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BIOINFROMATIC APPROACHES FOR BUILDING COMPARATIVE MOLECULAR DATABASE OF BACTERIOCIN PRODUCING SELECTED LACTIC ACID BACTERIA

Priyanka Gautam

Bioinformatics Lab, Dept. of Zoology, Dayalbagh Educational Institute,

(Deemed University) Dayalbagh, Agra-282005, India

ABSTRACT: Bioinformatics is marriage between the computer and biology. Present study deals with the screening of lactic acid bacteria having biopreservative capability to inhibit the wide range of pathogenic bacteria and for a value addition to the food as natural preservative. Bacteriocin producing lactic acid bacteria incorporate into milk products. Present study completed in three levels that is evolutionary analysis, genomic analysis and proteomic analysis. For the evolutionary analysis the phylogenetic model were prepared by MEGA 6 software. In genomic analysis secondary structure of RNA was predicted with the help of the RNAdraw software. In proteomic analysis secondary and tertiary structure prediction was done with the help of by Chou and Fasman method and by SWISS-MODEL package respectively. Passing with all the three levelsit was observed that of the screened 24 lactic acid bacteria only two species Lactobacillus acidophilus and Lactobacillus helveticus were showing the highest similarity. The combination of both of these lactic acid bacteria species may be used as a value additive in the food as these both have an antimicrobial activity against various pathogens.

KEYWORDS: Lactic acid bacteria, bacteriocin, phylogenetic analysis, genomic analysis, proteomic analysis

***Corresponding Author: Dr.Priyanka Gautam** Ph.D.

Bioinformatics Lab, Dept. of Zoology, Dayalbagh Educational Institute, (Deemed University) Dayalbagh, Agra-282005, India. * Email Address:drpriya18@gmail.com

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1. INTRODUCTION

Bacteriocin a natural preservative, an antimicrobial protenacious compound, has the antagonistic activity against the pathogenic bacteria, and it is produced by lactic acid bacteria [7]. Bacteriocin kills the pathogenic bacterial species such as Listeria monocytogenes, Escherichia coli, and Staphylococcus aureus. Bacteriocin initiates its antimicrobial activity by formation of pore in the cell membrane of the pathogenic bacteria that is followed by the cell death [2]. Bacteriocin producing lactic acid bacteria may present in the variety of products as milk, yoghurt, cheese, gastrointestinal tract, fermented plant product, meat product [9]. But for the present study we have selected the lactic acid bacteria those are present in milk and milk products and have the capability to produce bacteriocin.

Screening of the lactic acid bacteria with wide spectrum of antimicrobial activity was confirmed at the three level as by the evolutionary relationship based on 16S rRNA sequences and then by understanding the structural and functional relationship between the lactic acid bacterial (LAB) species in the area of genomics and finally on the basis of the structure of the bacteriocin produced by the concerned LAB in the area of proteomics.

Initially phylogenetic analysis is often studied by using fossil records, which contain morphological information about ancestors of current species and the timeline divergence. But now it was done by using molecular data as 16S rRNA sequences because the 16S rRNA gene is ribosomal RNA and in these gene very little amount changed over millions of year so it is a conserved sequence and these gene sequencing used for the identification and classification of the bacteria. These conserved regions of the 16S rRNA gene are not in use only in phylogenetic relationship [3, 4, 12], but also use in understand the structural and functional study of the species organism [10]. In present study by using 16S rRNA gene sequences we analysed and compared the lactic acid bacterial species by RNA secondary structure prediction. Proteomic is large scale of the protein dataset and these provide the information about the protein in which they include protein family, protein-protein interaction, and protein motif force and protein domain. It is also provide the structural information, expression level, pre, co, and post translation modification of the protein. Proteomic study is one of the major aspects of the bioinformatics. Proteomic deals with the structure, function and classification of the protein. The structural preparation of the proteins this procedure is called homology modelling. Homology modelling is a phenomenon related to protein structure which constructs the 3D structure of the protein.

2. MATERIALSAND METHODS

The data collection of the lactic acid bacteria was done by using the literature sites as PubMed and NCBI resource and other various search engines such as Google Chrome, Google Scholar. A total of 24 lactic acid bacteria were selected based on their activity of producing bacteriocin.

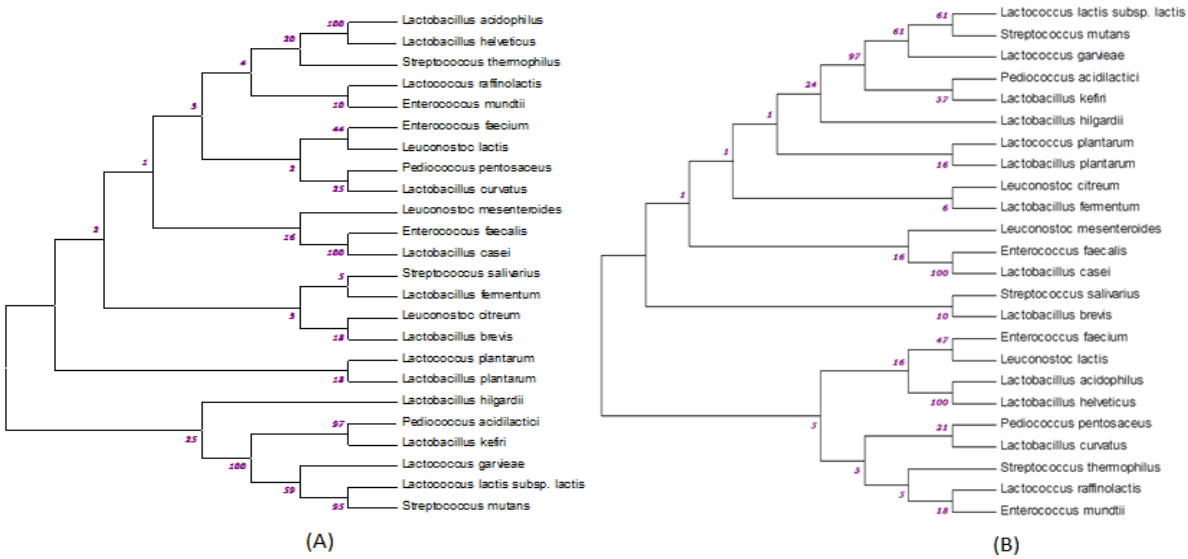
16S rRNA conserved sequences were retrieved from the NCBI source. Evolutionary models were

prepared by MEGA 6 using the four methods Neighor Joining (NJ), Unweighted Pair Group Method (UPGMA), Maximum Likelihood (ML) and Maximum Parsimony (MP).

Evolutionary models analysis followed by the genomics analysis to produce the secondary structure of the RNA using RNAdraw software. This work followed by the proteomic analysis to predict the structure of proteins. For this the Chou and Fasman method was used and the tertiary structure of the proteins was predicted by using the Swiss Model.

3. RESULTS AND DISCUSSION

In the present study there are three aspects of the results as phylogenetic analysis, Genomic analysis and Proteomic analysis. In the phylogenetic analysis the tree was prepared by four methods as UPGMA, NJ, ML and MP with boot strap statistical analysis method using MEGA 6 software. As observed in all the four method 90-100% similarity was found between four species of lactic acid bacteria shows with each other (Enterococcus faecalis and Lactobacillus casei), (Lactobacillus acidophilus and Lactobacillus helveticus). While the lesser similarity 37-95% was observed between (Pediococcus acidilactici and Lactobacillus kefiri) species and (Lactococcus lactis subsp. lactis and Streptococcus mutans) species with each other by all the four methods. According to the phylogenetic models, of the 24 LAB, we have found only 8 species of the LAB (above mentioned) those are showing the highest percent of similarity to each other **[Figure-1].**



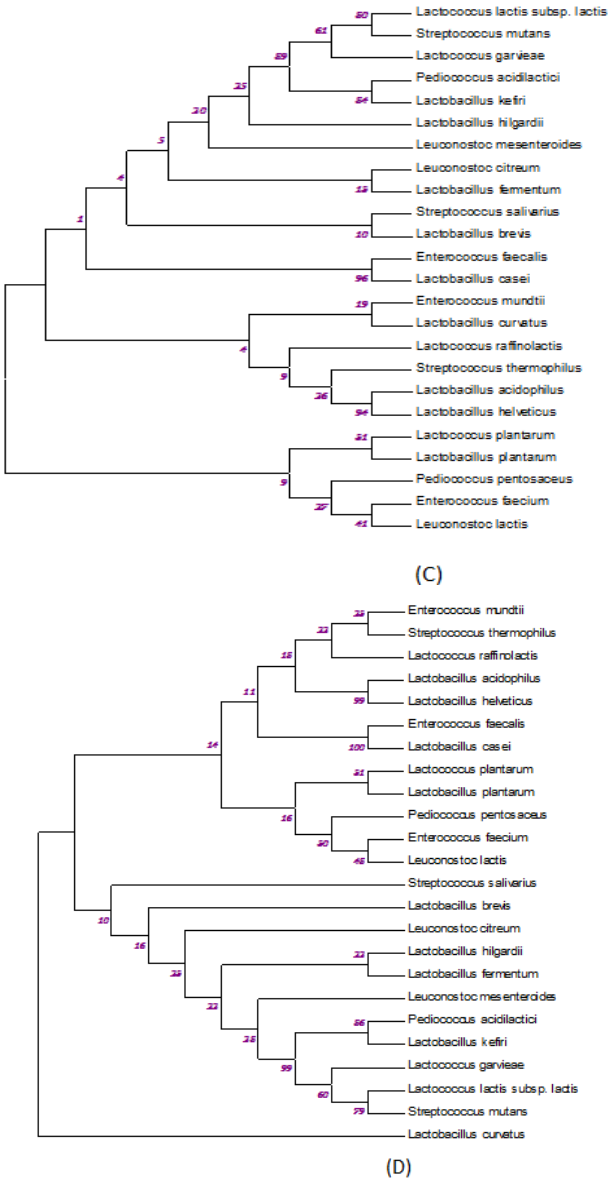


Figure.1. Phylogenetic tree of lactic acid bacteria prepared by (A) UPGMA, (B) NJ, (C) ML and MP method (Bootstrap test)

These phylogenetic results further confirm by the genomic analysis by predicting the secondary structure of these 8 species with the help of the 16S rRNA sequences by RNAdraw software package. In the secondary structure of the RNA various different kinds of loops were found such as loops multibranch loop, bluge loop, internal loop, hairpin loop and tetra loop. Multibranch loop it look like a junction and many branch arise from at one place. Bluge loop is form a bubble like structure in one side of the stems. Internal loop it form swallown like structure in middle of the stems. Hairpin loop it form edge of the all stems in secondary structure of the RNA and tetra loop form when the four internal loop was form at one branch it form a tetra loop in the structure. According to the observation of the lactic acid bacteria those are predicted by the RNAdraw software package, we observed that the out of 8 species of the LAB the 4 species of the LAB were showing the most structural similarity i.e. in between the (Enterococcus faecalis and Lactobacillus casei) and (Lactobacillus acidophilus and

Lactobacillus helveticus) The other four species were not showing the best structural similarity to each other so these species were eliminated by the genomics analysis results [Figure-2].

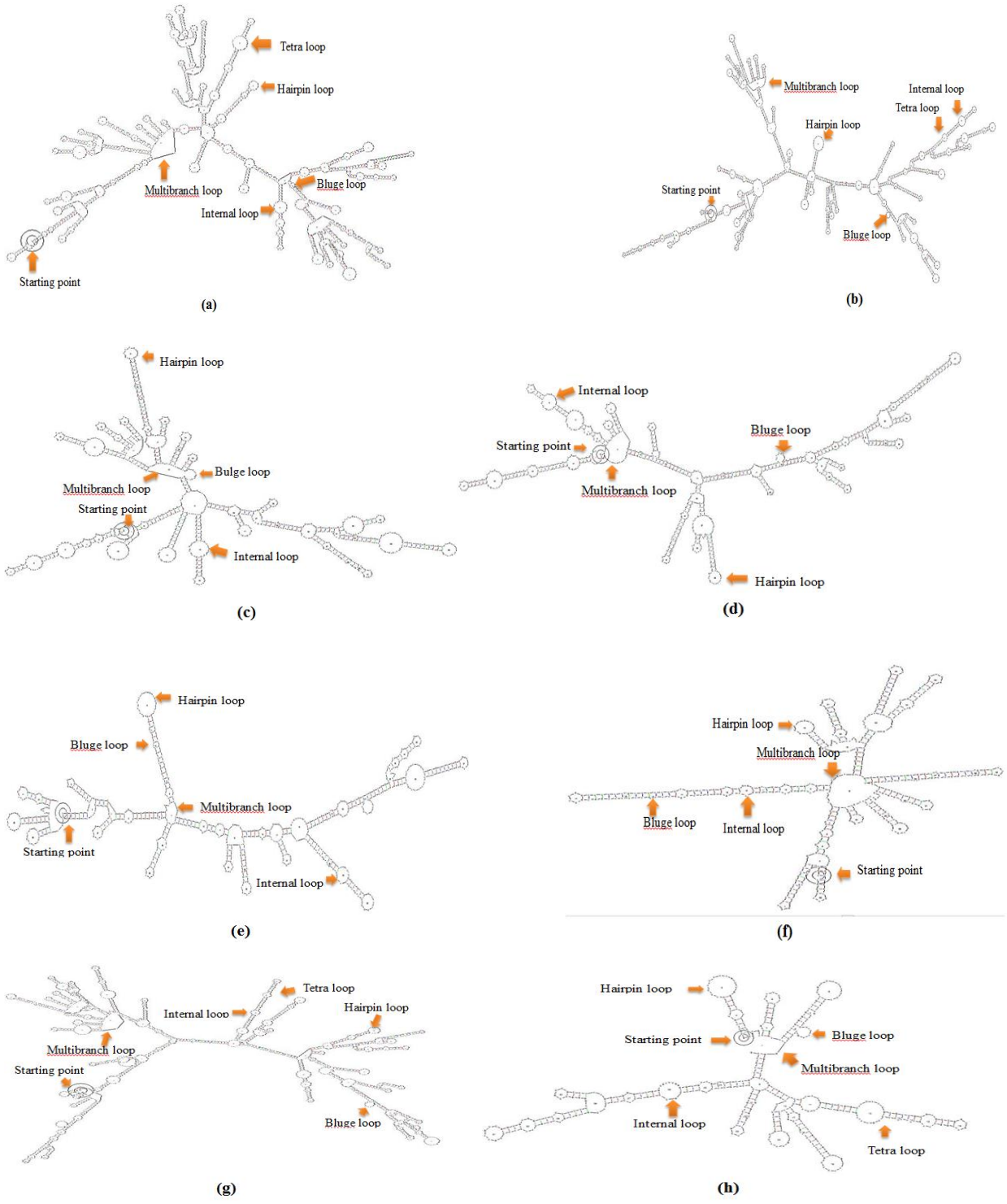


Figure: 2. Secondary structure of the 16S rRNA of lactic acid bacteria species (a) *Enterococcus faecalis* (b) *Lactobacillus casei* (c) *Lactobacillus acidophilus* (d) *Lactobacillus helveticus* (e) *Pediococcus acidilactici* (f) *Lactobacillus kefiri* (g) *Lactococcus lactis subsp. lactis* (h) *Streptococcus mutans*.

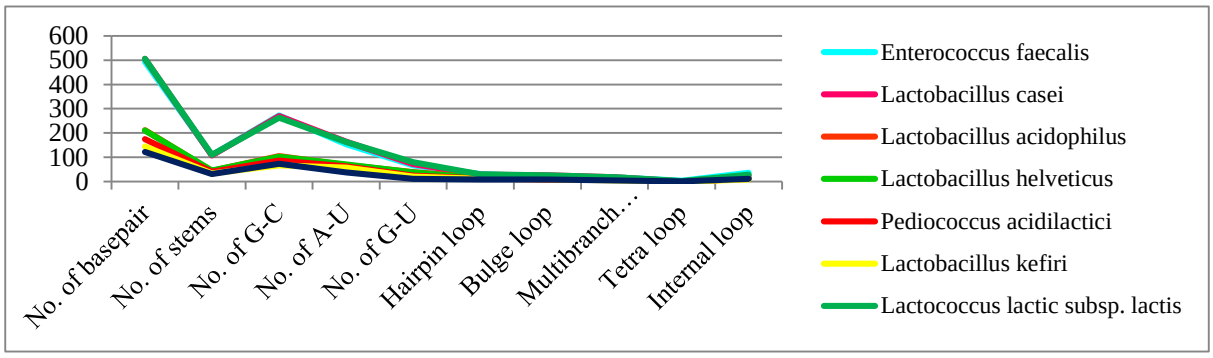


Figure: 3. Comparison between the different parameter the 8 lactic acid bacterial species

On the basis the different parameter in the secondary RNA structure the highest similarity was observed in between the (Enterococcus faecalis and Lactobacillus casei) species and (Lactobacillus acidophilus and Lactobacillus helveticus) species of LAB [Figure-3]. Further confirmation of result we had done proteomic analysis with these four species Enterococcus faecalis, Lactobacillus casei and Lactobacillus acidophilus, Lactobacillus helveticus. The secondary and tertiary structure of the lactic acid bacteria was prepared by chou and fasman [Figure-4to7] and swiss model method respectively [Figure-8and 9].

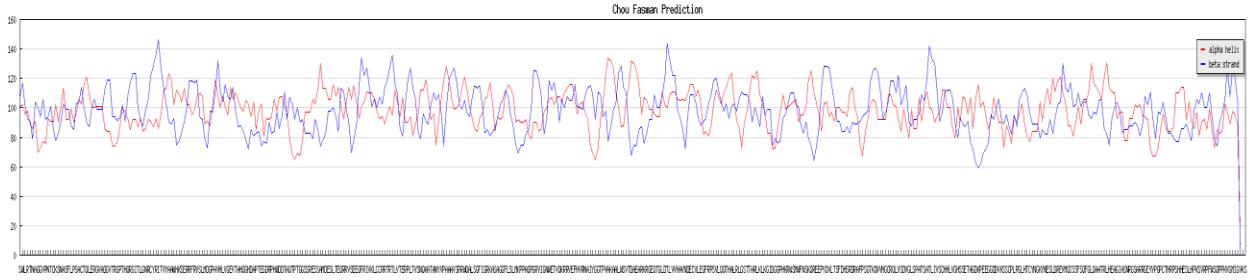


Figure: 4. Graphical representations of the protein sequences of *Enterococcus faecalis* by Chou and Fasman bioinformatics tool.

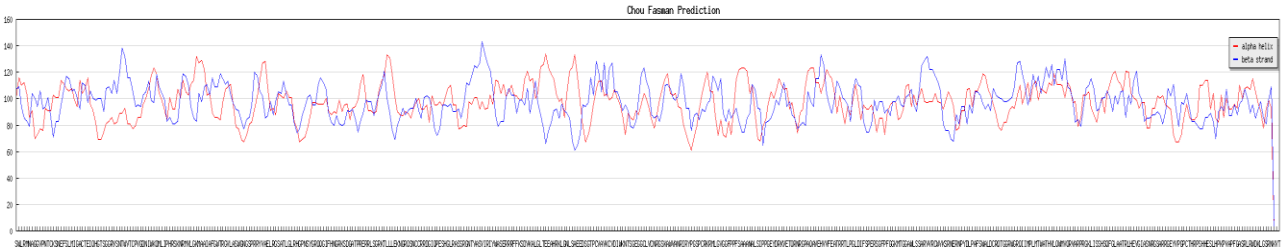


Figure: 5. Graphical representations of the protein sequences of *Lactobacillus casei*by Chou and Fasman bioinformatics tool.

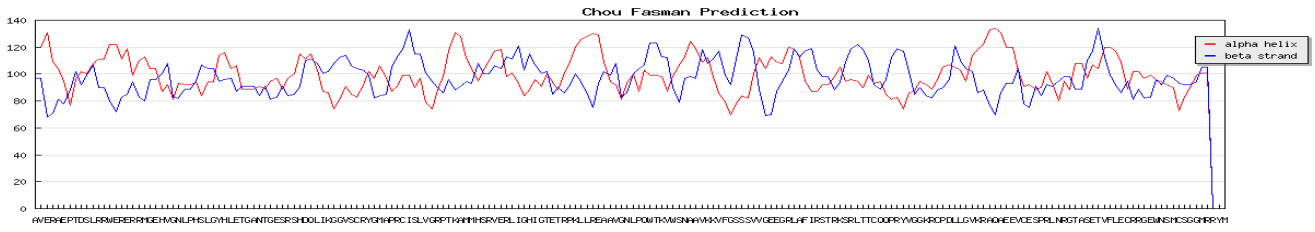


Figure: 6. Graphical representations of the protein sequences of *Lactobacillus acidophilus* by Chou and Fasman bioinformatics tool.

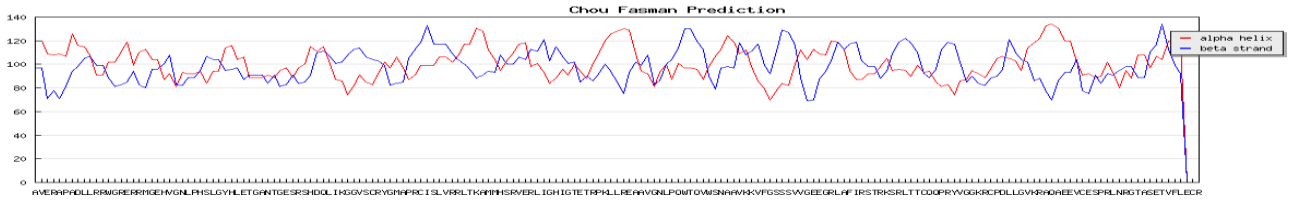
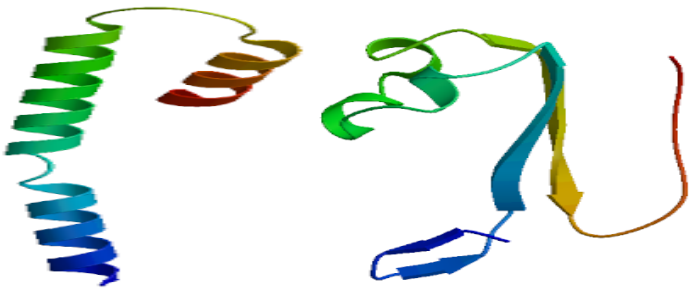
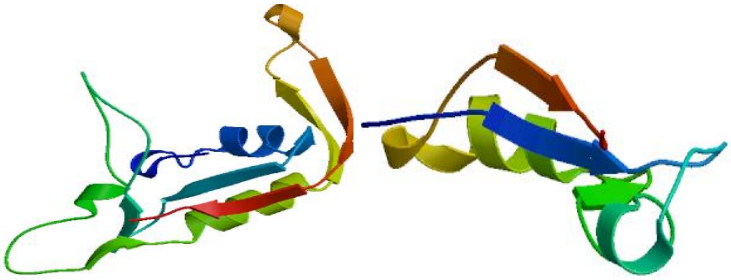


Figure: 7. Graphical representations of the protein sequences of *Lactobacillus helveticus*by Chou and Fasman bioinformatics tool.



(a) (b)

Figure: 8. Tertiary structure of the protein of (a) *Enterococcus faecalis*(b)*Lactobacillus casei*



(c) (d)

Figure: 9. Tertiary structure of the protein of (c) *Lactobacillus acidophilus* (d) *Lactobacillus helveticus*

These proteomics study were done by two ways asprediction of the primary and secondary structure. In secondary structure prediction by graphical representation the most similarity pattern observed between in Lactobacillus acidophilus and Lactobacillus helveticus on the basis of alpha and beta helix [Figure-4to7].

In the tertiary structure of the all four species we observed that there is lots of difference in the tertiary structure of (Enterococcus faecalis and Lactobacillus kefiri) as in the Enterococcus faecalis only α-helix was observed while in the Lactobacillus casei the α-helix and β-sheet both were present. While in the other two species (Lactobacillus acidophilus and Lactobacillus helveticus) much similarity was observed at a greater extend as both structure consist α-helix and β-sheet and also similarity was observed in number of fold [Figure-8 and 9]. So from the final analysis we found that of the 24 lactic acid bacteria only two species (Lactobacillus acidophilus and Lactobacillus helveticus) were shown the higher similarity by all the 3 methods as by phylogenetic analysis, genomics (RNAdraw) and proteomics (by prediction secondary and tertiary structure).

The bacteriocin produced by both lactic acid bacteria belongs (Lactobacillus acidophilus and Lactobacillus helveticus) to class-II bacteriocin [8]. Class-II bacteriocin is heat stable bacteriocin

and varies in size <10-KDa. Bacteriocins have four classes, Class-I, II, III and IV [5]. The bacteriocin of the Lactobacillus acidophilus show the antimicrobial effect against Salmonella species and Staphylococcus speciesand the LAB Lactobacillus helveticus shows the antimicrobial effect against Bacillus subtilis, Bacillus alvei and E.coli [6,11,1].

So our results shows that as the both lactic acid bacteria showing the higher level of similarity at various level as by phylogenetic analysis, at genomic and proteomic level. The antimicrobial compound (Bacteriocin) produced by them is also similar and shows effect against the different pathogenic bacteria. According to our result we can use their bacteria as alternative to each other as they are showing similarity at a greater extend. And a more potent drug can by formulated by the combination of both of these species with a wider spectrum of antimicrobial activity. There both can also give the more value addition to food to inhibit the wide range of bacteria. This study can further be proved by the wet lab analysis against the above mentioned pathogenic bacteria.

CONCLUSION:

Of the 24 lactic acid bacteria selected with the capability of the production of bacteriocin, only two species *Lactobacillus acidophilus* and *Lactobacillus helveticus* were showing the highest similarity at three levels i.e. phylogenetic analysis, at genomic and proteomic level. Therefore, the combination of both of these two lactic acid bacteria species may be used as a value additive in the food to decrease the growth of pathogenic microbes, as these both have an antimicrobial activity against various pathogens. The combination of both screened lactic bacteria species can be used to formulate a more potent drug as these two has a wider spectrum of anti-microbial activity against pathogenic microbes.

CONFLICT OF INTEREST:

The author confirms that this article has no conflict of interest.

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