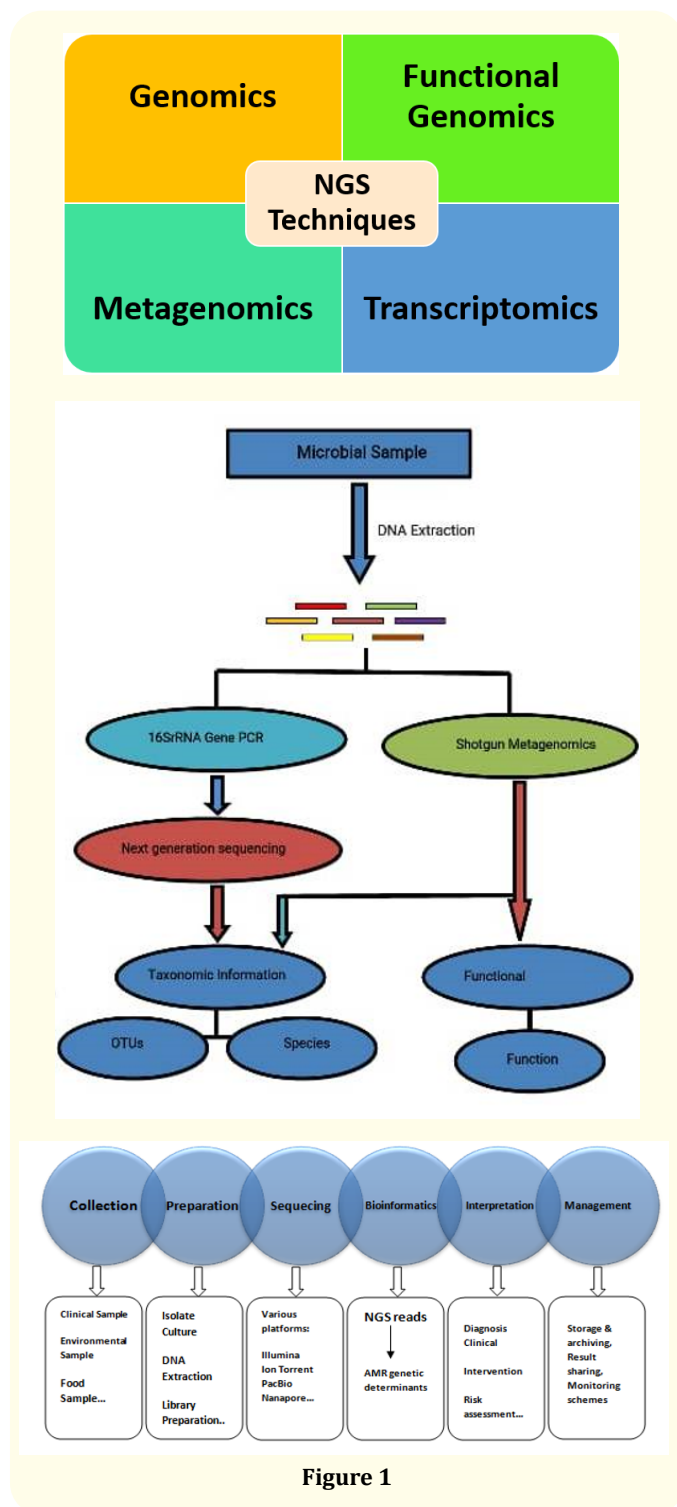


Figure 1

**Modern techniques for isolation and identification of probiotics**

The assessment, identification and characterization of the microbiome based on phenotypic properties has always been the focus of research and classification, e.g. morphology, growth at different temperatures, mode of glucose fermentation, fermentation of various carbohydrates and lactic acid configuration is now useless because some species are not easily distinguishable by pheno-

typic characteristics and the taxa produced do not correspond to the recommended phylogenetic relations and it was confirmed on the basis of comparative 16S ribosomal RNA sequencing analysis. By modern molecular techniques probiotic strains are now being identified and differentiated which include PCR based and molecular typing methods such as repetitive PCR, RFLP and PFGE [7]. In a particular environment we can assess the total genetic pool of all the microbes, in a culture independent manner and uncover novel genes with the help of sequence-based and functional metagenomics as functional screen for hemolytic clones in an *E. coli* metagenomic library helps in discovery of antibiotics turbomycin A and B. To identify and develop novel therapies and in solving of antibiotic resistance issues metagenomics can play a key role [8]. NGS analysis based on rRNA associated sequences helps in determining the probiotic supplements composition by decoding the probiotic strains genome sequence. It can also exposed genetic factors of genomic evolution and *Lactis* BB-12 stability of frozen dried stock 1995 when compared to the genome of reconstructed probiotic supplement strain of 2017 [9].

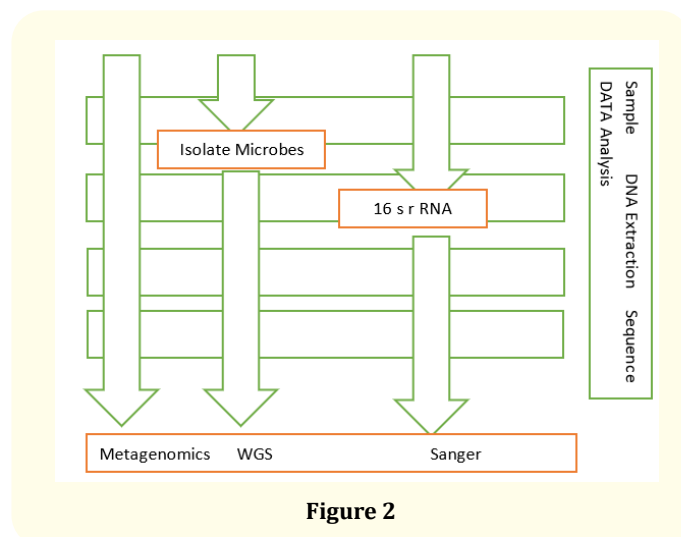


Figure 2

For characterization of bacterial communities illumina next generation sequencing was used for 3 fish groups and 8 distinguish eels' intestinal sites. This study helped the farmers by proving that rich *Spiroplasma* genus was found in the guts of stunted growth eels and is related with retardation in growth [10]. From faecal samples of dog probiotics like *L. johnsonii*, *L. reuteri*, *L. reuteri* AI were isolated and by using morphology and was confirmed by molecular characteristics through 16S rRNA gene sequence with the blast database. Their probiotic properties were screened by various tests such as bile salts, survival to gastric and pancreatic juices. Anti-pathogenicity and antibiotics resistance was also checked because control of pathogenic microorganisms of intestines in dogs is of great concern and to overcome some of the gut problems related with the modern domestication of animals is a valuable tool by

selection of autochthonous probiotic bacterial strains and can be used as nutritional supplements in feed [11]. By phylogenetic linear modelling the microbial genotype and phenotype relations can be determined. By using a summary of shotgun sequencing data the phylogenetic relatedness of gut prevalence was determined for thousands of species, and microbial genes that were involved in gut adaptation can be found, we may be able to exclude pathogens and support the growth of beneficial microbes. In an environment the prevalence and specificity of bacterial species can be estimated with the help of shotgun metagenomics data, like in the case of human gut, it should correlates their ability to colonize their specific environment and their adaptation status [12].

### Metagenomics

Functional capabilities and taxonomic composition of gut microbiota of freshwater carp (*Labeorohita*) is understood with the help of shotgun metagenomics. It was found that 3<sup>rd</sup>/4<sup>th</sup> of gut microorganisms belongs to Proteobacteria and were resistance against twenty four antibiotic types because 51 different types of

antibiotic resistance genes belonging to fifteen antimicrobial resistance gene families were present in flora and there is the possibility that horizontal gene transfer may occurred due to the restricted gut environment [13]. Deep shotgun sequencing can help in quantitative metagenomics study and development of new diagnostic tools and possible treatments due to identification of biomarkers for ankylosing spondylitis when metagenomics study was applied on gut microbial DNA from 211 Chinese individuals [14]. Metagenomics and analysis of 16S of the GI microbiota of grass carp (*Ctenopharyngodon idellus*) helps in identification of 5 AMPs that are small peptides group secreted by almost all creatures naturally and are toxic to many bacteria. For micro-ecologics or probiotic supplements they may be served as alternative pathogen antagonists (toxins) because in the gastrointestinal tract of grass carp and some commercially popular probiotics there was phylogenetic relationship was found between AMPs producing bacteria and also high similarities of previously reported bacterial toxins, such as antibiotic and class II bacteriocins were also reported [15].

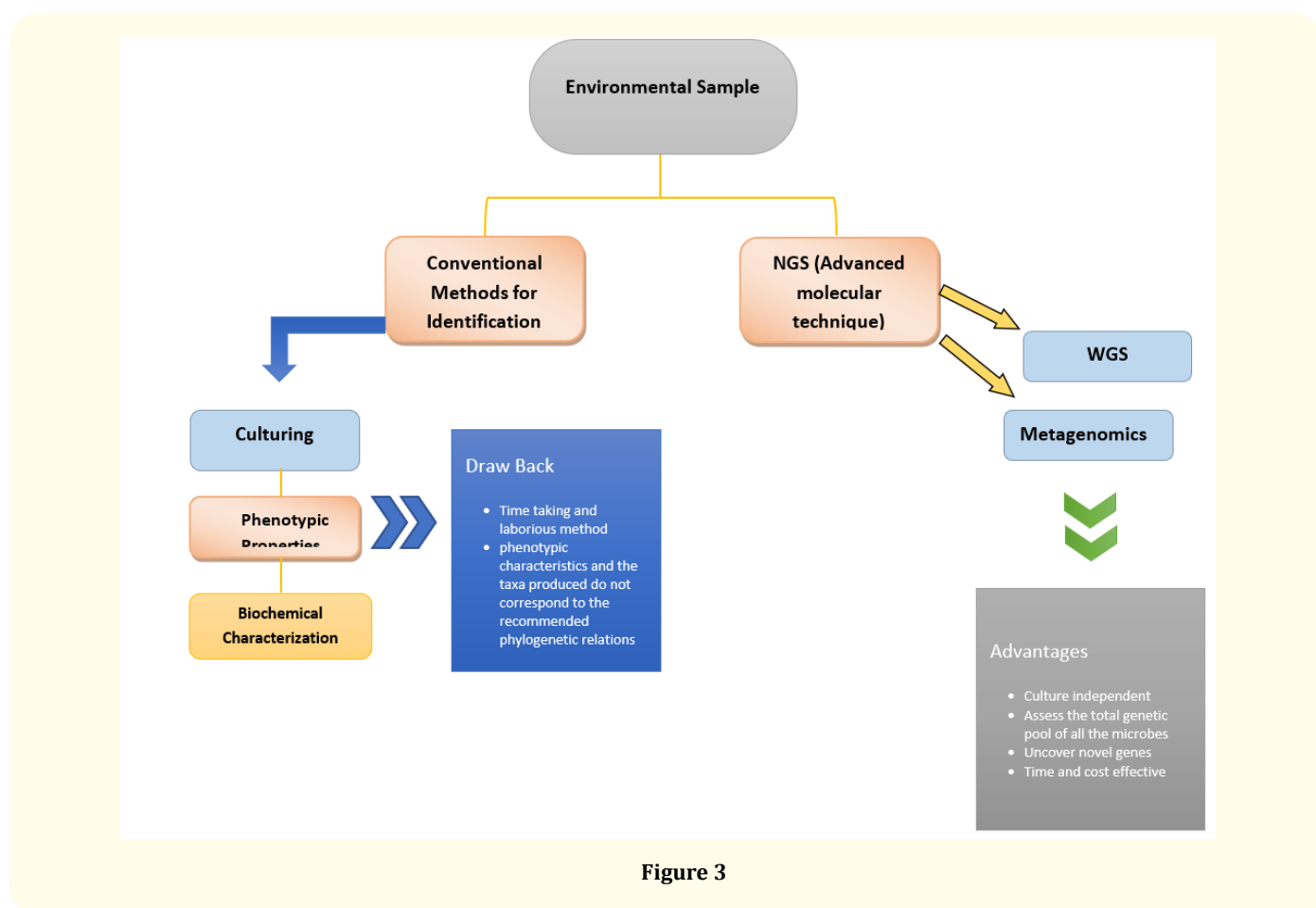


Figure 3

### Whole genome sequencing of microbial strains

During the past five years complete genome sequences of thirty microbial species have been determined, and work in progress show that the complete sequences of more than hundred further

microbial species will be available in the next 2 to 4 years. These results have exposed a great amount of information on the physiology and evolution of species of microbes and should provide novel approaches to the diagnosis and treatment of infectious disease.

For the practice of clinical microbiology whole genome sequencing promises to be transformative, and the rapidly decreasing cost and turnaround time mean that in diagnostic and reference labs this will soon become a viable technology [16]. We propose that the most important areas for the application of whole genome sequencing are molecular epidemiology conducted for surveillance and outbreak survey and the genotype antimicrobial susceptibility test for microbes that are hard to cultivate. The GIT of mammals is one of the richest and most diverse microbe communities in a heterogeneous ecosystem. The GI microbiota, with more than one hundred genes included in the human genome, offers the host positive functional features, such as nutrient metabolism, immune tolerance and resistance to colonization. Gut microbiota dysbiosis may have significant harmful effects on the host, such as cancer, obesity, neurological disorders, susceptibility to pathogens, inflammatory dysregulation and malnutrition. Identifying genes in the prokaryotic genome has progressed to a point where almost every protein coding region can be confidently recognized. Computational gene finders using Markov [17].

### Metatranscriptomics

Researchers and scientists use metatranscriptomics to test and analyze the expression profiles of mRNA in microbial communities. Gene recognition, genetic pathway and their regulation, associations between host and microbes and symbiotic relationships between microbes can be easily identified using the mRNA expression data. Metatranscriptome can be used in animal's microbiota to recognize endogenous probiotic yeast. The methods of sampling and molecular techniques should be improved for this purpose [18].

### Metabolomics

The study of the metabolites or final cellular products is called metabolomics. This is also considered as one of the useful and effective methods for determining a microorganism's probiotic potential within an animal's microbiota or selected biological sample [19]. The endogenous probiotic yeast ability can also be evaluated using this method. The role of metabolites in the form of probiotic potential and other inhibiting roles in metabolic compounds are still needed for complete understanding. Because metabolites vary in structure and function, comparative studies might be used for the species and populations. Metabolomics can be used to explore many species with high probiotic potentials [20].

### Conclusion

For the preparation of the unique probiotic product researcher should work on the isolation of microbial flora to conventional microbial molecular techniques merged with complex algorithms of computational efficiency for whole genome sequence analysis. Ruminant-gut microbiota is considered as a major source of unique

probiotic bacterial strains. Probiotics are "live microorganisms which when administered in adequate amounts confer a health benefit on the host".

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