ISSN: 0975-766X
CODEN: IJPTFI
Available Online through
www.ijptonline.com
Research Article

PHYLOGENETIC ANALYSIS AND HOMOLOGY MODELING OF BACTERIOCIN PRODUCED BY GRAM POSITIVE AND GRAM NEGATIVE BACTERIA

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Received on 12-07-2011 *Accepted on 28-07-2011*

Abstracts

Bacteriocins are therapeutic, biocontrolling and proteinaceous molecules produced by various lineages of Gram-positive and Gram-negative bacteria. It is synthesized under the control of genetic mechanisms and develops their lethal action on the microbial cell by multiples mechanisms. Various experimental data analyzed that indicate high polymorphisms among bacteriocin producing strains. In this study we evaluated the evolutionary relationship between bacteriocin producing gram positive and gram negative bacteria and modeling of their protein molecules. To find out these phylogenetic trees were constructed on the basis of homology between the protein sequences. Phylogenetic tree for gram negative sequences shown medium bootstrapped values even less than 50 and these strain were not much related with one another as in the case of gram positive sequences. In gram positive most were found with bootstrapped value equal to 100. Thus it was observed that they having intra high region and inter low region of homology between them as indicated by the bootstrapped values. It was concluded from modeling of proteins that were shown rich with alpha and beta sheets that leads stability of proteins. Thus coding sequences for alpha and B sheets remain more conserved in gram positive as compare to gram negative during period of evolutions.

Key words: Bacteriocin, Phylogenetic analysis, Protein modeling.

Introduction

Microbes produce an extraordinary array of microbial defense systems. These include classical antibiotics, metabolic by-products, lytic agents, antimicrobial compounds, including organic acids, hydrogen peroxide and proteins-bacteriocins, The term bacteriocin encompasses an array of structurally different molecules produced by a number of phylogenetically distinct Gram-positive and Gram-negative bacteria groups. Bacteriocins have been characterized as molecules of high antimicrobial property even at low concentrations, provoking the microbial survival inhibition by antibiosis.

(17,18) that can inhibit the growth of various pathogens. A number of studies have reported the antagonistic properties of bacteriocin against many common gastro enteric pathogens, e.g. *Salmonella* sp. **(16)**. *Escherichia coli* O157:H7 **(1)** *Clostridium perfringen, Campylobacter jejuni* **(14),** *Listeria monocytogenes* and *Helicobacter pylori* (**19**). Antagonist action exerted by bacteriocin producing bacteria on other bacteria inserted in the same environment is defined as Antibiosis **(15).** The possible mechanism of bacteriocin resistance of gram negative and some gram positive bacteria has been suggested to be associated with the barrier properties of the outer membrane and cell wall **(1)).** In addition, antimicrobial production by probiotic or lactic acids bacteria might play role during in vivo interactions occurring in the human gastrointestinal tract and other mucosal membranes hence contributing to gut health**.** The action of active metabolites occurs by binding with specific receptors present on the target microbial cell surface. After binding with these receptors, various mechanisms act, by isolated or concomitant way, causing the microbial cell killing **(12).** Most Bacteriocins are synthesized as biologically inactive prepeptides carrying an N-terminal leader peptide that is attached to the C-terminal propeptide. **(6).** Regulatory systems consist of signal – producing proteins, a membrane bound histidine protein kinase (HPK), and a cytoplasm response regulator (RR) **(8).** 16S rDNA has been used to classify ruminal bacteria, but some ruminal bacteria have outer membranes even though they are most closely related to Gram-positive species (e.g. *Selenomonas ruminantium* and *Megasphaera elsdenii*) **(10).**

The plasmids isolated vary greatly in size , ranging from 6.0 kb for the pediocin SJ-I-associated plasmid **(3)** to 131 kb for the plasmid associated with lactococcin A production in *L .lactis* **(21).** Colicins, those Bacteriocins produced by *E .Coli*, serve as a model system for investigations of Bacteriocins structure- function relationships, genetic organization, and their ecological role and evolutionary history. Colicins expression is often dependent on host regulatory **(4).** Sequence homologies between the genes and the peptide precursors of Nisin, Subtilin and Epidermin – Nisin, Subtilin and Epidermin are produced by *S. lactis* ATCC 11454, *B.subtilis* ATCC 6633 and *Staphylococcus epidermis Tu* 3298 respectively. These are all gram positive eubacteria that have evolved to fit very different ecological niches, but the similarities between the structures of these antibodies and unusual processing requirements suggesting a 'common ancestor'. The homologies of sequence and organization support the idea of common ancestor, but the differences in both amino acid and nucleic acid sequences indicate that they have been evolving separately for long time. Indeed, inspection of silent codon positions suggests that they have become completely randomized. **(21).**

Methodology

Collection of Sequences.

All the required sequences were taken from scientific sites NCBI (National Centre for Biotechnology Information) and databases like gene and protein database. Both 14 -14 sequences of gram positive species and gram negative species respectively were taken from NCBI which were available through **Entrez search engine. (2).** http://www.ncbi.nlm.gov/gene/?term=bacteriocin : The species and accession numbers of the corresponding database entries are listed in **Table. 1 and 2.**

Table-1:

S.NO.	GI No.	Name of Bacteria	YP No.
1	94993804	*Streptococcus pyogenes* MGA510750	601902.1
2	150388859	*Alkaliphilus Metalliredigens* QYMF	001318908.1
3	90962882	*Lactobacillus Salivarius* UCC118	536797.1

4	220929695	*Clostridium cellolulyticum* H10	002506584.1
5	222530573	*Anaerocellum Thermophilum* DSM 6725	002574455.1
6	152975898	*Bacillus cereus subsp. Cytotoxins* NVH 391-98	001375415.1
7	217966022	*Listeria monocytogenes* HCC23	002351700.1
8	148267524	*Staphylococcus aures subsp. Aures* JH9	001246467.1
9	116326531	*Lactococcus lactis subsp.cremoris* SK11	796464.1
10	169833112	*StreptococcusPneumoniaehungray* 19A-6	001693637
11	118480203	*Bacillus Thruingiensis strain* A	897354.1
12	195953370	*Hydroenobaculum sp.*YOAA51	002121660.1
13	222528358	*Anaerocellum thermophilum*	002572240
14	42518692	*Lactobacillus Johnsonii* NCC 533	964622.1

Gram positive species.

Table-2:

S.No.	**GI NoS.**	**Name of Bacteria**	**YP No.**
1	186683671	*Nostoc punciforme* PCC73102	001866867.1
2	218550450	*Cynothece sp.* PCC 7424	002378779.1
3	184157551	*Acinetobacter Baumannii* ACICU	001845890.1
4	121610805	*Verminephrobacter eiseniae* EF 01-2	998612.1

5	167627136	*Francisella philomiragia subsp. Philomiragia* ATCC 25017	001677636.1
6	237809814	*Salmonella enterica*	002894254
7	198244574	*Tolumonas anensis* DSM 9187	002894254
8	187930501	*Ralstonia picketti* 12 J	002218157.1
9	75812518	*Anabaena variabilis* ATCC 29413	320137.1
10	257058440	*Cynothece sp.* PCC 8802	003136328.1
11	42527932	*Treponema lanticola* ATCC 35405	973030.1
12	188535130	*Erewinia tasmaniensis* Et 1/99	001908927.1
13	189347152	*Chlorobium Limicola* DSM 245	001943681
14	162419349	*Yerseinia pestis angola*	001602741.1

Gram negative species.

Phylogenetic analysis

All the studies were done with the help of some phylogenetic techniques and software available online like CLUSTALX and PHYLIP. Tree construction was done with the help of the SEQBOOT program followed by PROTPARS and CONSENSE program and the tree. Moreover the BLAST (Basic local alignment search tool) program was also used to find out the homologous sequences. **(17)**

Multiple Sequence Alignment: CLUSTAL X 2.0.11

All the sequences were collected in FASTA format and multiple sequence alignment was carried out by using the CLUSTALW program, version 2.0.11 **(7)** which was down loaded from ftp://ftp.ebi.ac.uk/pub/software/clustalw2/2.0.11/

The steps briefly included as:

1. Perform pair wise alignment of all the sequences by dynamic programming.

2. Use the alignment scores to produce a phylogenetic tree by neighbor joining.

3. Align the multiple sequences sequentially, guided by the phylogenetic tree.

A page was generated showing the multiple alignments of sequences. **Fig.1**

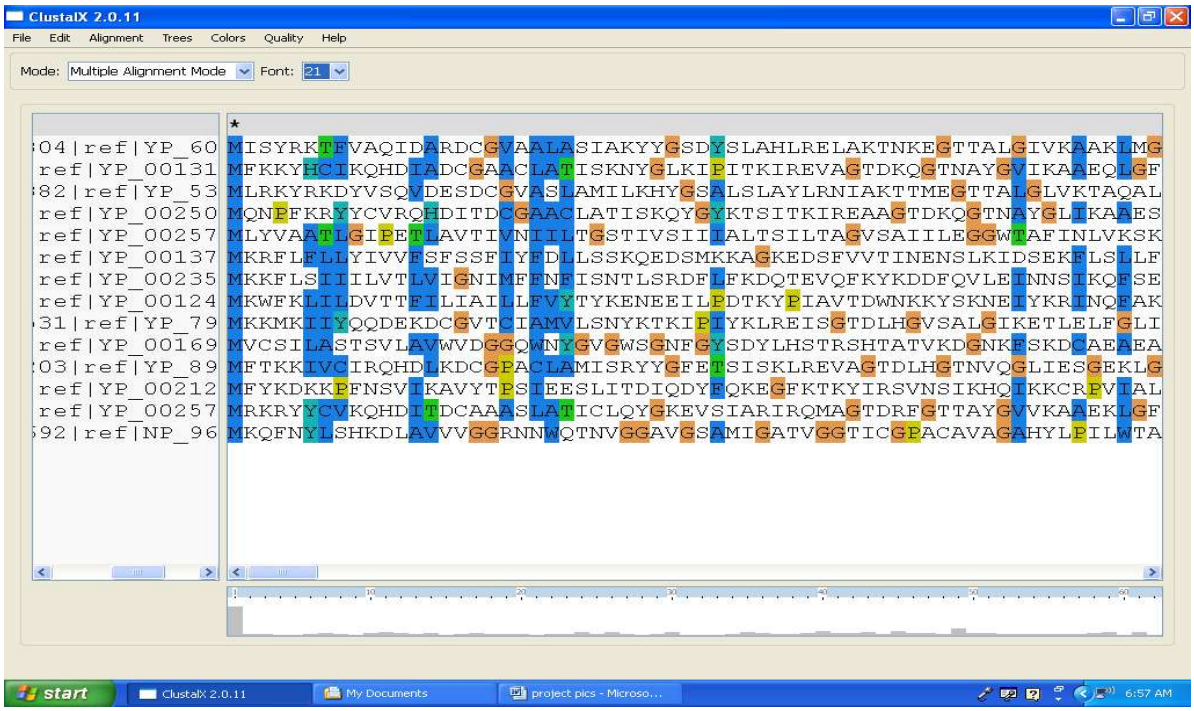

Figure 1: CLUSTALX software showing multiple alignment of the sequences. Clearly indicated Shaded regions shown the regions of homologies between the different sequences.

Use of PHYLIP program for phylogenetic analysis

The phylogenetic analysis was carried out by PHYLIP version 3.69 programs after running Clustal X program; all multiple sequence alignment data is available in PHYLIP format. PHYLIP is PHYlogeny Inference package, which is a free computational phylogenetics package of programs for inferring evolutionary trees (phylogenies) and protpars was run. http://evolution.genetics.washington.edu/phylip.html. The results were found as in **fig.2 and fig.3.**

Use of BLAST for Homology sequences.

BLAST (Basic Local Alignment Search Tool), is an algorithm for comparing primary biological sequence information http://blast.ncbi.nlm.nih.gov/Blast.cgi. The result of BLAST was found as in fig.4.

For Gram positive sequences:

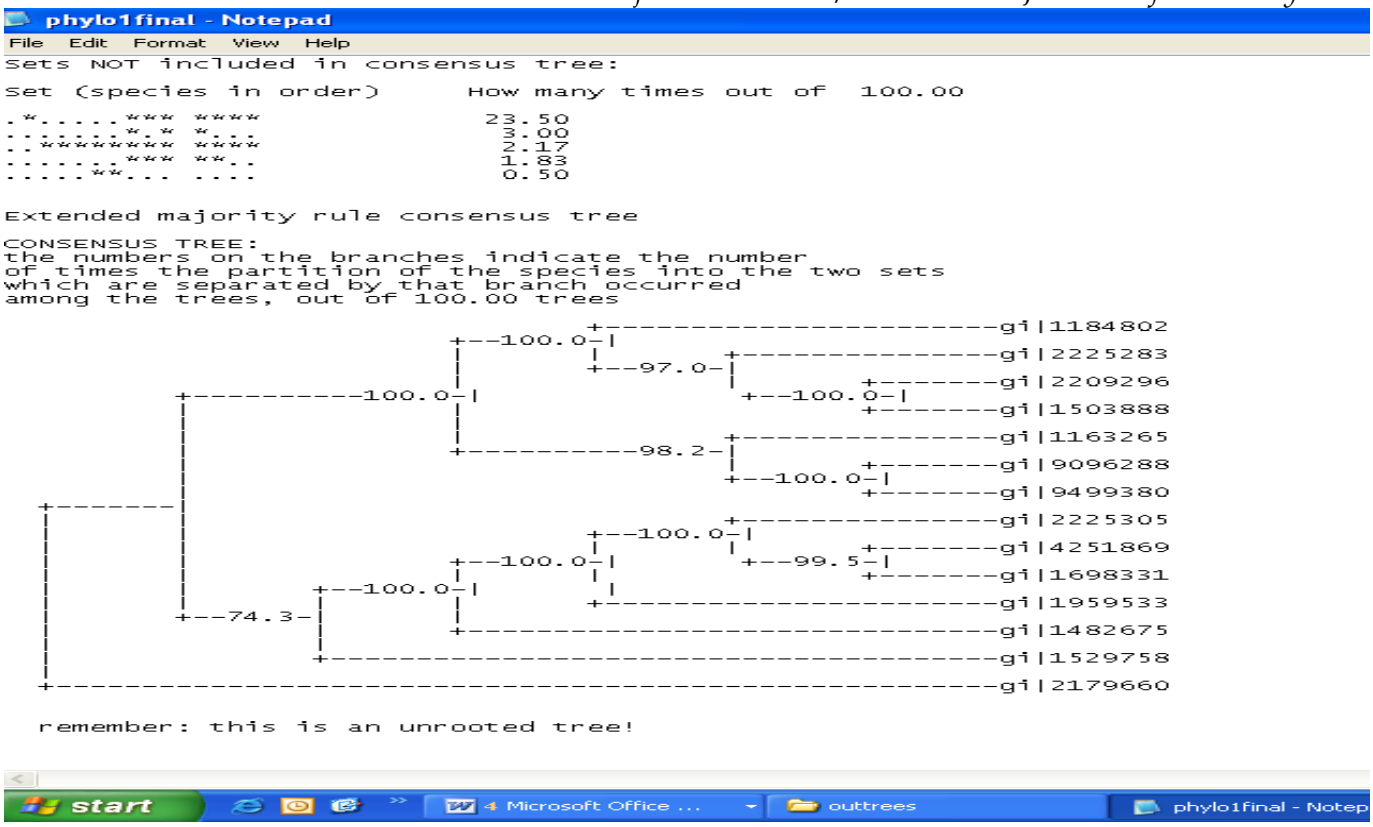

Figure 2: Figure shows the final output result of phylip program giving the consensus tree as phylogenetic tree for gram positive sequences.

For gram negative sequences:

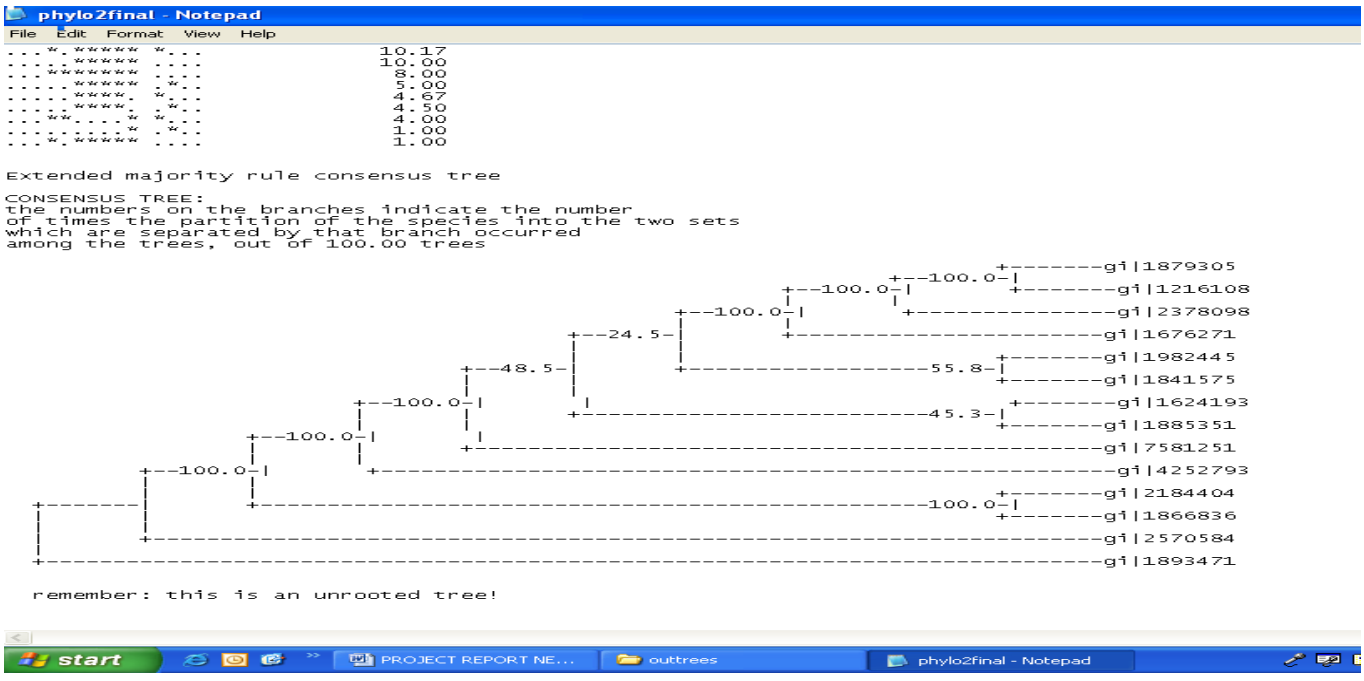

Figure 3: Figure shows the final output result of phylip program giving the consensus tree as phylogenetic tree for gram Negative sequences.

Result of BLAST

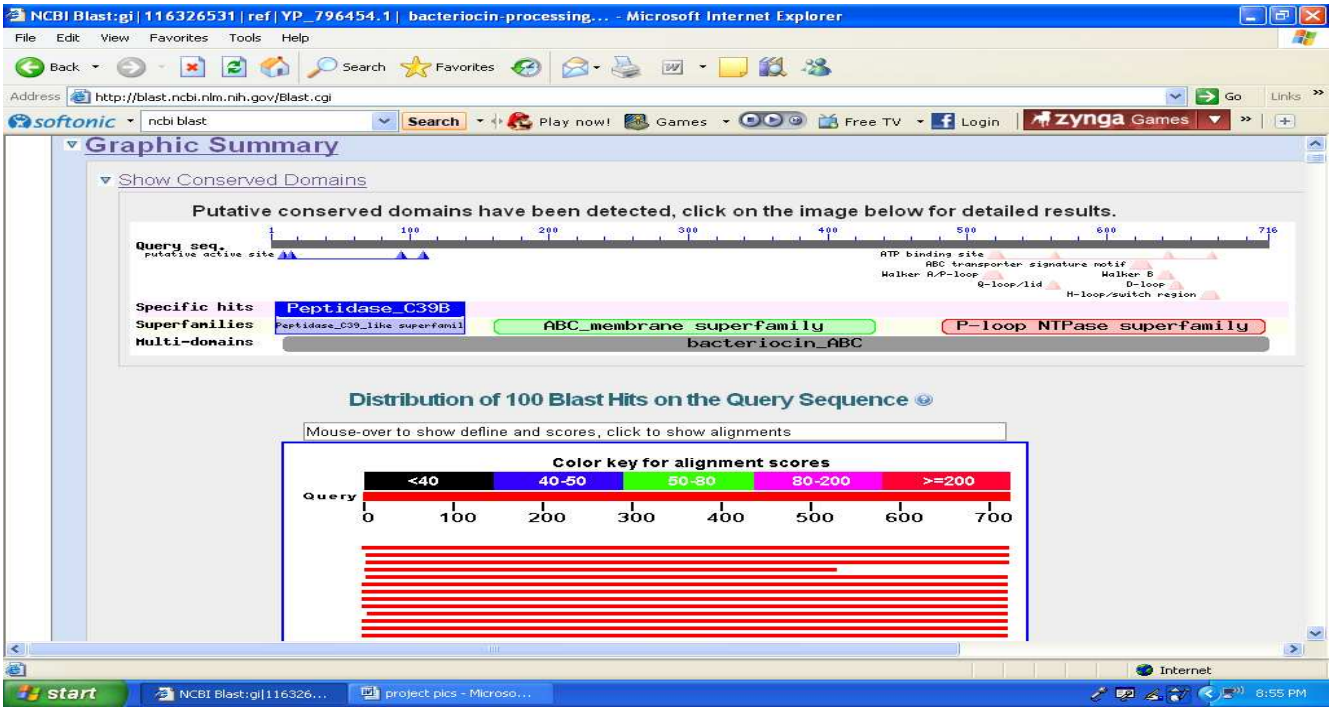

Figure 4: Figure shows the result of BLAST, which shows 100 blast hits that indicates 100 homology sequences in response to one particular submitted sequence.

Protein modeling – SWISS Model

Protein Homology modeling combines sequence analysis and molecular modeling to predict three dimensional structure was done by **SWISS-Model** downloaded from (htt://www.expacy.org/swissmod/SWISS-MODEL.htm http://swissmodel.expasy.org/workspace/index.php?userid=sajqazi@gmail.com&key=02e1fc871b1d868fc4016f89d2bd55e3&func=workspace_modelling&prjid=P000007**),**usingSWISS-MODEL program protein modeling of sequence of *Lactococcus Lactis* subsp. *Cremoris* SK11 having the GI No. 116326531 and ref/ YP No. 796454.1, for gram positive **fig. (5, 6 and 7)** and *Nostoc punciforme* PCC73102 GI No. 186683671 and ref/ YP. No. 001866867.1 for gram negative was generated **fig. (8 and 9).** The theoretical structure was then visualized with **Swiss-PDB Viewer** and **RasMol** to gain insight into the way in which its structure relates to its function**. (13, 20, 9).**

Result of SWISS-Modeling:

After submitting the sequence at workspace, the output result is as follows:

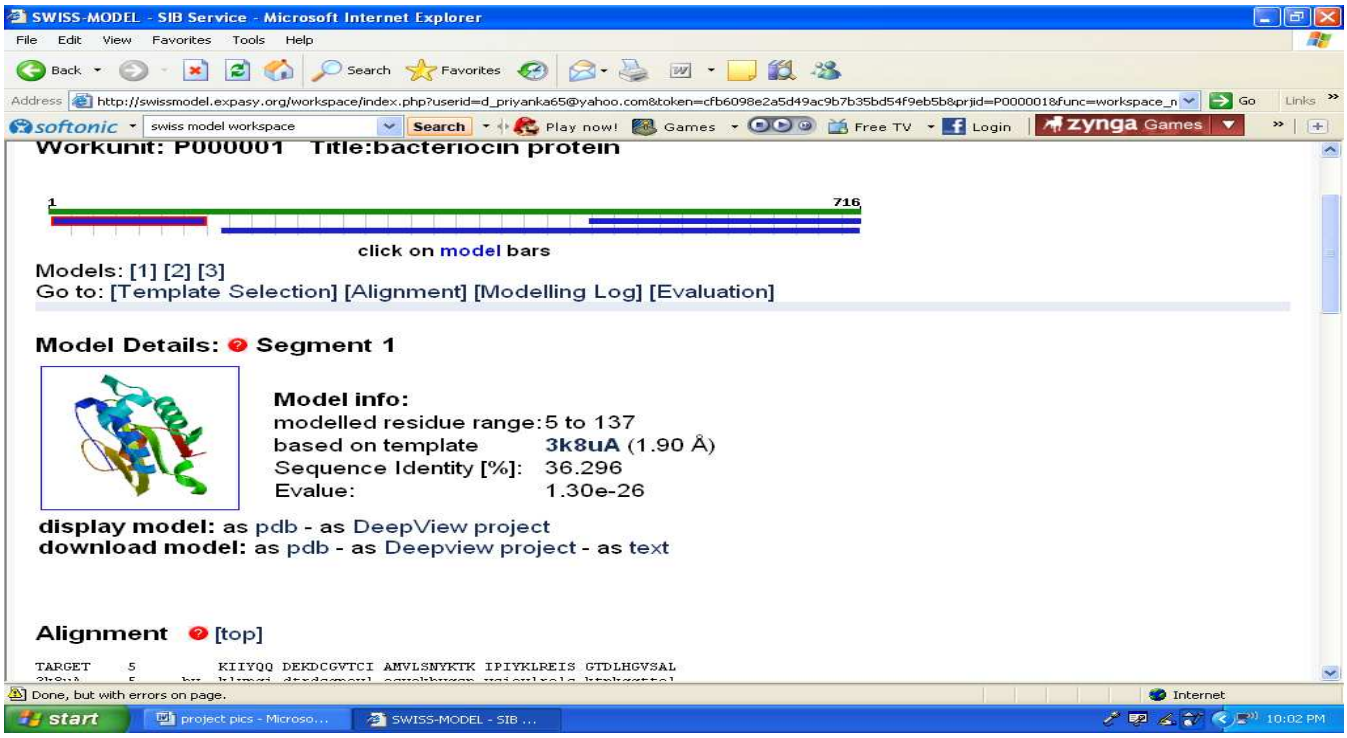

Fig.5. Swiss modeling.

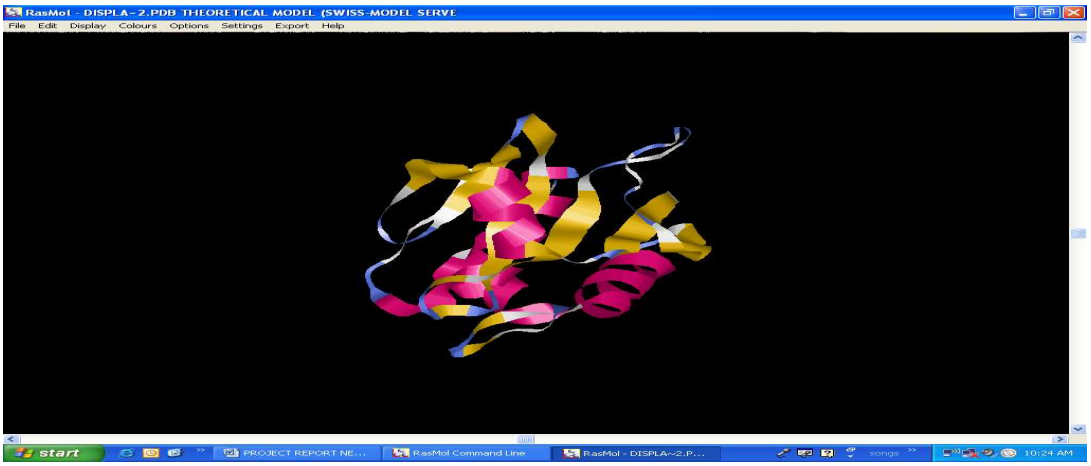

Figure 6: Figure shows the final predicted model of *Lactococcus Lactis* subsp. *Cremoris* SK11 by SWISS-model through RasMol.

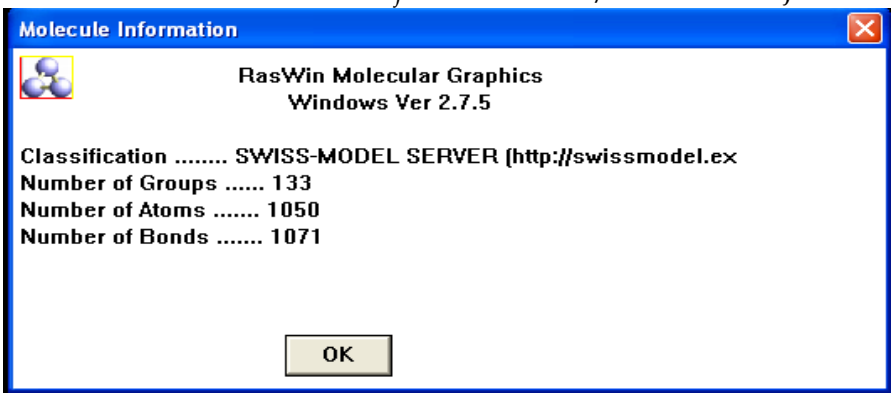

Figure 7: Description of the model

Modeling of Gram negative Bacteriocin

NCBI Reference Sequence: YP_001866867.1

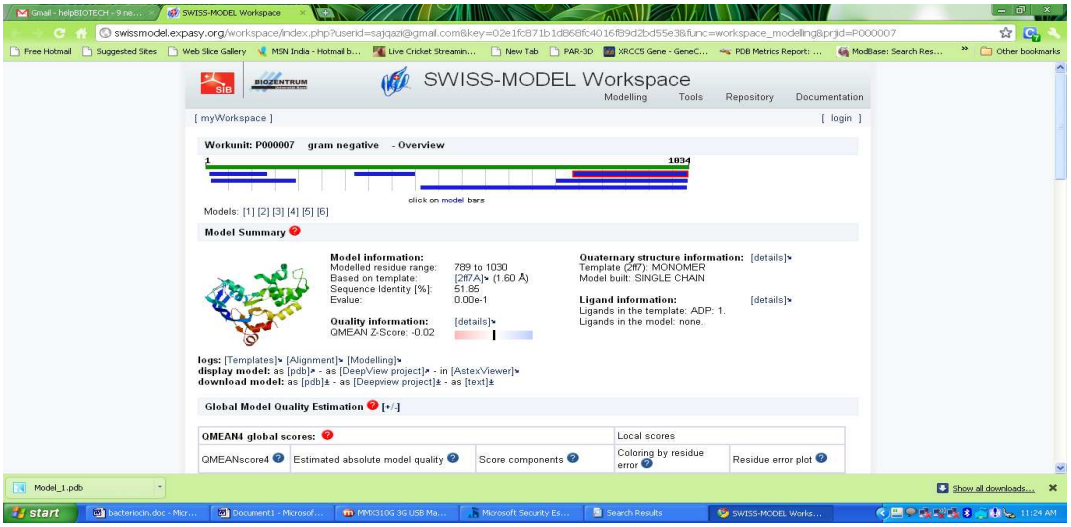

Fig.8. [*Nostoc punctiforme PCC 73102]*

Fig. 9.(*Nostoc punctiforme PCC 73102)*

Result and discussion

As mentioned before PHYLIP program was used for phylogenetic tree construction. Tree was constructed on the basis of homology between the protein sequences. For the tree construction using PHYLIP program it was required to run Seqboot, PROTpars and Consense Program. Consense program gives the consensus tree as 'out tree', which was the final tree with bootstrapped values as shown in fig.2 and 3. Phylogenetic tree of gram positive sequences shown high bootstrapped values which was clearly shown. All the bootstrapped values are greater than 50. As shown in tree the sequences with gi nos. 118480203 and 222528358, 220929695 and 150388859, 90962882 and 94993804, 222530573 and 169833112, 222530573 and 195953370, 222530573 and 148267524, 222530573 and 152975898, 118480203 and 94993804 shares common value of 100.0 which is the maximum value. Thus they are pretty much similar to one another and have large area of homology between them. Lowest value was 74.3, which was found between sequences with gi nos. 1184080203 and 152975898, indicating they were not much similar. Phylogenetic tree for gram negative sequences shown as all values are not high and found even less than 50. These low bootstrapped values indicate that they were not much related with one another as in the case of gram positive sequences. Protein modeling carried out by SWISS model showing presence of alpha and beta sheets in both the protein of gram negative and gram positive bacteria.

Conclusion

Collected information from NCBI database of gram negative and gram positive bacteria of bacteriocin producing were analysed by performing multiple sequence alignment Using Clustal X (version 2.0.11) software and Phylip version 3.69 software package Phylogram. Trees shown evolutionary relationship among the organisms and the distance shows the closeness among them. It was analyzed on the basis of phylogenetic trees and sequence analysis that all bacteriocin producing bacteria with high bootstrip value were highly homologous but both gram positive and gram negative were evaluated phylogenetically distinctly. From proteins modeling generated from SWISS model, it was noticed that due to the presence of more number of alpha and B sheets, the coding sequences remains conserved during period of evolutions.

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