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STRUCTURAL DYNAMICS OF *LACTOBACILLUS RHAMNOSUS* PROTEINS UNDER COPPER SULPHATE AND ZINC CHLORIDE STRESS

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ABSTRACT: *Lactobacillus rhamnosus* plays an important role as probiotic often associated with beneficial health effects. Although copper and zinc are trace metals, they are toxic at high concentrations. In this paper, bioinformatic tools and molecular modeling approach was adopted to explore the structure of *L. rhamnosus* under copper sulfate and zinc chloride stress. The differentially expressed proteins under stress were Aspartate kinase, Mannose-6-phosphate isomerase, Glutamate dehydrogenase, 30 S ribosomal subunit S19, 50 S ribosomal subunit L4, Pyruvate oxidase, Thymidylate synthase, and ATP dependent Clp protease ATP binding subunit ClpL. The homology models for these proteins were developed by using Modeller 9.5v. The models were validated by using protein structure checking tools PROCHECK. These structures will provide a good foundation for functional analysis of *L. rhamnosus* proteins against toxic metal pollutant. Another three differentially expressed proteins were also elevated namely Acetyltransferase, Alkaline shock protein, Cell division initiation protein Div IVA. But these protein structures were not predicted because Query coverage, identity, and E-values were not matched.

INTRODUCTION: Lactic acid bacteria (LAB) belong to the order Lactobacillales and produce lactic acid as a result of carbohydrate fermentation. They are widely used in the production of fermented food. They are heterotrophic and generally have complex nutritional requirements because they lack many biosynthetic capabilities. Because of this, LAB is generally abundant only in environments, where these requirements can be provided, such as animal oral cavities and intestines [1].

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Of the trace metals known to function in biochemical processes, iron, zinc, and magnesium are probably used by all bacteria, whereas nickel, cobalt, selenium, and molybdenum are only used by some. No function for copper or selenium has been identified in any member of the Lactobacillales [2]. Some trace metals, like iron, selenium, molybdenum, manganese, and copper, are often scarce in the environment, and it can be assumed that cells requiring these metals possess corresponding uptake systems. However, few of these have been characterized to date. For essential metals like copper, nickel, cobalt, and zinc, which can occur in widely different bioavailable concentrations in the environment, bacteria must have homeostatic control mechanisms that can deal with excess as well as with deficiency [3].

Unfortunately, the knowledge of metal homeostasis and defense against metal stress by LAB is still very limited. Of all the biologically relevant metals, copper by far has received the widest attention. It is well known that zinc is an essential element required by all living organisms. Zinc is essential for normal growth and development, and most aspects of reproduction. Next, to iron, it is the most abundant trace mineral in the body. It is a structural constituent of many enzymes and proteins, including metabolic enzymes, transcription factors, and cellular signaling proteins [4].

The interactions between metal ions and micro-organisms have been widely investigated in the previous years as their understanding may contribute to the development of suitable biotechnological approaches to tackle environmental problems associated with metal pollution. Homology modeling is an important computational technique, within structural biology, to determine the 3D structure of proteins. It uses available high- resolution protein structures to produce a model of a protein of similar, but unknown, structure [5].

Our attention in this paper is homology modeling studies of differentially expressed proteins of *Lactobacillus rhamnosus* under copper sulfate and zinc chloride stress. Three-dimensional structures of these proteins were not yet available.

Hence, we were concentrated on structural features for the understanding of molecular function. The model structures of these proteins were constructed.

MATERIALS AND METHODS:

Determination of Minimum Inhibitory Concentrations of Copper Sulfate and Zinc Chloride for *L. rhamnosus* and Evaluation of Growth Curves: Copper and zinc are essential metals, but if intake is excessive, they may be toxic. In our previous study, excess were taken. Minimum inhibitory concentrations for copper sulfate and zinc chloride for *L. rhamnosus* were done by using macro dilution method and growth curves of copper sulfate and zinc chloride stressed *L. rhamnosus* was evaluated at 40mM (MIC of both $CuSo_4$ & $ZnCl_2$) which was described in our previous research publication [6].

2-D Gel Electrophoresis and Image Analysis: The copper sulfate and zinc chloride stressed *L. rhamnosus* proteins were isolated and subjected to 2-D gel electrophoresis, which was described in our previous research publication [7].

MALDI-TOF Analysis and Protein Identification: Differentially expressed proteins were identified in *Lactobacillus rhamnosus* strain upon exposure to effective dose (at 50% growth inhibition) of metal ions (copper and zinc) by MALDI-TOF/ MS analysis and reported [8].

Homology Modelling: The sequences of copper sulfate and zinc chloride stressed proteins were obtained from mass spectroscopy data. The modeling step can be carried out by searching the metal stress expressed protein sequences against the databases of well-defined template sequences were identified by the BLAST program [9] against protein database (PDB), which shows the maximum identity with a high score and less e-value designated as a template. All sequence alignments were completed by using Clustal W.

The 3-D model was generated by using the academic version of MODELLER 9.5, based on the information obtained from sequence alignment. The 3-D structure obtained from modeler [10]. Further analyzed by Ramachandran's map drawn using PROCHECK v.3.0 and verified 3-D. These programs accessed from the SAVES online server and the model satisfying all the parameters after the evaluation was considered for the further process.

RESULTS AND DISCUSSION: Three-dimensional structures were predicted for proteins. There was a lack of experimental structures for these proteins considered. Out of six proteins, three-dimensional structures were modeled for five proteins since it has been reported that these proteins of *L. rhamnosus* altered in copper stress condition represented in **Table 1**.

The acetyltransferase protein structure was not predicted because Query coverage, identity, and E-value were not allowed to predict the structure. The modeling of the three-dimensional structure of the protein was performed by three homology modeling program MODELLER.

Out of five proteins, three-dimensional structures were modeled for three proteins since it has been reported that these proteins of *L. rhamnosus* altered in Zinc stress condition represented in **Table 2**. The Alkaline shock protein and Cell division initiation protein Div IVA proteins structures were not predicted because Query coverage, identity, and E-value were not allowed to predict the structure.

The phi and psi distribution of the Ramachandran's Map generated by non-glycine, non proline residues were summarized in the said **Table 3**. The final modeled structures were visualized by Swiss PDB Viewer that was shown in the following **Fig. 1**.

The phi and psi distribution of the Ramachandran's Map generated by of non-glycine, non proline residues were summarized in the said **Table 4**. The final modeled structures were visualized by Swiss PDB viewer that was shown in the following **Fig. 2**.

TABLE: 1 TEMPLATE SELECTION FOR PROTEIN SEQUENCES BY USING BLAST SEARCH AGAINST PDB

S. no.	Name of the differentially expressed protein	Q. coverage (%)	Identity (%)	E-value	Template PDB - ID
1	Aspartate kinase	96	38	1e-110	3tvi_A
2	Mannose-6-phosphate isomerase	97	45	4E-98	1QWR_A
3	glutamate dehydrogenase	99	55	1E-1692	2YFH_a
4	30S ribosomal protein S19	92	69	1e-39	2gy9_s
5	50S ribosomal protein L4	100	67	5e-101	3j3v_e
6	Acetyl transferase	15	38	0.073	4dlo_A

(Differentially expressed proteins of *L. rhamnosus* under copper sulfate stress)

TABLE: 2 TEMPLATE SELECTIONS FOR PROTEIN SEQUENCES BY USING BLAST SEARCH AGAINST PDB

S. no.	Name of the differentially expressed protein	Q. coverage (%)	Identity (%)	E-value	Template PDB ID
1	Pyruvate oxidase	97	51	0.0	1pow_a
2	Alkaline shock protein	45	25	1.3	3v0c_a
3	Thymidylate synthase	97	100	0.0	1lca_a
4	Cell division initiation protein DivIVA	60.1	46	8e-12	2wuk_a
5	ATP-dependent Clp protease ATP-binding subunit ClpL	87	44	4e-177	3j3t_A

(Differentially expressed proteins of *L. rhamnosus* under Zinc chloride stress)

TABLE: 3 RAMACHANDRAN'S PLOT CALCULATIONS COMPUTED WITH THE PROCHECK PROGRAM

Server	Copper sulfate stressed *L. rhamnosus* proteins	Residues in the most Favored Region (%)	Residues in the additionally allowed region (%)	Residues in the generously allowed region (%)	Residues in the disallowed region (%)
	Aspartate kinase	90.5	7.7	1.0	0.7
	Mannose-6 phosphate isomerase	90.1	8.4	1.1	0.4
	Glutamate DH	89.8	8.4	1.0	0.8
	30S ribosomal protein S19	90	8.8	1.2	0.0
	50S ribosomal protein L4	87.6	7.9	2.2	2.2

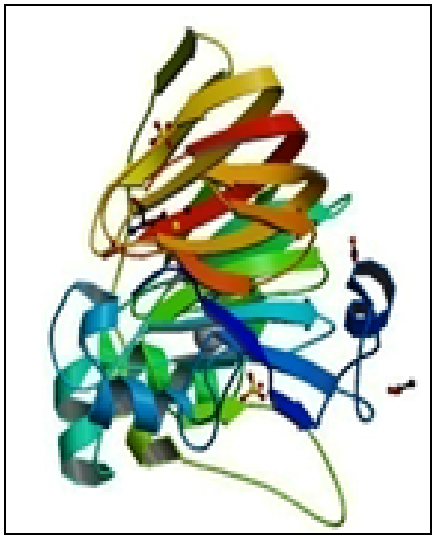
(Differentially expressed proteins of *L. rhamnosus* under copper sulfate stress)

TABLE: 4 RAMACHANDRAN PLOT CALCULATIONS COMPUTED WITH THE PROCHECK PROGRAM

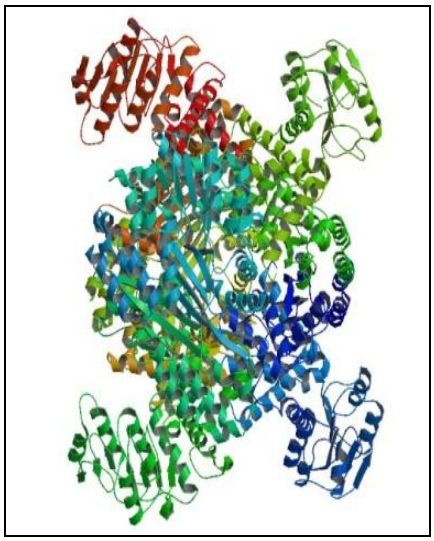
Server	Zinc chloride stressed *L. rhamnosus* proteins	Residues in the most Favored Region (%)	Residues in the additionally allowed region (%)	Residues in the generously allowed region (%)	Residues in the disallowed region (%)
	Pyruvate oxidase	93.0	6.2	0.6	0.2
	Thymidylate synthase	89.1	9.9	1.1	0.0
	ATP-depent Clp protease ATP-binding subunit ClpL	87.0	9.8	1.4	1.7

(Differentially expressed proteins of *L. rhamnosus* under zinc chloride stress)

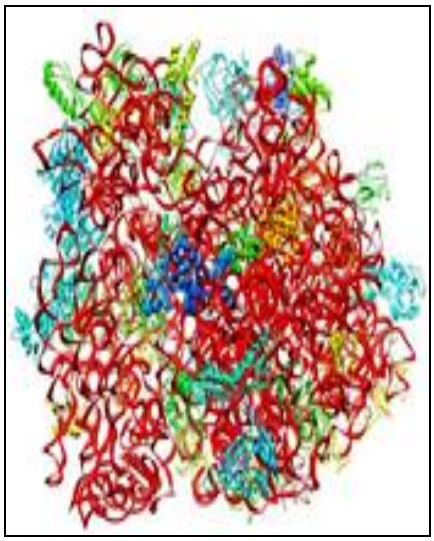
ASPARTATE KINASE



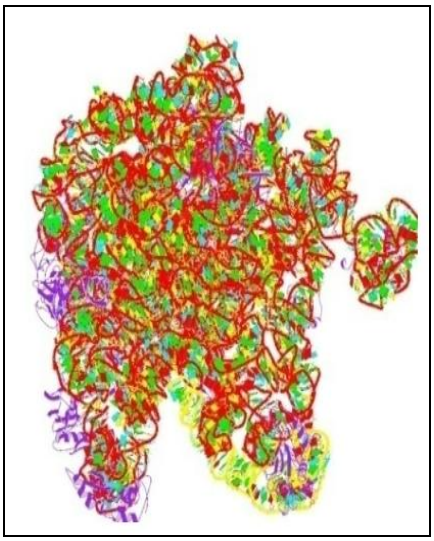
MANNOSE-6-PHOSPHATE ISOMERASE



GLUTAMATE DH

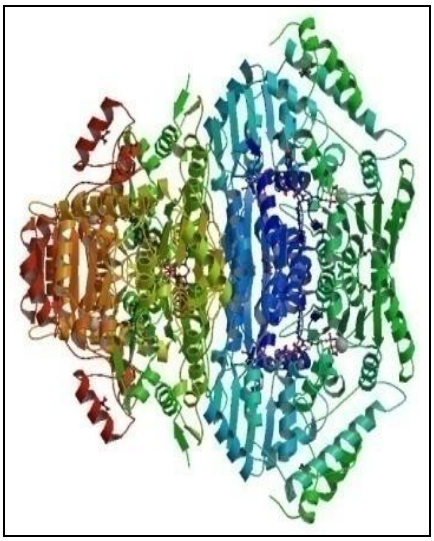


RIBOSOMAL PROTEIN S19

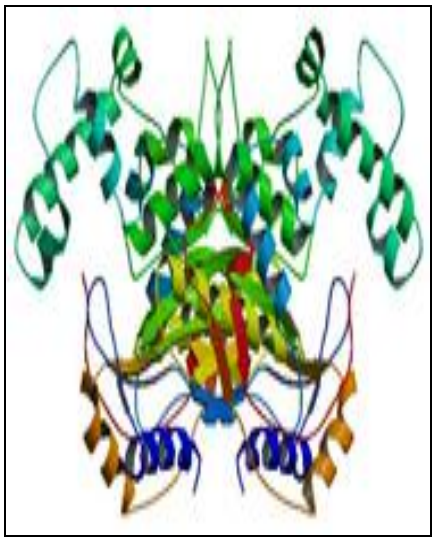


50S RIBOSOMAL PROTEIN L4

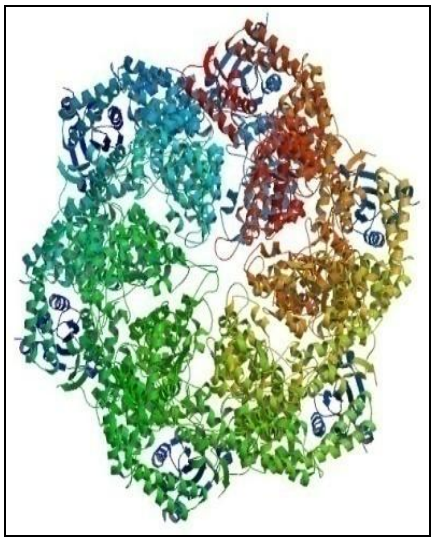
FIG. 1: MODELED STRUCTURE OF PROTEINS OF *L. RHAMNOSUS* UNDER COPPER SULPHATE STRESS



PYRUVATE OXIDASE

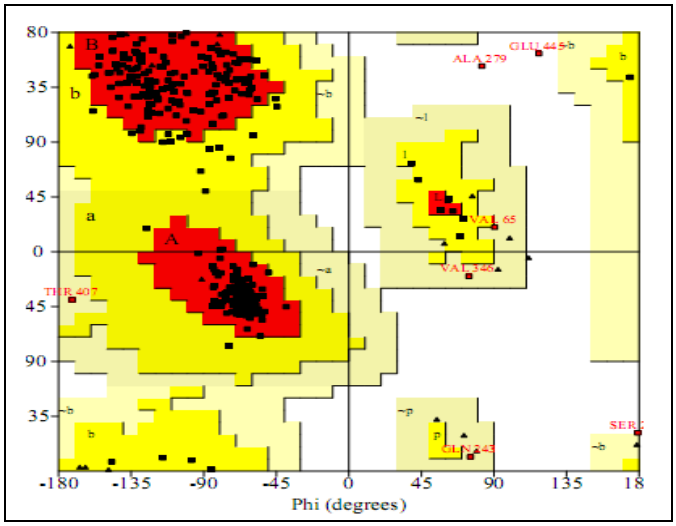


THYMIDYLATE SYNTHASE

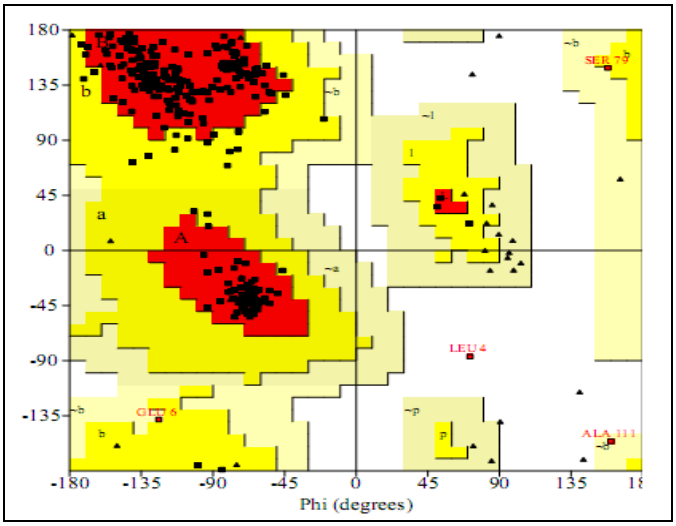


ATP DEPENDENT CLP PROTEASE ATP BINDING SUBUNIT

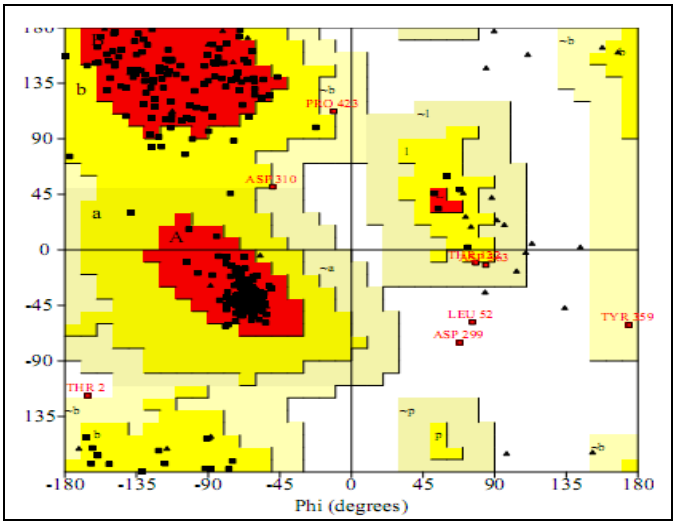
FIG. 2: MODELED STRUCTURE OF PROTEINS OF *L. RHAMNOSUS* UNDER ZINC CHLORIDE STRESS



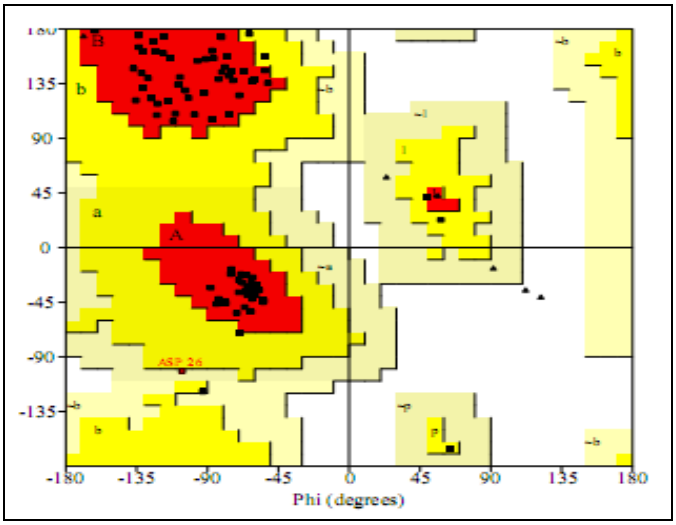
ASPARTATE KINASE



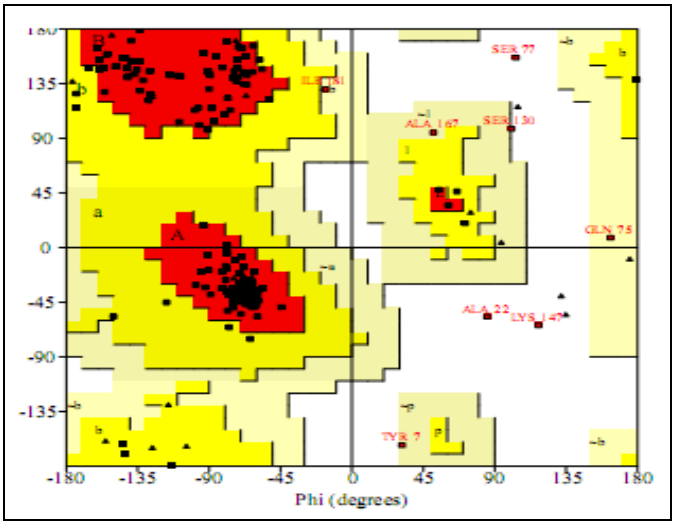
MANNOSE-6-PHOSPHATE ISOMERASE



GLUTAMATE DH



30S RIBOSOMAL PROTEIN S19



50S RIBOSOMAL PROTEIN L4

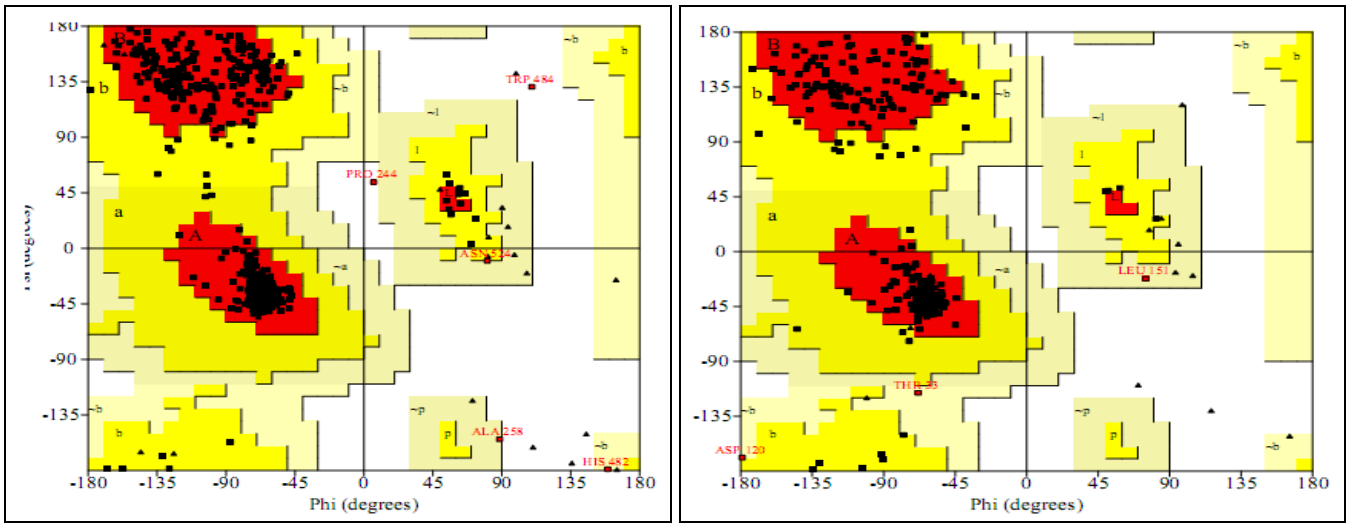
FIG. 3: RAMACHANDRAN'S MAP OF COPPER SULPHATE STRESSED *L. RHAMNOSUS* PROTEINS

The stereochemical quality of the predicted protein models and accuracy of the protein model was evaluated after the refinement process, by using Ramachandran's Map calculations were computed with the PROCHECK program. The assessment of the predicted models generated by MODELLER

9.5v was shown in **Fig. 3** and **4**. The main chain parameters plotted are Ramachandran's plot quality, peptide bond planarity, Bad non-bonded interactions, main chain hydrogen bond energy, C-alpha chirality, and over-all G factor. In the Ramachandran's plot analysis, the residues were classified according to its regions in the quadrangle.

The red regions in the graph indicate the most allowed regions, whereas the yellow regions represent allowed regions. Glycine is represented by triangles, and other residues are represented by squares. The result revealed that the modeled structure for Aspartate kinase, Mannose-6-phosphate isomerase, Glutamate dehydrogenase, 30s ribosomal protein S19, 50s ribosomal protein L4, Pyruvate oxidase, Thymidylate synthase and ATP dependent Clp protease ATP- binding subunit ClpL have 90.5%, 90.1%, 89.8%, 90%, 87.6%, 93.0%, 89.1% and 87.0% residue respectively in allowed region. The distribution of the main chain bond lengths and bond angles were found to be within limits for these proteins.

Such figures assigned by Ramachandran's plot represent the good quality of the predicted models.



PYRUVATE OXIDASE **THYMIDYLATE SYNTHASE**

FIG. 4: RAMACHANDRAN'S MAP OF ZINC CHLORIDE STRESSED *L. RHAMNOSUS* PROTEINS

CONCLUSION: In the present study, we described the Homology modeling of differentially expressed proteins of *L. rhamnosus* proteins. Homology modeling performed for the five structures of copper sulfate and zinc chloride stressed proteins, we got higher sequence similarity for Aspartate kinase, Mannose-6-phosphate isomerase, 30 S ribosomal subunit S19 and Pyruvate oxidase with template proteins.

All the results obtained from RMSD, verify3 - D and PROCHECK assembled showed the results for homology modeling. These structures are allowed as biomarkers and provide a good foundation for a finding new potential drug.

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CONFLICT OF INTEREST: Nil

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