Kiran Thakur, J Nutr Food Sci 2016, 6:4 (Suppl)
http://dx.doi.org/10.4172/2155-9600.C1.023

Riboflavin producing probiotic lactobacilli as a biotechnological strategy to obtain riboflavin enriched functional foods

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Riboflavin is an obligatory component of cellular metabolism, being as ultimate precursor of coenzyme FMN and FAD which are obtained by the phosphorylation of riboflavin in all living cells. It has been traditionally synthesized for food and feed fortification by chemicals means but past decade has witnessed a surge in information about commercial biotechnological processes. Hence this project was aimed at the isolation, identification and riboflavin operon characterization of lactobacilli from various niches. Among the 55 isolates bioprospected from dairy and non dairy sources, 16 isolates were found harboring complete *rib* structural genes. The cloning and sequencing of rib genes from one isolates was done for BLAST analysis. The isolates harboring both complete as well as incomplete operon were compared phenotypically for riboflavin production by chemical, fluorescence and microbiological based assays and the microbiological assay method was found most sensitive among these three methods. Among the 30 isolates tested for riboflavin production ability, 10 were found to be riboflavin producers. Among them, isolates viz., KTLF1, KTLF9, KTLP1, KTLF11 and KTLF16 have shown 1.89 mg/L, 1.5 mg/L, 1.456 mg/L, 1.19 mg/L and 1.67 mg/L respectively. The isolate KTLF12, LP13 and KTLF2 have shown 0.95 mg/L, 0.83 mg/L and 0.46 mg/L of riboflavin production. (These isolates were able to survive in medium devoid of riboflavin as well as they have supported the growth of riboflavin auxotroph (*L. casei* MTCC1408). Among the screened isolates on agar diffusion assay, the maximum increase in growth of auxotroph was observed in the presence of KTLF1 and KTLF16 respectively. Expression pattern of rib genes was studied in selected isolates viz., LF1, LF2, LF3, LF4, LP1 and MTCC8711. RNA was isolated at different intervals of time in MRS and Riboflavin assay medium (RAM), milk and whey based medium. The range of relative fold in mRNA expression in *Rib1* gene is 5 to 55 fold, 0.5 to 35 fold in *Rib2* gene, 0.2 to 6.5 fold in *Rib3* and 0.2 to 26 fold in *Rib4* in MRS and RAM over control culture. On the basis of fold increase in relative mRNA expression of all the *Rib* genes, the isolate KTLF1 was selected for expression studies in milk and whey. The fold increase observed was 0 to 1.1 fold in Rib1, 0 to 2 fold in *Rib2*, 0 to 2 fold in *Rib3* and 0 to 0.9 fold increases in mRNA expression in milk and whey. The riboflavin producers were further screened for *in vitro* probiotic, safety aspects as well as technological properties. Three riboflavin producing isolates KTLF2, KTLF5 and KTMUC were able to show potential probiotic and safety attributes, while KTLF5 was showing appreciable adhesion on HT-29 cell lines as well as hold the promises to be used as novel starter cultures. The expression profile has given the clear picture of variation in expression profile of *rib* genes at different intervals of time. All of the four genes have displayed significant difference with respect to media and time intervals. The isolate KTLF1, KTLP1 from human feces, KTLF16 from fermented bamboo shoot has shown highest riboflavin production. The study has generated the data for further exploration of these isolates endowed with appreciable starter as well as functional activities for industrial use as novel and native starter cultures to produce an essential vitamin *in situ* which would contribute significantly to the functional value of certain fermented foods.

Biography

Dr Kiran Thakur has completed Ph.D in Dairy Microbiology at National Dairy Research Institute (NDRI), Karnal, and Haryana, India. She is the member of Singapore Society for Microbiology and Biotechnology (SSMB) and International Scientific Association for Probiotics and Prebiotics (ISAPP). She has been conferred *BEST THESIS AWARD 2013-2014* for outstanding Doctoral Research work and Director's Gold Medal for the same. Her PhD work has been recognized at various national and international platforms. She has attended Annual meeting organized by (ISAPP) at Georgetown University, Washington DC, USA (May 18- 21, 2015) and International Seminar on Pharmaceutical Science and Technology at Padjadjaran University at Indonesia. She has been granted "Outstanding Oral Presenter" at 7th Asian Conference at Lactic Acid Bacteria held on 6- 8 September, 2013 at Indian habitat centre, New Delhi and also bagged Best Poster Presentation Award at National level. She has Presented Poste for Rowett-INRA 2014 Conference (co-hosted by ISAPP) "Gut Microbiology: from sequence to function", at Aberdeen, Scotland, UK on 16-19 June, 2014. She has attended workshop at National University of Singapore Sep, 2013. She has published 9 Research and Review articles with high impact international and national journals. She has published 12 popular articles. She has submitted 15 partial 16s DNA sequences in NCBI. She is the authors of many articles in newspapers and magazines. She has also contributed for technical manuals and compendium at national levels. She has authored two books for Lambert Academic Publishing, Germany. She is continuously seeking a dynamic & challenging career in Academics as well as Research and development in Food science and Technology and to promote the new research scenario as an ideal human resource.

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