ISSN NO. 2320-5407

*Journal homepage: http://www.journalijar.com*

*INTERNATIONAL JOURNAL OF ADVANCED RESEARCH*

**RESEARCH ARTICLE**

# MALDI-TOF mass spectrometry analysis for identification of differentially expressed proteins of copper sulphate and zinc chloride stressed proteins of *Lactobacillus rhamnosus.*

**Sreevani Sreemanthula, Chandra sekhar Kathera and Pramoda kumari Jasti***
Department of Microbiology, Sri Venkateswara University, Tirupati, Andhra Pradesh, India.

## *Manuscript Info*

***Manuscript History:***

Received: 08 November 2013
Final Accepted: 28 November 2013
Published Online: December 2013

***Key words:***
*Lactobacillus rhamnosus,* MALDI-TOF, Biomarkers**.**

## *Abstract*

**Aims:** The aim of our study is MALDI-TOF analyses of *Lactobacillus rhamnosus* strain upon exposure to effective dose (at 50% growth inhibition) of metal ions (zinc and copper) have been carried out. **Materials and Methods:** Copper sulphate and zinc chloride stressed *L. rhamnosus* strains were taken and subjected to MALDI-TOF analysis. **Results:** Six proteins in copper sulphate stressed and 5 proteins in zinc chloride stressed *L. rhamnosus* were differentially expressed. **Conclusion:** The majority of the obtained proteins were involved in aminoacid metabolism and the rest were in different biosynthesis processes. These changes in protein expression may serve as possible biomarkers for the usage of *L. rhamnosus* as probiotics.



## Introduction

Environmental pollution by metal ions is widespread receiving more attention as a global 'health-political-economical' concern due to their involvements in human health problem, their stockpiling in the food supply chain, and their spread out in water resources (Islam et al., 2007). Some elements play vital role in metabolism as essential metals but when the intake is excess they became toxic. Toxicity to the human-being comprises of several acute and chronic-diseases, nutritional deficit, hormonal imbalances, autoimmune and neurological disorders *etc* (Lynes et al., 2007). Presumptive explanations on the occurrence of such detrimental effects of metals can be drawn on; alterations on a balance of pro-oxidant and antioxidant systems, dislocations on a immense agglomerate of metabolic processes, and competitions with nutrient trace elements for binding sites on essential metallo enzymes, receptors, metal-binding transporter and storage systems (Bertin and Averbeck, 2006).

Investigations of detailed mechanisms on 'how metals exerted their toxic effects in biological systems' and 'how organisms acclimatized themselves in response to metal stress' are continuously carried out in several models e.g. plants, microbes, animals and mammalian cells (Yoon et al., 2008). Recent years have seen a growing interest in health beneficial bacteria, including commensal inhabitants of the gastro-intestinal tract (GIT) and probiotics. Recently, the knowledge accumulated on probiotic species, associated with the social desire to characterize health-promoting species, led to the exploration of the new mechanisms sustaining their probiotic activity, where extended knowledge will allow a more rational evaluation of health benefit claims.

Current research of the molecular biology of probiotic bacteria, the technique proteomics is widely used. Proteomics is a tool for studying the proteome, *i.e.*, the set of proteins expressed under a defined physiological condition in an organism. To date, the reported proteome studies of probiotic lactobacilli, including *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactobacillus casei*, and *L. rhamnosus*, have aimed to identify the mechanisms of probiotic functions using 2-DE based applications (Cohen et al., 2006). Several health beneficial experiments have been carried out on *L. rhamnosus.* However, the molecular mechanisms and underlying responses of *L. rhamnosus* cells against various metal ions are not yet completely understood. Therefore, in the present study, MALDI-TOF analyses of *L. rhamnosus* strain upon exposure to effective dose (at 50% growth inhibition) of metal ions (zinc and copper) have been carried out.

## Materials and Methods

**Determination of Minimum inhibitory concentration of copper sulphate and zinc chloride for *L. rhamnosus* and evaluation of growth curves:**

Copper and zinc are essential metals but if intake is excess they may be toxic. In our previous study excess were taken. Minimum inhibitory concentrations for copper sulphate and zinc chloride for *L. rhamnosus* were done by using macro dilution method and growth curves of copper sulphate and zinc chloride stressed *L. rhamnosus* were evaluated at 40mM (MIC of both $CuSo_4$ & $ZnCl_2$) (Sreevani et al., 2013a).

**2-D Gel Electrophoresis and Image analysis:**

The copper sulphate and zinc chloride stressed *L .rhamnosus* proteins were isolated and subjected to 2-D gel electrophoresis according to Sreevani et al., 2013b).

**Sample preparation for MALDI-TOF analysis:**

In-gel digestion of $CuSo_4$ & $ZnCl_2$ stressed *L. rhamnosus* proteins were carried out using MS-grade Trypsin Gold according to the manufacturer's instructions. Briefly, spots were cut out of the gel (1–2 mm diameter) using capillaries, and destined twice with 25 mM $NH_4HCO_3$/50% acetonitrile (ACN) at room temperature for 45 min in each treatment. This was followed by dehydration of the gels with 100%ACN for 5min. After dehydration and drying, the gels were pre-incubated in 10–20 µl trypsin solution (20ng/µl) for 1 h. Then samples were added in adequate digestion buffer (25 mM $NH_4HCO_3$/50% ACN) to cover the gels and incubated overnight at 37°C. Tryptic digests (peptides) were extracted twice with 50% ACN 5% trifluoroacetic acid (TFA) for 30min each time. The combined extracts were dried in a vacuum concentrator at room temperature. The extracted peptides were dissolved in 2.5 µl of 50% ACN/0.1% TFA, and then 0.8 µl of the digests were mixed with 0.8 µl of 5mg/ml alpha-cyano-4-hydroxy-cinnamic.

The samples were analyzed on a 4800 Proteomics Analyzer MALDI-TOF/MS mass spectrometer working in positive ion reflector mode. The instrument was calibrated to < 10 ppm accuracy using calibration mixture of known standard synthetic peptides in the mass range 800-4000 m/z.

***Protein identification***

PMF data was interrogated for protein identification with NCBI database for *L.rhamnosus* using Mascot search engine and analysis was done on global proteomic solutions (GPS) software automatically (Ventura et al., 2009). Database searches were carried out are summarized in the following Table-1.

**Table: 1 Mascot search parameters**

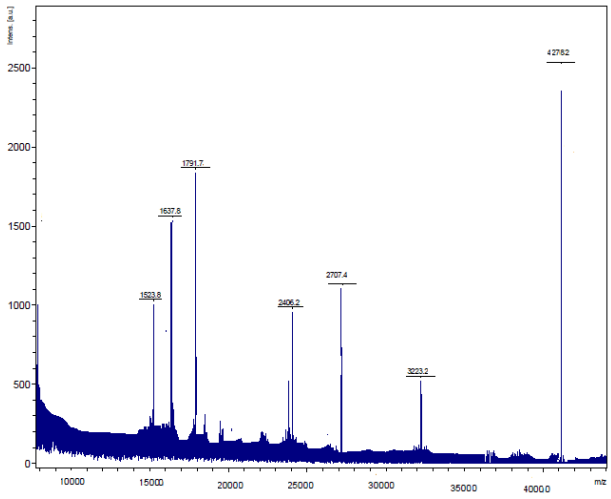
| **Parameter** | **Value** |
|---|---|
| Taxonomy | *L. rhamnosus* |
| Database | Swiss prot |
| Maximum missed cleavage | 1 |
| Fixed modification | Carbamido methyl (C) |
| Variable modification | Oxidation |
| Enzyme | Trypsin |
| Peptide tolerance | 200-1200 ppm |
| Peptide charge | +1 |
| Data format | Mascot generic |
| MS tolerance | 0.2-2 Da |

Searches were performed without constraining protein molecular weight or isoelectric point. Proteins with probability-based Molecular Weight Search (MOWSE) scores exceeding their threshold ($P$<0.05), number of matched ions, percent protein sequence coverage, and correlation of gel region with predicted MW and pI were collectively considered for each protein identification.

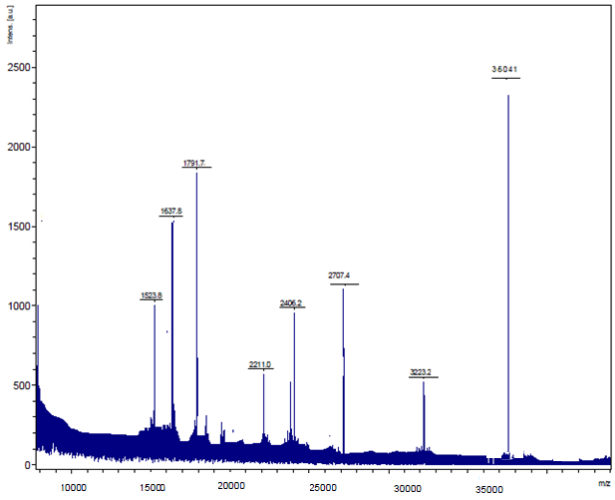
## Results and Discussion

In our previous study, the metal ion stress responses of *L. rhamnosus* was analyzed by using MIC, growth curves (Sreevani et al., 2013a) and 2-D gel electrophoresis (Sreevani et al., 2013b). By extending this with MALDI-TOF for complete proteome mapping of $CuSo_4$ & $ZnCl_2$ stressed *L. rhamnosus.*

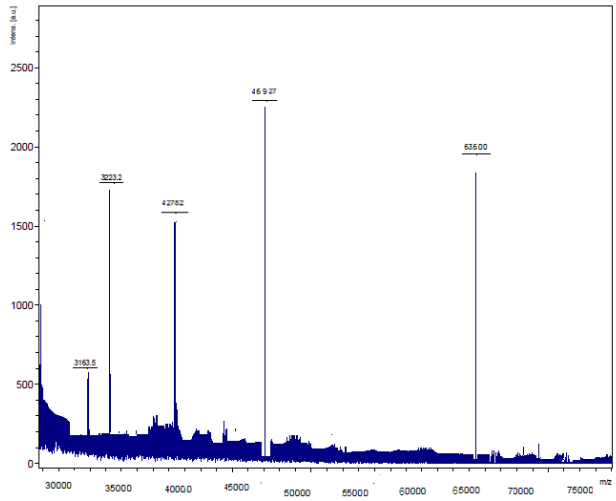
**Fig:1 MS spectra of for identification of differentially expressed proteins of *L. rhamnosus* under copper sulphate toxicity.**



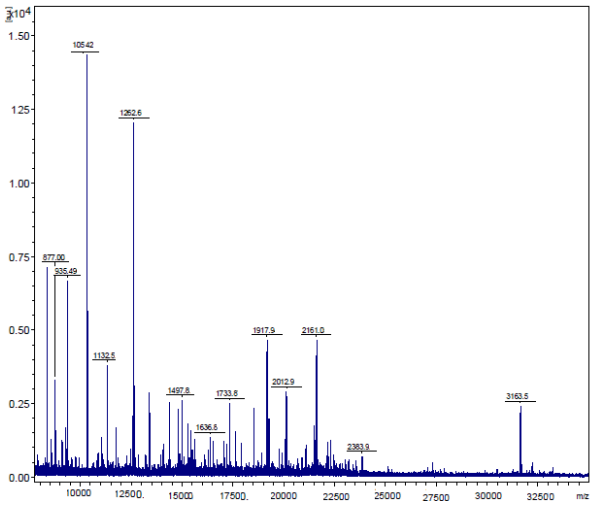

**Aspartate kinase**



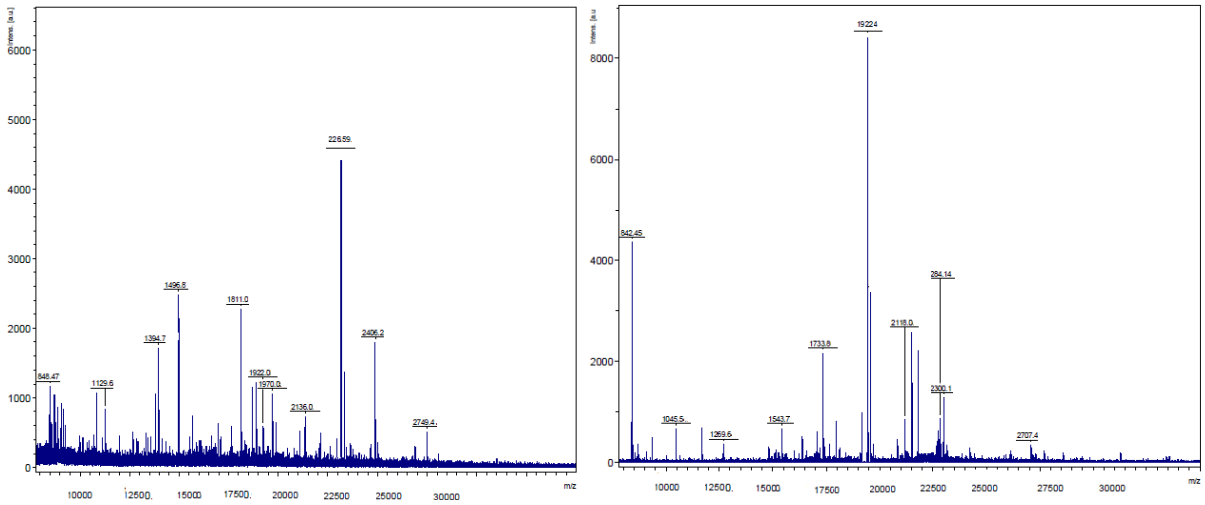

**Mannose-6-phosphate isomerase**




**Glutamate dehydrogenase**




**30S ribosomal protein S19**



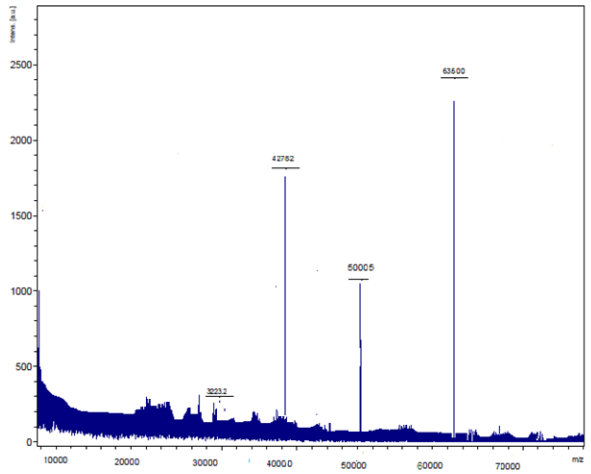

A MALDI mass spectrum of a whole-cell suspension of $CuSo_4$ stressed *L. rhamnosus* in alpha cyano-4-hydroxy-cinnamic as a matrix is shown in Figure 1. These peaks are highly reproducible if the same experimental protocol is observed. Results of the database search using the experimental masses as search parameters are summarized in Table 1. Upon exposure to copper sulphate, some proteins of *L. rhamnosus* were differentially expressed which were summarized in table-2.

**Table: 2 List of differentially expressed proteins of $CuSo_4$ stressed *L. rhamnosus***

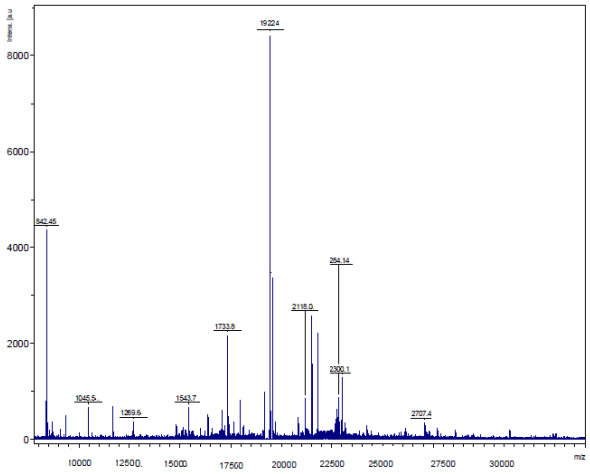
| Spot. No | Expression protein name | MW | Calculated p*I* value | Protein Score | Sequence coverage % | Matched Peptides |
|---|---|---|---|---|---|---|
| 1 | Aspartate kinase | 42782 | 5.47 | 84 | 21 | 6 |
| 2 | Mannose-6-phosphate isomerase | 36 041 | 5.36 | 71 | 36 | 11 |
| 3 | Glutamate dehydrogenase | 46927 | 5.68 | 74 | 6 | 2 |
| 4 | 30S ribosomal protein S19 | 10542 | 9.94 | 41 | 9 | 1 |
| 5 | 50S ribosomal protein L4 | 22659 | 9.76 | 56 | 23 | 4 |
| 6 | Acetyltransferase | 19 224 | 5.33 | 77 | 41 | 5 |

Aspartate kinase, is an enzyme that catalyzes the phosphorylation of the amino acid aspartate. This reaction is the first step in the biosynthesis of three essential amino acids: methionine, lysine, and threonine (Viola, 2001). Mannose-6 phosphate isomerase (MPI), alternately phosphomannose isomerase (PMI) is an enzyme which facilitates the inter conversion of fructose-6-phosphate (F6P) and mannose-6-phosphate(M6P). Glutamate dehydrogenase (GLDH) is an enzyme, which converts glutamate to α-ketoglutarate, and vice versa. Protein synthesis is a complex, multistep process that requires, in addition to the ribosome, several extrinsic GTP-hydrolyzing protein factors during each of the main stages of initiation, elongation and termination. The 30S ribosomal subunit has a crucial role in decoding mRNA by monitoring base pairing between the codon on mRNA and the anticodon on transfer RNA; the 50S subunit catalyses peptide-bond formation. Acetyl transferase catalyzes the transfer of acetyl groups in several energy metabolisms ((Donald Voet and Judith G Voet. 2010). Excess copper sulphate alters the proteins functions, which are mainly involved in the aminoacid and sugar metabolism in *L. rhamnosus.*

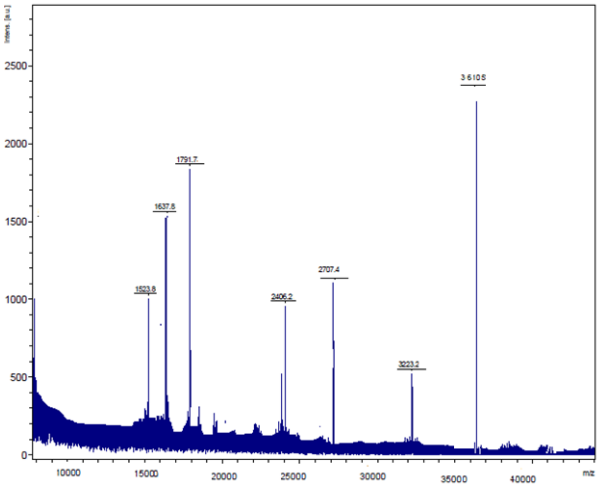
**Fig: 2 MS spectra of for identification of differentially expressed proteins *L. rhamnosus* under zinc chloride toxicity.**



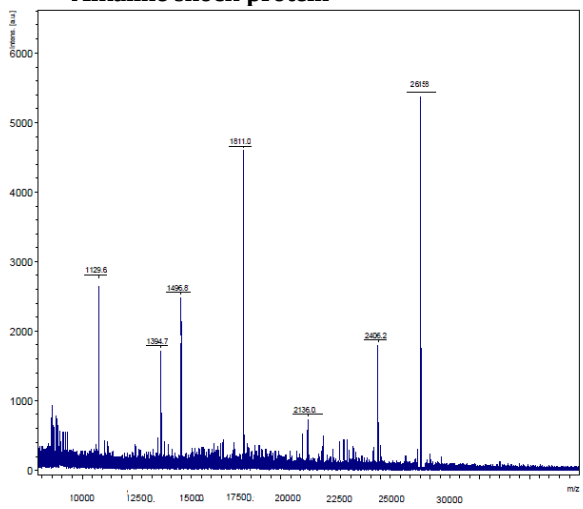

**Pyruvate oxidase**



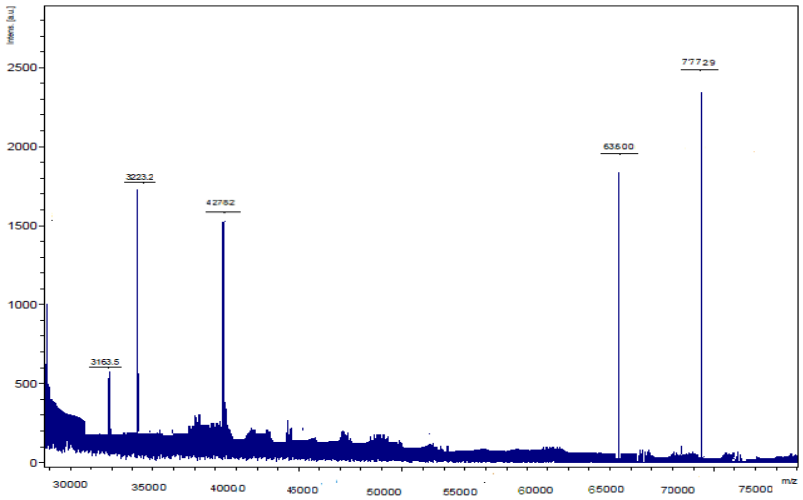

**Alkaline shock protein**




**Thymidylate synthase**




**Cell division initiation protein DivIVA**




**ATP-depent Clp protease ATP-binding subunit ClpL**

A MALDI mass spectrum of a whole-cell suspension of $ZnCl_2$ stressed *L. rhamnosus* in alpha cyano-4-hydroxy-cinnamic as a matrix is shown in Figure-2. These peaks are highly reproducible if the same experimental protocol is observed. Results of the database search using the experimental masses as search parameters are summarized in Table-2.

**Table: 3. List of differentially expressed proteins of $ZnCl_2$ stressed *L. rhamnosus***

| Spot. No | Expression protein name | MW | Calculated p*I* value | Protein Score | Sequence coverage % | Matched Peptides |
|---|---|---|---|---|---|---|
| 1 | Pyruvate oxidase | 63 600 | 4.94 | 68 | 14 | 6 |
| 2 | Alkaline shock protein | 17 556 | 5.11 | 56 | 30 | 5 |
| 3 | Thymidylate synthase | 36 105 | 5.52 | 70 | 27 | 7 |
| 4 | Cell division initiation protein DivIVA | 26 158 | 4.49 | 84 | 40 | 4 |
| 5 | ATP-dependent Clp protease ATP-binding subunit ClpL | 77729 | 5.44 | 89 | 46 | 3 |

A pyruvate oxidase is an enzyme that catalyzes the pyruvate, phosphate into acetyl phosphate, $CO_2$ and $O_2$. Alkaline shock protein plays a key role in maintaining the tolerance to alkaline pH. The enzyme, Thymidylate synthase which is involved in Pyrimidine catabolism and also essential for regulating the balanced supply of the 4 DNA precursors in normal DNA replication. Cell division initiation protein DivIVA involved in division site selection (Donald Voet and Judith G Voet, 2010). ATP-dependent Clp protease ATP-binding subunit ClpL involved in complete degradation of abnormal proteins (Daniel, 2013). Excess zinc chloride alters the proteins functions, which are mainly involved in the aminoacid metabolism and cell division in *L. rhamnosus.*

## Conclusion

We have established a specific proteomic pattern in *L. rhamnosus* that is indicative of $CuSo_4$ and $Zncl_2$ treated. Using 2-DE in conjunction with mass spectrometry, significant changes on the protein expression profiles of *L. rhamnosus* in response to trace metals have been explored. Study of toxic effects of trace metals on protein expression can be useful for gaining insight into the biomolecular mechanisms of toxicity and for identifying potential metal-specific protein markers of exposure and response. Proteomic studies on metal stress in *L. rhamnosus* bacteria are still at their beginning and one can hope at a great development on the knowledge of these bacteria and their rational use in near future. Ultimately, combining the proteomics technology with bioinformatics might lead to identification of new regulatory networks in the cell which will be useful for recognition of status of the cell, tissue or body.

## References

1. Bertin, G. and Averbeck, D. (2006): Cadmium: cellular effects, modifications of biomolecules, modulation of DNA repair and genotoxic consequences (a review). Biochimie., 88:1549-1559.
2. Cohen, D.P.A., Renes, J., Bouwman, F.G., Zoetendal, E.G., Mariman, E., de Vo, W.M. and Vaughan, E.E. (2006): Proteomic analysis of log to stationary growth phase *Lactobacillus plantarum* cells and a 2-DE database. Proteomics., 6 (24) :6485-93.
3. Daniel, A. (2013): Kraut protease function by slowing unfolding Slippery substrates impair ATP-dependent published online October 22, 2013 J. Biol. Chem. 1-13.
4. Donald, Voet. and Judith, G, Voet. (2010) VOET and VOET ISBN-13: 978-0470570951 | Edition: 4.

5. Islam, E., Yang, X.E, He, Z.L. and Mahmood, Q. (2007): Assessing potential dietary toxicity of heavy metals in selected vegetables and food crops. J Zhejiang Univ Scie., 8:1-13.

6. Lynes, M.A., Kang, Y.J, Sensi, S.L, Perdrizet, G.A. and Hightower, L.E. (2007): Heavy metal ions in normal physiology, toxic stress, and cytoprotection. Annals. New York, Acad Scie., 1113:159-172.

7. Sreevani, S., Chandra sekhar, K., Esther Lebonah, D. and Pramoda Kumari, J. (2013a): Noxious effect of trace metals on Probiotic "*Lactobacillus rhamnosus".* Int J Biol Scie and Technol., 3: 24-27.

8. Sreevani Sreemanthula., Chandra sekhar Kathera. and Pramoda kumari Jasti (2013b): preliminary protein profiling of copper and zinc treated *Lactobacillus rhamnosus.* Int J Lifescie and Pharma res., 3: (4) L-1-L-5.

9. Ventura, M., Turroni, F., Canchaya, C., Vaughan, E.E., O'Toole, P.W. and Van Sinderen D. (2009): Microbial diversity in the human intestine and novel insights from metagenomics. Front. Biosci., 14: 3214-3221.

10. Viola, R.E. (2001): The central enzymes of the aspartate family of amino acid biosynthesis. Acc Chem Res., 34 (5): 339–49.

11. Yoon, S., Han, S.S. and Rana, S.V. (2008): Molecular markers of heavy metal toxicity--a new paradigm for health risk assessment. J Envir biol., 29:1-14.