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ORIGINAL ARTICLE

Formation of volatiles and fattyacids of therapeutic importance in the probiotic *Lactobacillus plantarum* LPcfr adapted to resist GIT conditions

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Abstract Probiotics are the microorganisms that impart therapeutic effect and promote health by preventing various diseases. In the present work, the volatile compounds were studied in the native isolate *Lactobacillus plantarum* (LP) and after adaptation to resist gastro intestinal tract (GIT) conditions, which was coded as LPcfr. A number of therapeutically important compounds were present in LPcfr like butanediol (2.5%) and propionic acid (2.8%), which were not found in LP. Hexadecane (3%), butanoic acid (2%), dodecanal (2%), hexanal (7.5%), hexadecanoic acid (4%) and heptanal (6%) were found in higher concentrations in LPcfr as compared to the parent strain LP. Production of oleic acid (LP-19.2%; LPcfr -33.5%), known for reducing blood cholesterol and linoleic acid (LPcfr 2.3%), and a conjugated fatty acid known as a novel beneficial functional lipid was noticed. Linoleic acid was absent in LP. These important fatty acids were found in larger quantities in the probiotic adapted culture strain LPcfr as compared to the parent strain LP.

Keywords Volatiles · Fattyacids · *Lactobacillus plantarum*

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Introduction

Probiotics play a major role in health and well being beyond basic nutrition (Naidu et al. 2000). Probiotics are defined as live microorganisms, which when administered in adequate amounts confer a health effect on the host (FAO/WHO 2002). A natural cure to disease prevention is required and consumption of probiotic helps in imparting better health (Marteau and Boutron 2002). The role of probiotics in inhibiting toxic food pathogenic microorganisms has been demonstrated earlier (Haudault et al. 1997). Hypocholesterolemic and immunostimulatory effects of *Enterococcus faecium* have been studied in human beings (Gill 1998). Study of the flavour profile of *idli* batter prepared from defined microbial starter cultures like *Pediococcus pentosaceous* CFR 2123 and *Candida versatilis* CFR 505, where production of desirable flavour compounds like ketones, diols and acids up to 8 days of storage, has been reported (Agrawal et al. 2000). Volatile compounds of therapeutic importance were produced by *Leuconostoc mesenteroides* after adaptation to GIT conditions (Rani and Agrawal 2007). Formation of volatile compounds and fatty acids of therapeutic importance in probiotic strain (LPcfr) of *Lactobacillus plantarum* adapted to GIT conditions which will impart health benefits is presented in this work.

Materials and methods

Culture and inoculum preparation The parent culture LP, which was isolated from Bulgarian pickle and probiotic

Table 1 Yield of fatty acids in the parent culture (LP) and probiotic strain (LPcfr) of *Lactobacillus plantarum*

	Yield of fatty acids,wt%	
	LP	LPcfr
$C_{18:1}$ (Oleic acid)	19.2	33.5
$C_{18:2}$ (Linoleic acid)	nil	2.3
$C_{18:3}$ (Linolenic acid)	0.59	0.55
C_{12} (Lauric acid)	2.2	3.8
C_{14} (Myristic acid)	6.6	11.6
C_{16} (Palmitic acid)	44.0	4.2
C_{18} (Stearic acid)	14.5	34.0

culture (LPcfr), obtained after adapting LP strain to low pH (2.0) and high bile (4%), were grown individually for 18 h (exponential growth phase). The culture was maintained at 4 °C in MRS broth (Hi- Media Lab. Ltd., Mumbai, India) and subcultured at 15 days interval.

Extraction of fatty acids An aliquot (2 ml) of the actively growing cultures (LP and LPcfr) was centrifuged at 8000 rpm for 10 min at 4 °C and the pellet was washed twice with sterile saline (0.85%). Appropriate dilutions of cell suspensions were prepared in sterile saline to obtain an initial cell count of 2×10^{6}cfu/ml. To 2 ml of sample, methanol and chloroform were added in 2:1 ratio. The mixture was kept at 30 °C for 2 h for derivatization (Ext.1). For complete extraction, the pellet was again washed using a mixture of methanol: chloroform: water (2:1:0.8) and centrifuged at 5000 rpm for 30 min. The supernatant was collected and diluted with chloroform and water mixture (1:1). The chloroform layer was taken out and pooled with Ext. 1. Chloroform was evaporated and to this a mixture of hexane in 2 N methanolic KOH was added. The hexane layer was collected in a separate tube and the solvent was evaporated. For fatty acid analysis the samples were analyzed by GC (Shimadzu, Kyoto, Japan) Column; OV 351 (capillary), column temperature 220 °C, injector temperature 230 °C, detector temperature 240 °C with N_2 (flow rate 1 ml/min) as carrier gas. The fatty acids were estimated as per AOCS method (Walker 1983). A mixture of methyl esters of known standard compounds obtained from Sigma-Aldrich, USA was analyzed under the same operating conditions as those of the sample. The retention distance was measured as a function of the number of carbon atoms of the acids under isothermal conditions.

Extraction of volatiles For volatiles, the culture strains were grown to its exponential phase (18 h). An aliquot (10 ml, 1×10^{6}cfu/ml) was centrifuged at 6000 rpm for 30 min and the supernatant was discarded. The pellet was washed with saline and extracted in dichloromethane (10 ml). The solution was dried by adding anhydrous sodium sulphate. The sample was concentrated under nitrogen to 0.5 ml and was injected onto the GLC using column SE-30,3 M (0.5 mm id) column with a flame ionization detector and carrier gas N_2 (flow rate 30 ml/min). The oven temperature was programmed from 40 to 250 °C at 4 °C/min. The injector and detector temperatures were kept at 250 °C using FID detector. The oven temperature was programmed from 30 to 250 °C at 30 °C (6 min), 2 °C/min up to 100 °C; 4 °C/min up to150°C and 8 °C/min up to 250 °C.

The GC- MS analysis was carried out in a gas chromatograph mass spectrophotometer model QP- 5000 (Shimadzu, Kyoto, Japan) using a SE-30 column (25 Mx0.32 mm) and helium (99.9%) as the carrier gas. The injector and the detector temperatures were programmed from 30 to 250 °C at 30 °C (6 min), 2 °C/

Table 2 Volatile compounds produced by LP and LPcfr culture strains by GC-MS chromatography and their therapeutic uses

Compound	Mol. wt.	Yield, mg%		Fragmentation pattern
		LP	LPcfr	
Propionic acid	74	Nil	2.8	45, 74, 45, 57, 18
Butanoic acid	88	Nil	2.0	60, 73, 41, 55, 88
Butane diol	90	Nil	2.5	42, 57, 71, 53, 89
Hexanal	114	Nil	7.5	70, 55, 44, 81, 96
Heptanal	114	Nil	4.0	43, 56, 70, 83, 98
Heptanol	116	Nil	6.0	70, 56, 43, 31, 83
Dodecanal	184	Nil	2.0	43, 57, 68, 82, 96
Hexadecane	226	4.0	3.0	57, 43, 71, 85, 99, 113
Tetradecanoic acid	228	5.0	Nil	43, 73, 129, 185, 87
Hexadecanoic acid	256	Nil	4.0	43, 73, 29, 129, 157
Nonadecane	268	8.0	Nil	57, 43,71, 99, 127
Nonahexacontanoic acid	998	7.8	Nil	44, 57, 71, 85, 97

* indicates the expansion of LP and LPcfr, which was referred in Table 1

min up to 100 °C; 4 °C/min up to150°C and 8 °C/min up to 250 °C.

Volatiles were identified on the basis of their retention time and comparing the mass fragmentation pattern of standard compounds as given in the directory by Noever et al. (1988).The content is expressed by determining the percentage represented by the area of the corresponding peak relative to the sum of the areas of all the peaks.

Results and discussion

Bacterial growth and fatty acids An exponential growth (18 h; 1×10^{8} cfu/ml) culture for maximum volatile content was taken for fatty acid and volatile analysis. The probiotic adapted culture LPcfr showed high amount of oleic acid (33.4%), linoleic acid (2.3%), lauric acid (3.8%) and myristic acid (11.6%), as compared to its parent culture (LP; lauric acid 2.2% and myristic acid 6.6%) (Table 1). Production of free fatty acids with the addition of LAB is reported in the literature (Coskun and Ondul 2004). LABS also have the ability to produce conjugated linoleic acid and linolenic acid, considered to be beneficial functional lipids (Alosno et al. 2003, Ogawa et al. 2001, 2005).

Formation of C_{18} fattyacids in higher quantities in LPcfr than in LP strain is significant, as these have therapeutic properties. Antifungal properties were attributed to fattyacids produced from *Lactobacillus plantarum* Mi LAB 14 (Sjogren et al. 2003) and production of antifungal cyclic dipeptides cyclo-(1- phe-1-pro) and cyclo (1-phe-trans-4-OH-1-pro) and 3-phenyl lactic acid has been demonstrated in *Lactobacillus plantarum* Mi LAB 393 (Strom et al. 2002).

Volatile compounds Major differences were found in the formation of volatile compounds in LP and in LPcfr (Table 2). LP mainly produced hexadecane (4%), tetradecanoic acid (5%), nonadecane (8%) and nonahexacontanoic acid (7.8%). These compounds were not found in LPcfr, whereas, it produced many other compounds of therapeutic importance like propionic acid (2.8%), which is used as antimicrobial agent against microbes and fungi (Chamkha et al. 2001), butanoic acid (2%) used against colon cancer (Randazzo et al. 2007), hexadecanoic acid (4%) used as antifungal compound (Broberg et al. 2007), butane diol (2.5%), which has use in food formulations (Garg and Jain 1995), hexanal (7.5%) known to promote growth (Steinkraus et al. 1967), heptanol (6%) used for lowering foaming (Shakirova et al. 2007), heptanal (4%) and dodecanal (2%) known as sex attractants (Thierry and Maillard 2002) and hexadecane (3%), which is used to improve cardiovascular system (Hosono et al. 1974). Appearance of differences in the profile of compounds in LPcfr and LP shows the probability that both strains are utilizing either different substrates or undergoing different pathways. Formation of pentadecanoic acid has been studied (Brody et al. 1997) in *Sccharomyces cerevisiae*. Formation of propionic acid (1.3–3%) by *Lactobacillus casei* has been noticed (Bodie et al. 1987). Amino acids are utilized by various enzymatic reactions ultimately forming ketoacids that enter fatty acid synthetic pathway (Thierry and Maillard 2002). Fattyacids are synthesized as a result of amino-tranferase, aminoacid oxidase and dehydrogenase reactions of aminoacids to ketoacid and later forming benzaldehyde. Fatty acids play an important role in the development of ketones (Steinkraus et al. 1967).

Conclusion

Probiotic culture strain LPcfr of *Lactobacillus plantarum* has the capacity to retain in GIT conditions and the potential to form compounds of therapeutic importance. The volatiles and fatty acids produced by the adapted culture strain have produced beneficial compounds which may improve the health of the consumer. Probiotic which come under GRAS category seems to be a very optimistic approach of natural cure of many diseases. The culture strain can be utilized as such or may be supplemented in any food for imparting probiotic properties.

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