

**Original Research Paper**

## Screening of probiotic strains for development of ready- to -serve probioticated mango beverage

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### ABSTRACT

Out of the thirteen probiotic strains procured from different sources or isolated from the commercially available sachets, seven isolates showed growth in the ready to serve (RTS) mango beverage. Among the seven strains, only three strains, i.e., *Lactobacillus helveticus* MTCC 5463, *L. rhamnosus* MTCC 5946 and *Saccharomyces boulardii* showed significant growth in the mango beverage. These three strains were further evaluated for population build-up, physico-chemical and sensory evaluation parameters in the fermented mango beverage. Based on the results of sensory scores, minimum threshold population required for classification as probioticated beverage and physico-chemical characteristics, *L. helveticus* was used for probiotication of the RTS mango beverage. Mango beverage fermented with *L. helveticus* MTCC 5463 showed an average score of 7.34 on a hedonic scale of 9 for overall acceptability, had an acidity of 0.29%, sugar concentration of 7.6% and pH of 4.4. Probioticated mango beverage also had about 20 and 13% higher phenolics and flavonoids, respectively, compared to uninoculated RTS mango beverage. This study has shown that the RTS mango beverage inoculated with *L. helveticus* MTCC 5463 has potential for developing probioticated mango beverage.

**Key words:** Probiotics, Mango beverage, *Lactobacillus helveticus*, Non-dairy probiotics; Cell population; Sensory scores

### INTRODUCTION

Fruit and vegetable beverages are healthy and refreshing foods consumed globally. Probiotication is a means for further value addition to these products. Probiotics are microbes known to impart health benefits and probiotication refers to fortification of foods with sufficiently high population of probiotics. Intake of different probiotic organisms has been shown to have clinical benefits in various physiological or pathological situations. Probiotics exert a positive effect on health through modulation of intestinal microbiota and stimulation of immune system. High content of vitamins, mineral salts, dietary fibres and antioxidants together with the absence of competing starter cultures render the fruit beverages as good matrices for the addition of probiotics (Antunes *et al.*, 2013; Yoon, *et al* 2005, Yoon *et al.*, 2006). Development of fruit and vegetable-based probiotic foods also presents a great prospect for the development of low cholesterol, animal

derivatives and milk allergens free foods (Céspedes, *et al.*, 2013). Furthermore, the huge availability of different types of fruits and vegetables in India could permit the production of variety of beverages, satisfying the variety of consumer's preference. Because of their pleasant taste and acceptability among consumers, probioticated fruit juices hold promise in providing health benefits to the consumers in addition to their sensory attributes. Research on development of non-dairy based products has surged largely, because of the increase in the population of lactose intolerant individuals and allergies associated with the milk based products.

India ranks first among the world's mango producing countries, accounting for 54.2% of the total mangoes produced worldwide (Tharanathan *et al.* 2006). It is the most important commercial fruit crop grown in India with diverse genotypes with a wide variation in the size, shape, peel colour, pulp colour,

taste flavour and other physico-chemical characteristics. Ripe fruits generally are processed into canned and frozen slices, pulp, concentrate, nectar, jam, leather, bars, juices, puree, mango cereal flakes, toffee and various osmo-dried products. However, there is only a limited literature available on development of probioticated fruit beverages (Kumar *et al* 2015; Panghal *et al* 2017; Reddy *et al* 2015). Although the probioticated fruit beverages have distinct health benefits mentioned previously, the major impediments in developing probiotic fruit based products are low shelf life of the product even under refrigerated conditions, largely due to reduction in pH and accumulation of organic acids; typical organic acid flavours generally not appreciated by the consumers and maintenance of the population of probiotic strains. Probiotic viability is one of the important factors affecting the maintenance of the desired population levels of the selected probiotic strain. Karimi *et al* (2011) suggested consumption of at least 100 g/ ml of probiotic food so as to achieve a population of 10<sup>9</sup> CFU per serving. In order to improve the growth and population of the probiotics, researchers have studied the effect of addition of prebiotics, such as oat flour, brewer's yeast autolysate and cellulose in the fruit juices (Perricone *et al*, 2015). Commercially available products from companies like Pepsico and Danone are generally prepared from the juice concentrates through reconstitution or the mixture of different fruit purees to combat the impediments, mentioned in this paper. Therefore, an attempt was made in this study to screen different probiotic strains and evaluate the potential of the screened isolate for development of ready-to-serve (RTS) mango beverage.

## MATERIALS AND METHODS

Sixstrains used in this study (*Lactobacillus fermentum* MTCC1745, *L. brevis* MTCC 1750, *L. rhamnosus* MTCC 1423, *L. plantarum* MTCC 1407, *L. plantarum* 9510 and *L. fermentum* MTCC 8711) were procured from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, India. The strains were sub-cultured on the medium as per the instructions from IMTECH, Chandigarh. Four strains namely, *L. helveticus* MTCC 5463, *L. rhamnosus* MTCC 5462, *L. rhamnosus* MTCC 5945 and *L. rhamnosus* MTCC 5946 were procured from Anand Agricultural University, Anand, Gujarat, India. These four strains were maintained on the MRS medium.

The yeast strain *Saccharomyces boulardii* was isolated from a commercial formulation (Econorm), while the bacterial strain (*Bacillus clausii*) was isolated from *Enterogermina* sachets purchased from a medical store in Bengaluru, India. The contents from the Econorm sachet were suspended in the autoclaved yeast extract peptone dextrose (YEPD) broth and the inoculum from the broth was plated on to the YEPD medium plates, One millilitre from Enterogermina vial was suspended into the sterilized Nutrient broth and the inoculum was plated on to the Nutrient agar medium plates. The broth and the plates used for isolating the yeast and bacterial strain were incubated at 30 °C. All the dehydrated media were procured from Hi-Media Pvt Ltd, Mumbai, India while all the other chemicals used during analytical work were procured from Sisco research Laboratories (SRL), Mumbai, India.

## Preparation of RTS mango beverage

Ready to serve (RTS) mango beverage was prepared using the Mango pulp. Mango pulp was extracted from the sorted, washed, ripe mango fruits (variety Alphonso) harvested from the research fields of ICAR-Indian Institute of Horticultural Research (IIHR), Bengaluru, India in June 2018. Small cuts were made at the top and bottom and longitudinal cuts were made on either side of the fruit for removal of the stone. The fruits after preliminary operations were subjected to pulping in the pulper having 1 HP motor (Dharma Technologies, Tumkuru, Karnataka, India), and the pulp extracted was sieved through 1/16 sieve. Subsequently, the kernel along with the pulp adhering to it was subjected to pulping. Pulp collected from both the operations was mixed and passed through 1/32 sieve to obtain fine pulp. Ready to serve beverage was prepared using 20% pulp with total sugar concentration made to 15% (w/w) using refined sulphur free sugar (Madhur sugar obtained from Shree Renuka Sugars Ltd, Belgaum, Karnataka, India) and potable water (treated using reverse osmosis process). Initial sugar concentration in the Mango pulp was estimated using the Lane and Eynon method as mentioned by Ranganna (1995). Acidity in the final beverage was adjusted to 0.2% using citric acid. The beverage was bottled in 200 ml transparent glass bottles which were cleaned with potable water, followed by detergent wash and again potable water wash. The glass bottles with cotton plugs and aluminium foil were suspended in boiling

water and maintained for 10 minutes in the boiling water. The crowns for the bottles were placed in a beaker which was also immersed in the same crucible as were the glass bottles. The prepared beverage was bottled in the bottles which were crowned and pasteurized at 85 °C for 15 minutes, cooled and stored under ambient conditions for the preparation of probiotic beverage.

### Screening of the probiotic strains

In order to ensure a uniform colony count before screening, a loopful of inoculum from all the thirteen cultures was suspended in 100 ml pre-sterilized Ringer solution in 250-ml conical flasks separately. Inoculum (0.1 ml) from each of the flasks was pour plated on to the pre-sterilized media plates aseptically. The flasks after inoculation were stored in the refrigerator. Lactobacilli were plated on to the MRS medium plates, while the inoculum from the flasks having *Saccharomyces* and *Bacillus* cultures was plated on to the YEPD and NA medium plates, respectively. The plates were incubated at 30 °C in an incubator and removed from the incubator after 48h of incubation. Colony count from each plate was observed using the colony counter. The strain that showed the least growth was taken as a base and the dilutions were made for the remaining 12 flasks stored in the refrigerator, so that the CFU/ml was nearly same for all the strains. Thus, the inoculum from all the conical flasks having Ringer solution was equilibrated and used for screening of the strains in RTS mango beverage.

### Evaluation of the population dynamics of the probiotic strains in Mango beverage

Mango beverage prepared previously as described elsewhere in this paper was inoculated with 0.2 mL inoculum from each of the stored Ringer solution flasks containing the inoculum. Absorbance of the inoculated beverage at 600 nm was recorded using UV-Vis spectrophotometer (Shimadzu, Japan) at regular intervals of two days until eight days. For preliminary screening, an increase in OD was positively correlated with the growth of the organism. The uninoculated mango beverage was treated as control.

### Enumeration of probiotic population in mango beverage

Out of the 13 strains, only three strains showed an appreciable increase in the OD<sub>600</sub> values during incubation (mentioned elsewhere in this paper). Therefore, the selected three strains based on the increase in OD at 600 nm were inoculated into mango juice after equilibration using the procedure mentioned previously @ 10<sup>4</sup> cells per 200 mL beverage in triplicates. The bottles were incubated at 30 °C. The population build up was monitored at regular intervals until 8 days by serial dilution and pour plating on MRS agar for lactic acid bacteria and yeast extract peptone dextrose agar for *Saccharomyces boulardii* and the colony forming units (CFU) were enumerated after 48 h of incubation. Uninoculated mango beverage served as control.

### Sensory analysis of the probioticated RTS mango beverage

The probioticated beverages which permitted the build up of >10<sup>8</sup> CFU/mL were tested for their sensory acceptance by a panel of 15 semi- trained judges on a 9- point hedonic scale. The parameters considered for hedonic ranking were colour, taste, flavour & overall acceptance.

### Biochemical characteristics of probioticated RTS mango beverage:

The pH of uninoculated juice and probioticated beverages was analyzed using a pH meter (Elico Ltd, New Delhi, India), and total acidity was measured by titrating against 0.1N sodium hydroxide. Total sugars were estimated by Nelson Somogyi method (Nelson, 1944). Total carotenoids, ferric reducing antioxidant capacity (FRAP) and total phenols were estimated spectrophotometrically by standard methods (Ranganna, 1986), Benzie & Strain, (1996) and Singleton & Rossi (1965), respectively.

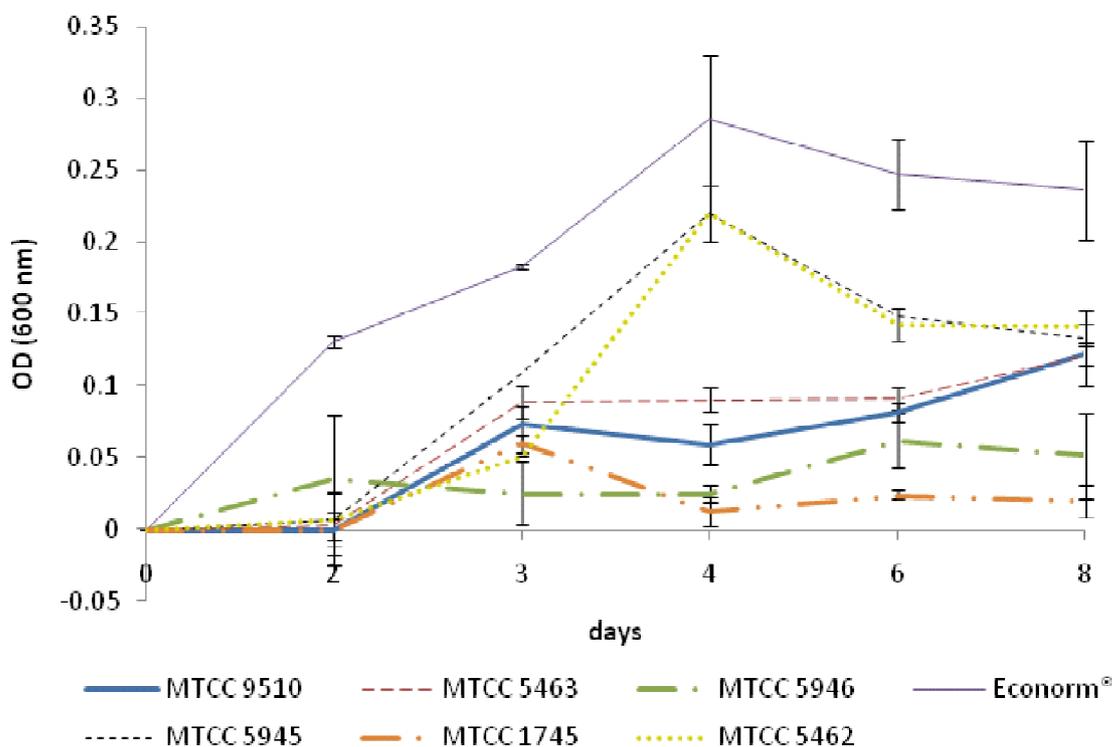
## RESULTS AND DISCUSSION

### Screening of strains in the RTS Mango beverage

Increase in absorbance is an easy method used traditionally to screen microbial growth in a medium. Although seven strains showed an increase in their

population as is evident from the results of **Fig.1**, only three strains showed an OD of 0.15 or above. The remaining six strains did not show any growth even after eight days of incubation. It is therefore clear that out of the thirteen strains used in this study, only three could grow in the RTS Mango beverage, while all of the thirteen strains earlier had shown population build-up in the selective media. This could largely be due to the (i) acidity build up in the beverage during growth (ii) nutrient limitation and (iii) absence of proper

conditions for growth of the cells. It is also evident from Fig.1 that there was a steep fall in the OD values after four days of incubation. This could be correlated with an increase in the acidity in the beverage or exhaustion of nutrients or both. Similar results have been reported previously by Brizuela *et al* (2001). On the basis of the results of Fig.1, only three strains, i.e., *L. helveticus* MTCC 5463, *L. rhamnosus* MTCC 5946 and *S. boulardii* were used for further studies.



**Fig 1. Change in absorbance values of mango beverage after inoculation with different probiotic strains**

### Evaluation of population dynamics of the screened strains

As mentioned previously, three strains i.e., *L. helveticus* MTCC 5463, *L. rhamnosus* MTCC 5946 and *S. boulardii* were inoculated separately in the RTS Mango beverage. They were evaluated for population build-up, acidity, total sugar concentration and pH in the beverage. It is clear from the results presented in **Table 1** that *L. helveticus*, MTCC 5463 and *L. rhamnosus* MTCC 5946 attained a population level of  $>\log 8$  CFU/mL on the second day of incubation, while the yeast *S. boulardii* needed three days to reach a similar population level. Different growth potential of bacterial strains to grow in fruit juice media

has been reported previously and reasons for the difference in levels of survival and growth was attributed to the sensitivity of the strains to natural antimicrobials in plant based foods (Sheehan *et al.* 2007 & Sagdic *et al* 2011). Mango pulp is a rich source of different phytochemicals like phenolics, flavonoids, terpenoids etc (Masibo and He, 2009) and phenolics and flavonoids are known for their antimicrobial characteristics. The results in Table 2 suggest that the acidity build up in the beverage prepared using *L. rhamnosus* MTCC 5946 and *S. boulardii* was substantial in comparison to that in the beverage inoculated with *L. helveticus* MTCC 5463 after four days of incubation. It is a well established fact that higher acidity and lactic acid odour alter the sensory

**Table 1. Population build up of probiotic strains in mango RTS beverage**

Strain	Population (cfu/mL)		
Incubation period (days)	2	4	6
<i>L. helveticus</i> MTCC 5463	8.25±0.21	9.07±0.5	9.1±0.32
<i>L. rhamnosus</i> MTCC 5946	10.82±0.03	10±0.20	10.11±0.12
<i>S. boulardii</i>	53.2±0.21	8.04±0.38	9.36±0.14

Values represented are for Mean ±SD for n-3

parameters of the beverages. Increase in acidity could also be directly correlated with the fall in the pH levels in the beverage. Compared to control (uninoculated beverage), drop in the total sugar concentration ranged between 34 and 43% approximately for the three strains evaluated in this study. However, the sugar consumption by the three strains varied by about 9% among the strains (Table 2). It is also clear from the results of Table 2 that the *Lactobacilli* strains

consumed and metabolized sugars more efficiently in comparison to *S. boulardii*. Kumar *et al* (2015) reported a decrease of more than 40% in total sugar concentration and increase up to 0.66 in acidity levels after 72 h of incubation of Mango juice inoculated with *L. plantarum* NCDC LP 20. It is noteworthy to mention here that the sugar-acid ratio is one of the important parameters having a profound effect on the sensory attributes of any beverage. Therefore, it is

**Table 2. Physico-chemical analysis of mango juice probioticated with two *Lactobacillus* and a *S. boulardii* strains**

Probiotic strains	Sensory score	pH	Acidity (% lactic acid)	Total Sugar (%)
Control	8	4.5±0.2	0.20 (as citric acid)	13.5±0.7
<i>L. helveticus</i> MTCC 5463	8	4.4±0.2	0.29±0.00	7.6±0.2
<i>L. rhamnosus</i> MTCC 5946	5	3.2±0.0	1.03±0.08	8.1±1.0
<i>S. boulardii</i>	5	3.4±0.1	0.68±0.03	8.9±0.6

Values represented are for Mean ±SD for n-3

important to maintain an ideal sugar-acid blend throughout the shelf life of the product for consumer acceptance. The results of our experiment also emphasise the need for a precise strain selection for endurance in fruit media and interaction with the different food matrices.

### Sensory evaluation of the probioticated Mango beverage

Organoleptic quality of a product is the stimulus to popularise a novel product in food market. Mango beverage inoculated with *S. boulardii* showed a typical odour and flavour of ethanol and hence was not liked by any of the panelists. Therefore, it was decided to carry out the comparative evaluation of the beverage probioticated with the two *Lactobacillus* strains. The results in Table 3 show a significant difference in the overall acceptance parameters among the Mango

beverage probioticated with two different *Lactobacillus* strains. Though the panelists rated both the beverages comparable for the colour, scores for flavour and taste were significantly different. It is clear from the results of Table 3 that the organoleptic quality of the beverage prepared using *L. helveticus* MTCC 5463 was acceptable, whereas, the one prepared using *L. rhamnosus* MTCC 5946 was more towards unacceptability (Table 3). Better organoleptic properties may be correlated with a better balanced higher sugar –acid ratio. Panghal *et al* (2017) obtained a score of 7 on a 9 point hedonic scale for probioticated beetroot drink prepared using *L. rhamnosus*, *L. delburcki* and *L. plantarum*. Based on these observations, it was decided to use *L. helveticus* MTCC 5463 for probiotication of RTS mango beverage. Antioxidant properties of the RTS mango beverage inoculated with *L. helveticus* MTCC 5643 were compared with that of the uninoculated beverage.

**Table 3. Mean sensory scores for probioticated Mango beverage prepared using *Lactobacilli***

Strain	Colour	Flavour	Taste	Overall Acceptability
<i>L. helveticus</i> MTCC 5463	7.38±0.76	7.19±0.90	7.30±0.75	7.34±0.68
<i>L. rhamnosus</i> MTCC 5946	7.42±0.81	5.38±1.32	5.38±1.50	5.65±1.24

### Antioxidant properties of the probioticated mango beverage

The results of Table 4 clearly indicate a significant increase in the total phenol and flavonoid content in the probioticated mango beverage, compared to control. This could largely be due to the release of phenolics and flavonoids from the dietary fibre present in the complex form in the fruit juices, through the action of secondary metabolites (enzymes) and by the

acids produced by the fermenting microbial strains. There have been reports about the production of phenolics by the *Lactobacillus* strains. Coupled with the release of phenolic compounds due to the action of enzymes and acids as mentioned previously, production of phenolic compounds by the *Lactobacillus* strains could have led to higher concentration of phenolics in the probioticated beverage (Table 4). Fras *et al* (2014) reported production of volatile phenolic compounds in red wine inoculated with

**Table 4. Antioxidant properties of mango beverage probioticated with *Lactobacillus helveticus* MTCC 5463**

S.No.	Assay	Control (Uninoculated beverage)	Probiotic RTS mango beverage
1.	Total Carotenoids (mg/100ml)	0.23	0.20
2.	β-Carotene (mg/100ml)	0.20	0.18
3.	Total phenols (µg/ml)	140.07±3.15	169±2.01
4.	Flavonoids (µg/ml)	45.81±1.09	51.17±2.02
5.	FRAP (mg/ml)	0.09	0.09
6.	DPPH (mg/ml)	0.17	0.15

Values represented are for Mean ±SD for *n*-3

*L. plantarum*. A decline in the total carotenoid and β-carotene concentration in the probioticated beverage could be due to their oxidation at incubation temperatures. Chen *et al* (2018) also reported a decline in the carotenoid and vitamin C content in the probioticated papaya juice. No significant difference in the antioxidant concentration was observed between the control and the probioticated sample as is evident from the FRAP and DPPH values (Table 4). Mousavi *et al* (2013) reported a significant increase in the antioxidant values for probioticated Pomegranate juice, compared to uninoculated juice. Panghal *et al* (2017) reported an increase in the DPPH values in the probioticated beetroot juice. However, Chen *et al* (2008) reported a decline in the antioxidant activity during fermentation with *Lactobacillus acidophilus*, whereas the same authors reported an increase in the

antioxidant activity during fermentation with *L. plantarum*. We are now evaluating the different characteristics of probioticated RTS mango beverage during its storage.

### CONCLUSIONS

This study has shown that inoculation of ready to serve mango beverage with *Lactobacillus helveticus* MTCC 5463 has the potential for development of probioticated mango beverage. Compared to the other probiotic strains used in this study, *Lactobacillus helveticus* MTCC 5463 showed an appreciable population build-up, an insignificant increase in the acidity levels and a good sugar-acid blend in the fermented beverage. Mango beverage fermented with the screened *L. helveticus* strain showed good sensory attributes for colour, taste and

flavour. In addition to good sensory attributes, fermented beverage showed higher concentration of phenolic compounds and flavonoids after fermentation of the Mango beverage, compared to control. Further studies are needed to evaluate the shelf life of probioticated mango beverage prepared using *L. helveticus* MTCC 5463 for cell population, acidity, sugar concentration, phenolic and flavonoid profile during regular intervals during its storage.

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