ACTA SCIENTIFIC NUTRITIONAL HEALTH (ISSN:2582-1423)

Volume 4 Issue 7 July 2020

Review Article

Whole-Genome Sequencing of *Lactobacillus fermentum* and its Application as Probiotic in Poultry Feed

Rafia Sameen[1] and Shakira Ghazanfar[2]*

[1]*Department of Animal Genomics and Biotechnology, PIASA National Agriculture Research Centre, Islamabad, Pakistan*

[2]*National Institute of Genomics and Agriculture Biotechnology (NIGAB), National Agriculture Research Centre, Islamabad, Pakistan*

***Corresponding Author:** Shakira Ghazanfar, National Institute of Genomics and Agriculture Biotechnology (NIGAB), National Agriculture Research Centre, Islamabad, Pakistan.

Received: May 22, 2020

Published: June 19, 2020

Abstract

Aviculture is the efficient animal production system and good source of animal protein worldwide. Poultry gastrointestinal tract houses certain microbial communities with bacteria being dominant above all. These bacteria produce beneficial products and result in non-pathogenic immune response providing nutrition and protection for animals. Antibiotic treatment causes reduction of beneficial bacterial population in intestine which can be controlled by probiotic supplements. Probiotics play their role to control intestinal pathogens by competing for adhesion sites and nutrients, producing anti-bacterial substances. Lactic acid bacteria could be a good probiotic for animal use among which *Lactobacillus fermentum* is major heterofermentative specie found to have probiotic potential and can be used in supplements for animal feed. Its probiotic potential was well studied by its tolerance to inhibitory substances like bile and salt, its antimicrobial activity and evaluation by supplementing it in poultry feed. To identify probiotic properties of *Lactobacillus fermentum*, its whole-genome was sequenced and analysed. Whole genome sequencing is a DNA sequencing technology that has revolutionized genomic research. Whole genome is sequenced, assembled and annotated using bioinformatics tool and then analyzed. Galaxy is one of most commonly used genome analysis tool that provides data analysis support through framework, to give simple interfaces to certain powerful tools and automatically manage computational details. Following to evaluation of probiotic potential through certain parameters and whole genome sequencing, antimicrobial resistance can also be evaluated to make a decision about safety of *L. fermentum* use as probiotic in poultry feed. Administration of *L. fermentum* to poultry showed beneficial effects on their growth.

Keywords: Probiotics; Lactobacillus fermentum; Whole Genome Sequencing (WGS); Galaxy

Introduction

Aviculture forms basis of protein production worldwide and efficient animal production system [1]. The gastrointestinal track of poultry is highly populated with microbial communities including fungi, bacteria, archaea, viruses and protozoa with bacteria being dominant above all [2]. Bacteria colonizing GI track produce short chain fatty acids (butyric acid, propionic acid and acetic acid), vitamin B group and vitamin K, organic acids e.g. lactic acid, lower triglycerides, antimicrobial compounds (bacteriocins) and result in non-pathogenic immune response, providing protection and nutrition for animal [3]. On other side, GI track can also house bacterial pathogens (*Salmonella* and *Campylobacter*) which can pose threat for food safety and public health by disseminating to human or acting as a pool of antibiotic resistance and transmission [4,5]. The GI microbiota can be more deeply classified into luminal and mucosal microbiota. Available nutrients, antimicrobial concentration and

feed passage rate are important determinants of composition of luminal microbiota while mucosal-attached microbiota composition is controlled by certain host factors like expression of specific sites for adhesion on enterocyte surface, secondary immunoglobulin secretion, and production rate of mucous. Bacterial count is 10^8 to 10^9 cfu/g in crop, 10^7 to 10^8 cfu/g in gizzard, 10^8 to 10^9 cfu/g in small intestine and 10^{10} to 10^{11} in caeca [6]. *Lactobacillus* species are dominant in crop as well as in small intestine where they account for 70% of total [7]. *Salmonella enterica, Campylobacter, Clostridium perfringens* and *Escherichia coli* are human disease causing taxa reported in chicken microbiota [8]. Antibiotic treatment causes reduction of *Lactobacillus* population in intestine [5] which can be controlled by probiotic supplements [1].

Poultry industry is striving to find alternatives to antibiotics that could maintain performance and also economically feasible to meet market standards and demands of international health organisation. Probiotics are a good option among these products [9]. Probiotics play their role to control intestinal pathogens by competing for adhesion sites and nutrients, producing anti-bacterial substances (acetic acid, lactic acid, antibiotic-like substances), alteration (increase/decrease) in enzyme activity, higher antibody level and enhanced macrophage activity [10]. Lactic acid bacteria could be a good probiotic for animal and human use. To declare a LAB strain as potential probiotic (1) it should be generally recognised as safe (2) it should be bile and acid tolerant (3) should have ability to adhere to host intestinal epithelium (4) it should exhibit antagonistic activity in opposition to pathogenic bacteria (5) have ability to be viable during processing and storage [11].

An important genus of lactic acid bacteria is gram positive *Lactobacillus* widely found in gastrointestinal track of animals among which *Lactobacillus fermentum* is major heterofermentative specie [12] found to have probiotic potential and can be used in supplements for animal feed. *Lactobacillus fermentum* have been shown to have antimicrobial activity and effects similar to antibiotics in feed [13]. *Lactobacillus fermentum* grows at 45°C. Type strain of *Lactobacillus fermentum is ATCC* 14931 and genome has been sequenced from IFO 3956 having 2.09 Mb size with GC content of 52 - 54% [14]. *L. fermentum* strains have shown bile and acid tolerance, survival in GI conditions and antagonistic effects against *Salmonella* spp., *Citrobacter* spp., *Klebsiella* spp., *Escherichia coli, Staphylococcus aureus* and *Shigella sonnei* [9,11]. *Lactobacillus fermentum* TMU12 have been identified to have high tolerance to bile salt and high cell surface hydrophobicity [15].

Next generation sequencing (NGS) is a DNA sequencing technology that has revolutionized genomic research. Certain NGS platforms are available using different technologies for sequencing [16]. Analysis of whole genome sequencing has revolutionized the safety related to food [17]. To identify probiotic properties of *L. fermentum,* its whole-genome has been sequenced and analysed (Figure 1).

Figure 1

Probiotic analysis

Probiotic properties of *Lactobacillus fermentum* was evaluated by inoculating the sample taken from chicken gut in MRS sterilized broth. Decimal dilutions of samples were prepared and suspended in broth for enrichment purpose and incubated under anaerobic conditions for 48hrs at 37°C. Identification of selected strain was carried out by physiological characters, motility, staining and biochemical assays and identified as *Lactobacillus fermentum* showing single, short and square bacilli, producing gas from glucose, and grow well at 45°C [10,13].

Tolerance to inhibitory substances

When 1% test organism was inoculated with MRS broth containing 0.3 or 10% bile, 4 - 8% sodium chloride and 0.3 or 0.4% phenol and incubated for 72h at 37°C, strains tolerate 0.3 - 10% bile 0.3 - 0.4% phenol but not with 8 percent salt [10]. For determination or acid tolerance, cells from overnight culture was inoculated with crop fluid content and incubated for 3h at 37°C. Results showed better acid tolerance by *L. fermentum* [9].

Antimicrobial activity

Inoculate 1% of growing culture in sterile MRS broth having pH 6 and incubate for 24h at 37°C. Fermented broth was then centrifuged to obtain test material and remove microbial cells. The remaining liquid was dried under vacuum using rotary evaporator and 45°C water bath, then re-suspended in water and filtered through 0.45 mm membrane filter. Antimicrobial activity was checked against *Escherichia coli, Staphylococcus aureus and Salmonella typhimurium* grown in nutrient broth at 37°C for 24 hours and then poured to plate with agar and allowed to solidify. Test material was diluted with equal volume of molten agar and 0.2 ml was added through pipette in 1 cm wide cut across centre of agar dish and plate was incubated and zone of inhibition was measured [10]. *L. fermentum* strains have inhibitory effects on pathogenic bacteria which may be due the production of organic acids, H_2O_2 and specific bacteriocins [11,13].

Whole genome sequencing

Genome of *L. fermentum* was sequenced using combination of illumine paired-end sequencing and 454 sequencing technology. Genome libraries with 3kb insert were constructed and 49.643 single end and 174,200 pair end reads were generated by GS-FLX system giving coverage of 28.8-fold genome [19]. Rapid progress in sequence technology and development of bioinformatics tolls has revolutionized the field being reachable for individual research groups [20]. For whole genome sequencing, intact high quality, non-degraded DNA is required in sufficient amount. For sequencing, ~1 mg DNA is required as starting material [20]. DNA is extracted by centrifuging liquid culture media and re-suspend pellet in PBS. Cells are lysed by lysozyme, vortex and incubated. DNA is extracted by DNeasy® Blood and Tissue Kit Quick-start protocol. DNA is eluted and treated with RNase and incubated at room temperature for 1h [21]. DNA is fragmented before sequencing via enzymatic digestion, high frequency sound waves, or transposase, or binding pools of amplicon to DNA fragments for amplification of target region going to be sequenced in parallel PCRs [22]. Libraries are prepared and amplified. Pooled amplified library is diluted to form diluted amplified library. MiSeq sample sheet is generated using Illumina experiment manager. DAL is transferred to MiSeq reagent catridge and insert into MiSeq instrument for sequencing to start (Figure 2) [21].

Figure 2: Process of obtaining whole genome sequence data from bacteria culture.

Genome assembly

Amount of data generated in genome sequencing is staggering up to several hundred gigabytes. An appropriate data management system is needed is needed at start of project. Bioinformatics experts provide a link between researchers and computing grid sys-

tem experts [23]. Before assembly of data, quality of sequencing data, repeat abundance GC content or fraction of duplicate reads should be checked. A number of software is available for *de novo* assembly of whole genome sequencing data. After initial contig building, contigs are combined into scaffolds by using read-pair information from long inserts. In many commonly used programs for assembly, step for scaffolding is already included. While choosing software for assembly, both the sequencing data amount and available computational resources should be considered. It should also take into account whether to invest in commercial software or use freely available program [20].

Genome annotation

Genome sequence is annotated with biologically relevant information ranging from functional information and gene models to microRNA and epigenetic modifications to harness its full potential. Automated gene annotation is possible for newly sequenced species where lack of pre-existing gene models exists [24]. Annotation has conceptually two phases, a "computational phase", in which initial gene and transcript prediction is created using evidence from other genomes or specie-specific transcriptome data. In second "annotation phase", all information is synthesized into gene annotation [20].

Galaxy; a genome analysis tool

Galaxy is software that provides data analysis support through framework, to give simple interfaces to certain powerful tools and automatically manage computational details. Galaxy is available both as publically available Web service providing tools for genomic, functional genomics and comparative genomic data analysis, or can been downloaded for use in individual laboratories. It allows performing large scale analysis without programming expertise and informatics with just a Web Browser [25]. Computer with modern web browser supporting HTML5 and JavaScript is required to use Galaxy interface. Recent internet browsers such as Chrome, Firefox, Opera and Safari are supported [26]. Galaxy has different protocols to cover basic functionality aspects. Basic Protocol 1 is introduction to Galaxy and Basic Protocol 2 is data manipulation to find coding exons with SNPs. Basic Protocol 3 describes how to generate work flow from history and Basic Protocol 4 describes workflow generation from scratch. Basic Protocol 5 describes extracting alignments and sequences with Galaxy. Galaxy is progressing rapidly on monthly basis by adding new features and tools [25] (Figure 3).

Figure 3: Galaxy: A genome analysis tool (Blankenberg D., *et al.* 2014).

Identifying antimicrobial resistance genes for safety

Assembled genome is BLASTed with genes from ResFinder database and best matching genes are given as output if present. To report a gene as resistant, it should cover 2/5th of resistance gene length in database. To detect a % identity threshold is possible [27].

Evaluation of probiotic activity in chicken

L. fermentum has been evaluated as probiotic for poultry feed supplement. In a study, 120 chicks were evaluated for effect of *L. fermentum*. *L. fermentum* were administered for 12 weeks in feed without any antibiotics in feed. After 12 weeks' chicks were administered for body weight and results showed that *L. fermentum* have similar effects like antibiotics manifested by efficiency of feed in chick growth [13]. According to study (Khan 2007), single dose of intragastrically administered *L. fermentum* can improve food conversion efficiency and weight gain of broiler chicken [18].

Conclusion

Lactobacillus fermentum found to have probiotic potential and can be used in supplements for animal feed. Whole genome sequence, tolerance to high concentration of salt and bile and antimicrobial activity has illustrated its safety and beneficial effects.

Administration to poultry clearly showed it ability to confer good health benefits. *L. fermentum* could be proved to be a major probiotic feed supplement to avoid extensive use of antibiotics and maintain healthy gut microbiota.

Bibliography

1. Clavijo Viviana and Martha Josefina Vives Flórez. "The gastrointestinal microbiome and its association with the control of pathogens in broiler chicken production: a review". *Poultry Science* 97.3 (2018): 1006-1021.

2. Wei S., *et al*. "Bacterial census of poultry intestinal microbiome". *Poultry Science* 92.3 (2013): 671-683.

3. Yegani M and DR Korver. "Factors affecting intestinal health in poultry". *Poultry Science* 87.10 (2008): 2052-2063.

4. Kumar Sanjay., *et al*. "Effect of antibiotic withdrawal in feed on chicken gut microbial dynamics, immunity, growth performance and prevalence of foodborne pathogens". *PLoS One* 13.2 (2018).

5. Mancabelli Leonardo., *et al*. "Insights into the biodiversity of the gut microbiota of broiler chickens". *Environmental Microbiology* 18.12 (2016): 4727-4738.

6. Shang Yue., *et al*. "Chicken gut microbiota: Importance and detection technology". *Frontiers in Veterinary Science* 5 (2018): 254.

7. Han Geon Goo., *et al*. "Relationship between the microbiota in different sections of the gastrointestinal tract, and the body weight of broiler chickens". *Springerplus* 5.1 (2016): 911.

8. Oakley Brian B., *et al*. "The chicken gastrointestinal microbiome". *FEMS Microbiology Letters* 360.2 (2014): 100-112.

9. Idoui Tayeb. "Probiotic properties of Lactobacillus strains isolated from gizzard of local poultry". *Iranian Journal of Microbiology* 6.2 (2014): 120.

10. Reque Elizete de F., *et al*. "Isolation, identification and physiological study of Lactobacillus fermentum LPB for use as probiotic in chickens". *Brazilian Journal of Microbiology* 31.4 (2000): 303-307.

11. Lin Wen-Hsin., *et al*. "Different probiotic properties for Lactobacillus fermentum strains isolated from swine and poultry". *Anaerobe* 13.3-4 (2007): 107-113.

12. Dickson EM., *et al*. "A novel species-specific PCR assay for identifying Lactobacillus fermentum". *Journal of Medical Microbiology* 54.3 (2005): 299-303.

13. Raja M Mohamed Mahroop., *et al*. "Lactobacillus as a probiotic feed for chickens". *International Journal of Poultry Science* 8.8 (2009): 763-767.

14. Ibrahim Salam A. "Lactic Acid Bacteria: *Lactobacillus* spp.: Other Species" (2016).

15. Torshizi MA., *et al*. "Screening of indigenous strains of lactic acid bacteria for development of a probiotic for poultry". *Asian-Australasian Journal of Animal Sciences* 21.10 (2008): 1495-1500.

16. Behjati Sam and Patrick S Tarpey. "What is next generation sequencing?". *Archives of Disease in Childhood-Education and Practice* 98.6 (2013): 236-238.

17. Pightling, Arthur W., *et al*. "Interpreting whole-genome sequence analyses of foodborne bacteria for regulatory applications and outbreak investigations". *Frontiers in Microbiology* 9 (2018): 1482.

18. Khan M., *et al*. "Growth-promoting effects of single-dose intragastrically administered probiotics in chickens". *British Poultry Science* 48.6 (2007): 732-735.

19. Sun Zhihong., *et al*. "Complete genome sequence of the probiotic *Lactobacillus fermentum* F-6 isolated from raw milk". *Journal of Biotechnology* 194 (2015): 110-111.

20. Ekblom, Robert and Jochen BW Wolf. "A field guide to whole-genome sequencing, assembly and annotation". *Evolutionary Applications* 7.9 (2014): 1026-1042.

21. Gautam Sanjay S., *et al*. "A step-by-step beginner's protocol for whole genome sequencing of human bacterial pathogens". *Journal of Biological Methods* 6.1 (2019).

22. Dunn Paul., *et al*. "Next generation sequencing methods for diagnosis of epilepsy syndromes". *Frontiers in Genetics* 9 (2018): 20.

23. Lampa Samuel., *et al*. "Lessons learned from implementing a national infrastructure in Sweden for storage and analysis of next-generation sequencing data". *Gigascience* 2.1 (2013): 2047-217X.

24. Yandell Mark and Daniel Ence. "A beginner's guide to eukaryotic genome annotation". *Nature Reviews Genetics* 13.5 (2012): 329-342.

25. Blankenberg Daniel., *et al*. "Galaxy: a web-based genome analysis tool for experimentalists". *Current Protocols in Molecular Biology* 89.1 (2010): 19-10.

26. Blankenberg Daniel and Jennifer Hillman-Jackson. "Analysis of next-generation sequencing data using Galaxy". Stem cell transcriptional networks. Humana Press, New York, NY (2014): 21-43.

27. Zankari Ea., *et al*. "Identification of acquired antimicrobial resistance genes". *Journal of Antimicrobial Chemotherapy* 67.11 (2012): 2640-2644.