Indian Journal of Applied Microbiology
ISSN (Online): 2454-289X, ISSN (Print): 2249-8400

Volume 21 Number 2
July - September 2018, pp. 11-19

Functional traits of exopolysaccharide (EPS) producing lactic flora from feces of Irula and Urban community of Tamil Nadu

Jayamanohar Jabastin, Palanisamy Bruntha Devi, Rajendran Suresh and Venkatesan Brindha Priyadarisini*

Department of Microbial Biotechnology, Bharathiar University, Coimbatore 641046, India

Abstract: There is a significance importance on functional products from food to gut to enrich the indigenous flora to protect the host system. A relative assessment of functional metabolite producing lactic flora from feces of Irulas and Urban community were done. One twenty three from Irulas and seventy seven from urban, EPS producing lactic flora were isolated by appearance of different phenotype (i.e. mucoid, slimy and watery). Further, the strains were subjected for selenite to Se^o reducing capacity. Among these, 112 strain from Irulas and 43 from urban were appeared as red colour colonies. Further, 58 isolates from Irulas and 5 from urban showed positive for the production of Gamma amino butyric acid (GABA). Overall screening, Irula tribals possessed high lactic flora consortium with multiple functional traits in the intestine. Further, the Irula tribal isolates could be screened for probiotic attributes and can be used as functional microflora.

Keywords: EPS-lactic flora, Irula tribes, GABA, Se^o strain

Introduction

At present, gut microbiota are leading microbial community to enhance host metabolism and it can differ in diverse geographical origin by special lifestyles, environments, genetic history or nutritional behavior [1]. Diet is a crucial component which has a close gastro intestinal (GI) tract microbiota relationship. Such diets together with either plant materials or animal product as a prime component may influence a person's health [2]. Previously, many studies could be

*Author for Correspondence. E-mail: brindha02@buc.edu.in

authorized by high plant food present in diet. It influences lactic acid associated with functional bacteria (i.e. bacterioides) and product (i.e. short chain fatty acids (SCFA) respectively [3]. Irulas is a well authorized scheduled tribes by Government of India [4]. They use whole plants for the treatment of common symptoms (i.e. fever, cold, and cough) and their sources of the food are totally depended on forest [5]. Especially, wild yams i.e. *Dioscorea sp* are greater importance for food and medicine for their social and religious belief [6, 7]. Similar to nicobarese tribes, these tribals specifically use carbohydrates (i.e. *Dioscorea alata*), whole grains, and fruits of *Pandanus tectorius* as primary feed to fermentation for diet to influence saccharolytic bacteria i.e. bacterioides to generate SCFA and also increase in the certain friendly bacteria [3]. Accordingly European Food Safety Authority (EFSA) framed, dietary intake of 25 g/day or above of the traditional diet of humans, it improves bowel movement, reduce Coronary Heart Disease (CHD) and type-II diabetics [8].

Although, exopolysaccharide (EPS) producing strain escaped from human physiological stress and reach the gut [9, 10]. That EPS attained various functions like antitumor, antioxidant, immunomodulatory and immune-stimulatory activities, cholesterol lowering properties, modulation of gut microbiota and protection of epithelial cell against intestinal pathogenic microorganisms [11]. Previously, many studies can explore functional traits based on EPS and less extends on its EPS-lactic flora. Additionally, certain bacteria from gut produces neuroactive compounds serotonin, gamma aminobutyric acid (GABA), dopamine, norepinephrine, acetylcholine and histamine [12]. Among that GABA plays a role in the gut to influence the brain–intestine–microbiome axis [13]. GABA is important for regulating physiological and psychological process like anxiety, depression, blood pressure, heart rate and hypertension respectively [14]. Similar to GABA, different selenoprotein and enzymes by diverse physiological processes compounds possess higher bioactivity compared to inorganic derivatives of Selenium (Se) [15, 16]. Previously, insoluble red elemental Se was investigated by several *in vitro* and *in vivo* studies and showed lower level toxicity than selenite [17]. Therefore, the present study aim is to evaluate the functional attributes of EPS-lactic flora from feces on its healthy volunteers of Irula and Urban community of Tamil Nadu.

Materials and methods

Collection and isolation of EPS producing Lactic acid bacteria (LAB)

The fecal samples were collected from healthy volunteers of urban and Irula tribes of Tamil Nadu. The samples were collected from the regions of Tamil Nadu where Irula tribals were located densely. For urban, samples were also collected from the urban community from the same regions where Irula samples were collected. Fecal samples were collected from all age groups of human volunteers, viz. male and female infants (1 month – 3 yrs), children (3 – 16 yrs), adults (16 – 59 yrs) and elders (60 – 85 yrs). Five samples were collected from each category. Samples were collected in sterile clincon (HiMedia, India) and transported immediately for further enumeration. About, one gram of feces from each volunteer was homogenized in sterile saline solution (0.85% NaCl) and agitated for 20 min in orbital shaker at 150 rpm. After that fecal suspension was

serially diluted and plated on MRS agar with 2 % sucrose as individual carbon source with 0.5% CaCo3, 10 mg/L of cycloheximide, 10 mg/L sodium azide to avoid fungal and yeast contamination and then incubated at 30^{o}C for 72 h [18]. Colonies with strong mucoid, slimy and watery surfaces were selected for EPS producing lactic acid producers. Single colonies were isolated, sub-cultured and stored in 80% glycerol stock at 4^{o}C for further uses.

Qualification of functional metabolites producing strain

(a)GABA producing strain by pH method

The GABA producing strains were qualified using pH method by Yang et al. [19] with minor changes. The isolates were revived and inoculated into 50 ml MRS broth and incubated at 30 °C under static condition for 24 h. Then the grown strains were centrifuged at 5000 rpm for 15 min and washed thrice with 0.85% NaCl to remove extracellular debris. Further the cells (10W/V) were dissolved into 10% glutamic acid solution and adjust its pH to 4.7 by 1N HCl and NaOH. Then the solution was incubated at 37°C for 24 h. After incubation, the supernatant were re-collected by centrifugation at 5000 rpm for 5 min. About 500µl of supernatant were added and allowed to react with complex pH solution to convert colorless to colored solution (i.e. chlorophyll green, yellow, pale yellow and magenta). Then GABA producing positive strains were classified into chlorophyll green (strong producer), yellow (weak producer), pale yellow (weak producer) and magenta (negative) respectively. Subsequent with 72 h, the strains showing non disappearance of chlorophyll colour could be used for further studies.

(b)Selenium accumulating strains by plate method

The isolated strains were studied for selenium accumulation by visualization method as described by Shakitab et al. [20]. Briefly, the overnight strains were spreaded on MRS agar supplement with 1.26mM selenium dioxide and then incubated at anaerobic cabinet for 37°C at 72 h incubation. The selenium accumulation was assessed based on growth rate and rapid appearance of red colour colonies on MRS agar plate.

3. Results and discussion

3.1. Isolation of EPS producing strains

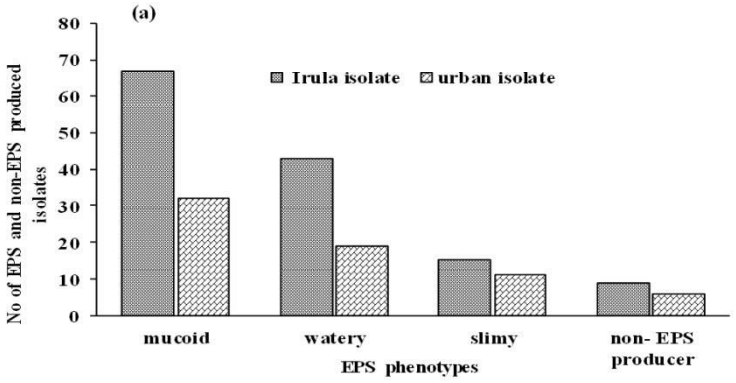
In the screening test for EPS production, one hundred and twenty three different lactic acid bacterial flora were positive from Irula tribes and 77 from urban by the appearance of EPS phenotype through MRS with 2% sucrose at 37 C for 24-48 h incubation respectively. As shown Fig. 1a and b**,** the most of colonies possess capacity to excrete different forms of EPS phenotype like mucoid, watery, and slimy colonies. Compared to Irula tribes, the lowest numbers of EPS producing strains (6.65-8.83cfu/ml) were noticed from urban isolates in the Table 1. Previously, Price et al. [21] studied metabolites present in the *D. elephantipes,* it showed high monosugars (i.e. glucose). Additionally, Anwesh et al. [3] studied nicobarese tribes who used *D. alata*, whole grain and *Pandanus tectorius* as a primary carbohydrate source. Therefore, it strongly influenced carbohydrate metabolism by the existence members of *Bacteroidetes* and *Firmicutes* phyla to

produce more SCFA. It reabsorbed into host system and can serve as an energy source for other friendly bacteria. This may be due to presence of carbohydrate metabolism in food system to induce or increase the number of EPS producing strains.

Table 1. Enumeration of EPS producing gut lactic acid bacterial flora from different healthy volunteers of Irula tribes and urban peoples

Categories	Irula tribes (cfu/g)	Urban community (cfu/g)
Infant	8.83	6.23
Childhood	7.78	5.04
Adults	8.21	3.67
Elders	6.65	3.23

Fig. 1 a) Colony morphology of different EPS phenotype strains obtained from human volunteers b) EPS producing strains (black arrow-mucoid; red arrow-non-EPS; blue arrow- slimy; pink arrow- watery)



(b)

3.2. Screening of functional metabolites strains

3.2.1. Selenite to Se^0 reducing strains

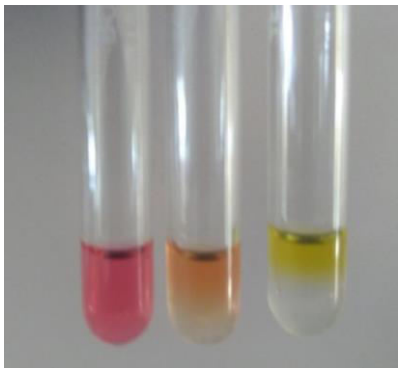
The EPS produced isolates possessed capacity to transform inorganic selenium compound and it can to tolerate growth on MRS agar plate with 1.26mM sodium selenite as shown in the Fig. 2**.** Among foregone strains, 112 accumulated selenium from Irulas and lesser on urban (43) by the appearance of the red colour colony respectively. As compared to Irula and Urban community, Irula strains showed high number of encompassed in the selenium particles on the EPS whereas weaker or growth inhibition or delayed growth from urban strains. According Saini et al. [22] it showed less significant number of strains accumulated selenium from lactobacillus isolates from different origin respectively. This is probably the presence of selenium elements in food i.e. *Dioscorea* (0-13.62 mg/kg) as more enough than every day RDA mentioned pattern of selenium per day [23]. Through on its continuous exposure of selenium were present in the regular diet through food by enrichment of selenium on Irula strains. This might due to reduction to Se oxyanions for requirement of diverse bacteria for growth and metabolism [24]. Additionally, the Se oxyanions accept electrons on terminal position and their reduction paired by oxidation of diverse organic compound such as acetate, lactate and aromatics [25]. Moreover, other mechanisms were reduction of the system of bacteria by siderophore reduction, abiotic reduction by H_2S to sulfate, thio-redoxin reductase system and painter-type reaction by thiol groups it resulted on detoxification and reduction of selenite to Se^o [24]. This may be influence of diverse reducing mechanism by bacterial system respectively.

Fig. 2. Sodium selenite into Se^o reducing strain a) control strain, (b) Se^o EPS strain, (c) and (d) Se^o non-EPS strain

3.2.2. GABA producing strains

It has been steered that microbially produced GABA in the intestine may influence the brain–intestine–microbiome axis [13]. For this, the above isolates were subjected for screening of GABA production. Only fifty eight strains showed GAD activity from Irula isolate by appearance of green colour using pH indicator method whereas five on urban isolates as shown in the Fig.3. The Irula isolate were showed high GAD activity at 72 hrs. while disappearance of green colour within 24 h on urban strains. This is due to induction of frame shift mutation on LAB intracellular system to causes insertion or deletion of DNA nucleotide (A and T) and unfunctionalized translational process [26]. Previously, Price et al. [21] revealed GABA play a response to drought tolerance by existence of GABA on ground vegetation (i.e. *D. elephantipes*). This might be high existence of GABA on *Dioscorea* sp. through regular to explore GABA strains by Irula tribes.

Fig.3 Screening of GAD producing strains by GAD→ GABA using pH indicators method (chlorophyll green colour – positive; yellowish orange – moderate positive; magenta – negative)



Conclusion

EPS producing lactic bacteria were screened for the other functional traits such as, GABA production and Selenium accumulated isolates from the Irula and urban community. Irula community possessed many functional isolates compared to urban isolates. Further studies were ongoing on the probiotic attributes of Irula isolates.

Conflict of Interest

Authors do not have any conflicts of Interest

References:

1. La-ongkham, O., Nakphaichit, M., Leelavat charamas, V., Keawsompong, S., and Nitisin prasert, S. 2015, ''Distinct gut microbiota of healthy children from two different geographic regions of Thailand'', *Arch. Microbiol,* 197, pp.561-573.
2. Ruengsomwong, S., La-Ongkham, O., Jiang, J., Wannissorn, B., Nakayama, J., and Nitisin prasert, S., 2016, ''Microbial community of healthy thai vegetarians and non-vegetarians, their core gut microbiota, and pathogen risk'', *J Microbiol Biotechnol*, 26, pp.35.
3. Anwesh, M., Kumar, K. V., Nagarajan, M., Chander, M. P., Kartick, C., and Paluru, V., 2016, ''Elucidating the richness of bacterial groups in the gut of Nicobarese tribal community–perspective on their lifestyle transition'', *Anaerobe,* 39, pp.68-76.
4. Rasingam, L., 2012, ''Ethnobotanical studies on the wild edible plants of Irula tribes of Pillur Valley, Coimbatore district, Tamil Nadu, India'', *Asian Pac J Trop Biomed*, 2,pp. 1493-1497.
5. Muthuraj, K., Abdul Kaffoor, H., and Nagarajan, N., 2018, ''Establishment of in vitro protocol and impact of mycorrhization with phosphobacteria on micro propagated Pogostemon mollis Benth. (Lamiaceae)", *J Taibah Univ Sci*, pp.1-10.
6. Barman, P., Bhat, K. V., and Geeta, R., 2018, ''Phylogenetic analysis of Indian Dioscorea and comparison of secondary metabolite content with sampling across the tree'', *Genet. Resour. Crop Evol*. 65, pp.1003-1012.
7. Ravishankar, T., 2007, ''Ethnobotany of Dharmapuri and North Arcot Districts in Tamil Nadu, South India'', *Biodiversity*, 8, pp.12-20.
8. Shen, Q., Zhao, L., and Tuohy, K. M., 2012, ''High-level dietary fibre up-regulates colonic fermentation and relative abundance of saccharolytic bacteria within the human faecal microbiota in vitro'', *Eur J Nutr,* 51, pp.693-705.
9. Y. Wang, Z. Ahmed, W. Feng, C. Li, S. Song, Physicochemical properties of exopolysaccharide produced by *Lactobacillus kefiranofaciens* ZW3 isolated from Tibet kefir, *Int. J. Biol. Macromol. 43 (2008)* 283-288.
10. Brink, M., Todorov, S. D., Martin, J. H., Senekal, M., and Dicks, L. M. T., 2006, ''The effect of prebiotics on production of antimicrobial compounds, resistance to growth at low pH and in the

presence of bile, and adhesion of probiotic cells to intestinal mucus'', *J. Appl. Microbiol*, 100, pp.813-820.

11. Looijesteijn, P. J., Trapet, L., de Vries, E., Abee, T., and Hugenholtz, J., 2001, ''Physiological function of exopolysaccharides produced by *Lactococcus lactis*'', *Int J Food Microbiol*, 64, pp.71-80.
12. Evrensel, A. and Ceylan, M.E., 2015, The gut-brain axis: the missing link in depression, *Clin Psychopharmacol Neurosci*, 13, p.239.
13. Bienenstock, J., Forsythe, P., Karimi, K., and Kunze, W., 2010, ''Neuroimmune aspects of food intake'', *Int. Dairy J*., 20, pp.253-258.
14. Barrett, E., Ross, R. P., O'Toole, P. W., Fitzgerald, G. F., and Stanton, C., 2014, ''This article corrects: c-Aminobutyric acid production by culturable bacteria from the human intestine'', *J. Appl. Microbiol.*, 116, pp. 1384-1386.
15. Yang, J., Huang, K., Qin, S., Wu, X., Zhao, Z., and Chen, F., 2009, ''Antibacterial action of selenium-enriched probiotics against pathogenic *Escherichia coli*'', *Dig. Dis. Sci.,* 54, pp.246-254.
16. Sun, Y., Nie, Y., Wang, Z., Hua, C., Wang, R., and Gao, J., 2017, ''Bio-macromolecule-Directed Synthesis and Characterization of Selenium Nanoparticles and Their Compatibility with Bacterial and Eukaryotic Cells'', *Nanosci Nanotechnol Lett,* 9, pp. 1987-1991.
17. Pophaly, S. D., Singh, P., Kumar, H., Tomar, S. K., and Singh, R., 2014, ''Selenium enrichment of lactic acid bacteria and bifidobacteria: a functional food perspective'', *Trends Food Sci Technol*, 39, pp. 135-145.
18. Manini, F., Casiraghi, M. C., Poutanen, K., Brasca, M., Erba, D., and Plumed-Ferrer, C., 2016, ''Characterization of lactic acid bacteria isolated from wheat bran sourdough'', *LWT-Food Sci Technol,* 66, pp.275-283.
19. Yang, S. Y., Lu, Z. X., Lu, F. X., Bie, X. M., Sun, L. J., and Zeng, X. X., 2006, ''A simple method for rapid screening of bacteria with glutamate decarboxylase activities'', *J Rapid Methods Autom Microbiol*, 14, pp.291-298.
20. Shakibaie, M., Mohammadi-Khorsand, T., Adeli-Sardou, M., Jafari, M., Amirpour-Rostami, S., Ameri, A., and Forootanfar, H., 2017, ''Probiotic and antioxidant properties of selenium-enriched *Lactobacillus brevis* LSE isolated from an Iranian traditional dairy product'', *J Trace Elem Med Biol,* 40, pp.1-9.
21. Price, E. J., Wilkin, P., Sarasan, V., and Fraser, P. D., 2016, ''Metabolite profiling of Dioscorea (yam) species reveals underutilized biodiversity and renewable sources for high-value compounds'', *Sci. Rep*., 6, pp.29136.
22. Saini, K., Tomar, S. K., Sangwan, V., and Bhushan, B., 2014, ''Evaluation of lactobacilli from human sources for uptake and accumulation of selenium'', *Biol. Trace Elem. Res*., 160, pp.433-436.
23. Otegbayo, B. O., Oguniyan, D. J., Olunlade, B. A., Oroniran, O. O., and Atobatele, O. E., 2018, ''Characterizing genotypic variation in biochemical composition, anti-nutritional and mineral

bioavailability of some Nigerian yam (Dioscorea spp.) land races'', *J Food Sci Technol*, 55, pp.205-216.

24. Nancharaiah, Y. V., and Lens, P. N. L., 2015, ''Ecology and biotechnology of selenium-respiring bacteria'', *Microbiol Mol Biol Rev*, 79, pp.61-80.
25. Stolz, J. F., and Oremland, R. S., 1999, ''Bacterial respiration of arsenic and selenium'', *FEMS Microbiol Rev*, 23, pp.615-627.
26. Li, H., and Cao, Y., 2010, "Lactic acid bacterial cell factories for gamma-aminobutyric acid'', *Amino acids*, 39, pp.1107-1116.