Abstract

Plant probiotics are plant associated elite bacteria which have multiple abilities like protecting plants from diseases and improving the nutritional quality of the plant. Junnar, Ambegaon and Khed talukas were the target locations based on productivity from Pune district. Total 266 presumptive Lactic acid bacteria isolates were obtained from ten different vegetables from fields by cluster sampling using the randomized block design. The vegetable samples were cabbage, cauliflower, gherkins, cluster beans, cow pea, french beans, tomato and cucumber. These vegetable provide suitable microenvironment for colonization of Lactic acid bacteria. Identification of isolates was done using Matrix Associated Lesser Desorption Ionization Time-of-Flight (MALDI-TOF), Microlog Gen III and 16S r RNA gene sequencing. Fresh vegetables harbour Lactic acid bacteria and the predominant genera were Lactobacillus, Enterococcus, Pediococcus, Lactococcus, Leuconostoc and Weissella. Lactic acid bacteria colonization appeared to preferably occur on tomato, cauliflower, fenugreek and gherkins. Lactobacillus plantarum was the common predominant genus.

Probiotic potential of these isolates in terms of acid tolerance, tolerance to bile salt and pancreatin was at par with reference strain Lactobacillus plantarum MCC2156 and strains selected from other sources (Human origin and fermented food). This shows that isolates from unconventional sources particularly non-intestinal isolates are equally potent in their probiotic properties as compared to the isolates of intestinal origin. Cellular extracts of several isolates displayed antioxidant activity. In vitro safety tests were performed on peripheral blood mononuclear cells alone and on peripheral blood mononuclear cells exposed to oxidant. Comet assay was used to assess if DNA damage was induced by any of the Lactobacilli. It was found that none of the Lactobacilli induced DNA damage and on the contrary Lactobacillus plantarum AG40V offered DNA protection comparable to that provided by quercetin. Two strains Enterococcus sp. ID11V and Enterococcus sp. ID19V offered best protection from oxidative DNA damage.

Some of the isolates also displayed antimicrobial activity against plant and human pathogens. Lactobacillus sp. J129V, Lactobacillus sp. J23V, Lactobacillus sp. ID12V, Enterococcus sp. ID11V and Enterococcus sp. ID19V showed maximum growth inhibition of plant and human pathogens.
Lactobacillus plantarum AG40V, Enterococcus sp. ID11V and Enterococcus sp. ID19V were synthesized the acetic acid and propionic acid, type of short chain fatty acids which exerts many positive effects on host such as in providing source of energy and in intestinal inflammation and gut cancer.

Based on bioactive properties; Lactobacillus plantarum AG40V, Enterococcus sp. ID11V and Enterococcus sp. ID19V were further selected for their ability to modulate the expression of genes in gut cancer cells Caco-2. The gene expression data shows the ability of the bacteria to drive the cells towards apoptosis and found their role in anti-inflammatory, which could offer a clue to the use of probiotics in curing gut cancer and gut inflammation.

To test the efficacy of these isolates in gut inflammation, four probiotic formulations were prepared and tested on animal model. Lactobacillus plantarum AG40V, Enterococcus sp. ID 11V and Enterococcus sp. ID19V were the vegetable isolates selected for probiotic formulation preparation. According to FSSAI and ICMR-DBT guidelines the bacteria used in probiotic formulation preparation was tested for the antibiotic susceptibility pattern. Lactobacillus plantarum AG40V, Lactobacillus sp. HO17HF and Lactobacillus sp. SK2FB was sensitive to β-lactam group of antibiotics, Enterococcus sp. ID 11V and Enterococcus sp. ID19V exhibited sensitivity towards β-lactam and aminoglycoside antibiotics; indicating their safety for use as probiotics. Oral dosing of probiotic formulation on Sprague–Dawley rats found non toxic in acute and sub-acute oral toxicity test. In the efficacy testing of probiotic formulations, Trinitrobenzenesulfonic acid was used to induce gut inflammation in male and female Sprague-Dawley rats. All probiotic formulations could aid in recovery of the animals from the gut inflammation. The recovery was at par with the recovery observed in animals treated with the standard drug prednisolon. Probiotic formulation with Lactobacillus plantarum AG40V and Enterococcus sp. ID11V and the formulation with Lactobacillus plantarum AG40V and Lactobacillus sp. HO17HF gave the best recovery. There was significant decrease observed in inflammatory markers such as nitric oxide, catalase, lipid peroxidase, myeloperoxidase, fibrinogen and C-reactive protein.

Biotically potent isolate Lactobacillus plantarum AG40V when exploited for preservation, offered protection on fresh cut vegetables and sprouts from spoilage. The Lactobacillus plantarum AG40V found helpful in protecting the fresh fruits and vegetables from pathogen attack such as Pseudomonas sp., Yeast and moulds. No
substantial decrease was observed in the LAB count in the test fresh tomato and lettuce as well as sprouts (Matki) treated with *Lactobacillus plantarum* AG40V.

Conclusively, Lactic acid bacteria isolated from vegetables are suitable probiotic candidates because they exhibited probiotic potential in addition to anti-oxidant, anti-microbial, anti-inflammatory activity *in vitro* and *in vivo*. Bioactive compounds secreted by these isolates proves nutraceutical property and health application. This study is evident for probiotic application of *Enterococcus* sp. Additionally the stain tested *Lactobacillus plantarum* AG40V exhibited good preservation ability against tested vegetables. These isolates could be tested for several microbiome-based therapies in the near future.