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Design of Experiment Approach for Developing Probiotic and Prebiotic Fortified Fermented Food Product

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Objective: Human gut microbiota plays a significant role in determining health and disease status. Probiotics may help maintaining favorable composition of gut microbiota, and can even reverse the ill effects of dysbiosis thus, contributing towards sustaining good health. Several of the potential health benefits of probiotics are well established, and numerous are still under investigation, and wanting more evidences. Probiotics show immense diversity with respect to their health benefitting attributes i.e. health benefits earmarked in one strain/species cannot be extrapolated to others without active experimentation. Thus, bioprospecting of novel efficacious probiotic strains is a continuous practice. Dairy products have widely been used as probiotic vehicles, however, recently earned some criticism due to high fat content, lactose intolerance, and allergies. Therefore, alternative probiotics-vehicles are being explored. Cereals could be one of the good choices as probiotic carriers. The current study aimed at isolating efficient probiotics, their functional characterization, and application for development of oat-based fermented food product using design of experiment (DOE) approach.

Methods: Probiotic bacteria (mostly lactic acid bacteria) were isolated from various sources based on enrichment in MRS. Isolates were examined for tolerance against gastrointestinal (GIT) conditions, and those capable of withstanding, were characterized for desired functional attributes like hydrophobicity, auto-aggregation, co-aggregation, extracellular enzyme activity, antibacterial activity, antibiotic susceptibility, among others. The isolate which showed most of the desired functional features (M-13) was, identified based on 16S rDNA sequence analysis, used for development of oat based fermented food product.

Results: Among 39 LAB isolates, 12 showed tolerance under GIT conditions, and were characterized for various functional properties of probiotics. The isolate M-13 identified as *Lactobacillus plantarum* (designated as *L. plantarum* M-13), was selected for developing oat based fermented food product. Optimization of process variables like concentration of oat, and honey, and incubation time was executed using Box-Behnken design of response surface methodology. Optimum level of variables which supported maximum growth of *L. plantarum* CFU/ml (15.98) was as follows: oat 8.0 %, w/v, honey 3.0 % w/v, and incubation time 48 h. Furthermore, growth of *L. plantarum* M-13 in modified MRS in which glucose was replaced with various prebiotics (inulin, lactulose, fructooligosaccharides, and xylooligosaccharides) showed that most of prebiotics including the one *in-house* generated (xylooligosaccharides) supported generous growth of bacterium. Among process variables incubation time was the most effective, and was followed by honey, and oat; interactive effect of honey and incubation time was maximum on growth of bacterium, and was followed by that of oat and honey, and oat and incubation time. The probiotic-prebiotic-fortified food product developed was studied for shelf life.

Conclusion: It may be concluded that *L. plantarum* have most of the desired probiotics properties, and may potentially be exploited as probiotic. Probiotic-prebioitc-fortified oat based food product (oat +honey) could be a very health carrier of probiotics as it includes all health effects of oats, and that of probiotics, and of course of preobiotic honey.