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Studies on traditional Indian (turmeric) pickle as probiotic pickle for therapeutic uses in view of COVID-19 pandemic

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The aim of present study was to assess therapeutic potential of traditional turmeric pickle by characterizing the potential health boosting probiotics present in it and recommend it as an attractive food supplement. Probiotics TP1, TP2 and TP3 isolated from turmeric pickle had shown good antagonistic activity against all test pathogens i.e., *Listeria monocytogenes*, *Bacillus cereus* and *Staphylococcus aureus* with 100% inhibition. Most potential strain amongst them was identified as *Enterococcus durans* TP2 strain by 16S rRNA gene technique. Further safety assessment was confirmed by evaluating haemolytic activity, DNase and gelatinase activities and all the three probiotic isolates TP1, TP2 and TP3 had shown -ve results, thereby proving their completely safe status. The strain exhibited high tolerance to acid with minimum cell survival of 39.24% after 180 min at pH 2.0, high bile salt tolerance expressed cell survival of 93.69% after 8 h at 2.0% bile salt concentration, exhibited satisfactory auto aggregation potential after 5 h i.e., higher than 40% and reveal the strong hydrophobicity. The strain TP2 also exhibited good antioxidant activity, depletion of sodium nitrite and cholesterol reduction and was found to be susceptible to most of the antibiotics used. These observations designate that *Enterococcus durans* TP2 [MH916769] as a good probiotic candidate for enhancing the further therapeutic potential of well known immunity booster – Turmeric in the fermented pickle, thus recommending it as highly beneficial product for health.

Keywords: *Enterococcus durans* TP2 [MH916769], Fermentation, Probiotic, Turmeric pickle

IPC Code: Int. Cl.[20]: A23L 33/135, A61K 35/741, A61K 36/9066

Recently, amidst the outbreak of pandemic – COVID 19, immunity enhancing products have become of high interest amongst the consumers. Turmeric is one of the well- known immunity enhancers worldwide[1]. Repeated recommendations of Ayush to consume turmeric in different forms viz., golden milk and dried powder etc. are a few examples of them. In the present study a new product i.e., turmeric pickle can have tall claims over the other products due to presence of not only raw turmeric chunks added in it but also being doused with additional immunity enhancing probiotics in it, thus highly recommending it as one of the best amongst the list of immunity booster products available commercially at economical rates and can be prepared at home also due to its easy fermentation process. Probiotic is 'live microorganisms which provides immense benefits to the health of human beings. Probiotics have great potential in medicine, prevention and treatment of gastrointestinal infections, inflammations and allergic reactions to enhance immunity[2]. Beneficial effects on the host health such as anticarcinogenic, antioxidants properties, cholesterol lowering properties, antiinfection properties, immune system improvement, minimizing lactose intolerance and enhancement of nutritional level have strongly been attributed to probiotics. Probiotic-based natural health products are mostly available commercially in the form of fermented products as well as dietary supplements and lactic acid bacteria are predominant flora among them due to their good probiotic activity and WHO designated GRAS status[3]. The majority of the probiotic enriched food products is classified as functional foods and represents a remarkable segment well recognized as immunity booster of upcoming market. The need of probiotic enriched functional foods especially in current scenario of Covid-19 is increasing rapidly due to arising awareness of consumers. In this scenario, traditional Indian turmeric pickle has immense potential to be considered as a strong immunity booster along with its appetizing taste. So keeping in view the medicinal properties of turmeric, the present

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study was undertaken to prepare turmeric pickle as well as to establish therapeutic effect of potential fermenting probiotic strains present in it by their isolation, identification and characterization.

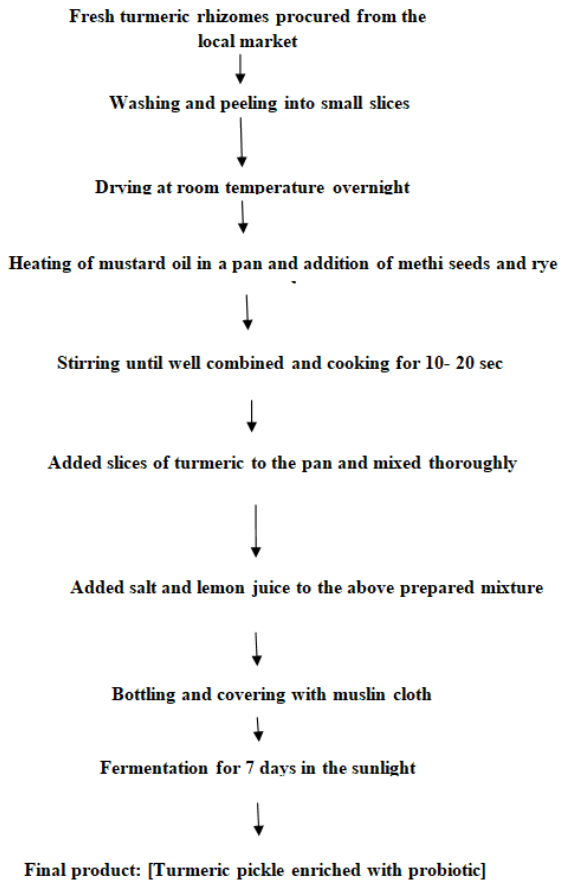
Material and methods

Process for the preparation of turmeric pickle

Ingredients used

Fresh turmeric rhizomes, mustard oil, lemon juice, methi seeds, rye seeds and salt

Preparation



Flow sheet of Turmeric pickle preparation method

Isolation of fermenting lactic acid bacteria

Isolation of fermenting lactic acid bacteria was done from turmeric pickle by using standard spread plate method by preparing serial dilution range of 10^{-1} to 10^{-9}. 1ml sample from each dilution was mounted on sterilized MRS agar medium containing petriplates to isolate lactic acid bacteria as well as assess microbial load.

Antimicrobial activity

Spoilage causing bacteria viz. *Bacillus cereus* CRI (CRI, Kasauli), *Listeria monocytogenes* MTCC 839 (IMTECH, Chandigarh) and *Staphylococcus aureus* (IGMC, Shimla) were used to study their antagonistic potential. Three potential lactic acid bacteria i.e., TP1, TP2, and TP3 were isolated from turmeric pickle. Antimicrobial activity of isolates TP1, TP2 and TP3 were studied by the spot on lawn method[4].

Bacteriocin production: Well diffusion method

Isolated lactic acid bacteria TP1, TP2 and TP3 were further subjected to test their bacteriocin production by well diffusion method against test indicators i.e., *Listeria monocytogenes* MTCC 839, *Staphylococcus aureus* IGMC and *Bacillus cereus* CRI. 1.0 OD culture of each indicator (1 ml) was swabbed on nutrient agar plates by using sterilized cotton buds. Well of 7 mm diameter and 5 mm depth were prepared on the lawns laid in the nutrient agar plates. The culture supernatant was poured into the wells and the method was repeated with crude preparations of isolates against their respective indicators. The plates were kept for incubation at 37^{o}C for 24 h. The inhibition zones appeared around the wells were measured[5].

Molecular identification of isolate TP2 by 16S rRNA technique

On the basis of highest antimicrobial activity and bacteriocin production molecular identification of potential lactic acid bacteria TP2 was done by applying 16S rRNA technique. Bacterial isolate TP2 was grown overnight at 30^{o}C in MRS broth. The DNA was isolated by using GeNei Genomic DNA isolation kit. Partial DNA fragment of the 16S rRNA was amplified by polymerase chain reaction (PCR). The universal primers used for amplification were 27F (5' AGAGTTTGATCMTGGCTCAG 3') and 1492R (5' TACGGYTACCTTGTTACGACTT 3'). The phylogeny and family of the bacterial strain was accessed using BLAST search and the sequences so obtained were submitted in Gen bank, National Center for Biotechnology Information (NCBI), US.

Characterization of E. durans TP2 as potential probiotic and therapeutic agent

A. Evaluation of safety assessments of Enterococcus durans TP2 [MH916769]

DNase production

DNase agar medium plates were prepared and spot inoculation of isolates was done and incubated at 37^{o}C for 24 h.

Gelatinase production

Gelatinase enzyme production was confirmed by inoculating 20 µL of 12 h old cultures on MRS agar

plates supplemented with 3% gelatin and incubated at 37°C for 24 h. Plates were flooded with ammonium sulphate solution.

Haemolytic activity

Haemolytic activity was assessed by spot inoculation of bacterial cultures on sheep blood agar plates enriched with 5% human blood and incubated at 37°C for 24 h.

B. Primary characteristics of E. durans TP2 [MH916769]

Acid tolerance of E. durans TP2

Acid tolerance of isolate *E. durans* TP2 was measured following the method of Guo *et al.*[5].

Bile salt tolerance E. durans TP2

Bile salt tolerance was done following the method of Ramos *et al.*[6].

Antibiotic Sensitivity Test

The antibiotic sensitivity was measured towards antibiotics viz. Nystatin (100 units), Ampicillin (10 µg), Ofloxacin (10 µg), Augmentin (10 µg), Co-trimoxazole (25 µg), Nalidixic acid (10 µg), Erthromycin (15 µg), Tetracycline (30 µg), Gentamycin (10 µg), , Novobiocin (10 µg), Fluconazole (25 µg), Kanamycin (10 µg), Ampicillin (10 µg), Sulphatriad (10 µg), , Vancomycin (10 µg), Chloramphenicol (10 µg) and Amoxicillin(10 µg). Antibiotic discs were placed on seeded plates and the zone of inhibition was observed after 24 h of incubation at 35°C.

Auto-aggregation and Co aggregation

Auto-aggregation and co aggregation assay was performed as described by Zommiti *et al*.[7].

C. Therapeutic potential of E. durans TP2 [MH916769]

Bacterial adhesion to solvents: Hydrophobicity

Microbial adhesion to hydrocarbons was measured by the method of Rokana *et al.*[8].

Antioxidant properties: DPPH free radical scavenging assay

Antioxidant properties of the cell free extract was measured and absorbance was read at 517 nm after 30 min of incubation. The antioxidants activity was calculated by applying the equation [Ab_{517} C – Ab_{517} S / Ab_{517} C] x100[9].

In vitro cholesterol lowering property

The capability of isolates to assimilate cholesterol was assessed by using Liong and Shah (2005) method[10].

Estimation of Siderophore production

Screened probiotic isolates were checked for siderophore production by following the method of Schwyn and Neilands (1987)[11]. Observations were recorded. Formation of yellow orange zones around the colony on blue background was taken as positive result[11].

Determination of sodium nitrite depletion

Sodium nitrite depletion by the isolates in MRS broth was determined. Nitrite depletion (%) = (1-C_1/C_0) x100, where C_0 and C_1 are the concentration of nitrite present at time= 0 and time= 48 h, respectively[12].

Bile salt hydrolase activity

Isolates were cultivated in medium supplemented with 0.2% glycocholic acid (GCA) and incubated at 37°C for 48 to 72 h. Plates were observed for the white precipitates.

Results and Discussion

Preparation of turmeric pickle and isolation of fermenting lactic acid bacteria from turmeric pickle

Food fermentation is a traditional process of food preservation used since ancient times. Fermentation also improves the nutritional value of foods, increases digestibility of food and enhances flavor thus making these traditional foods more popular all over the world. India is well known for its fermented foods especially a variety of fermented pickles. Turmeric pickle is one of them. Turmeric itself has therapeutic properties and especially its most active concentrated compound curcumin has many scientifically proven health benefits such as being very powerful antioxidant, anti-inflammatory in nature and also beneficial to prevent heart disease and cancer. In the present study, turmeric pickle was prepared by using fresh rhizomes of turmeric collected from local market by the process of natural fermentation followed by isolation of many potential probiotic strains - TP1, TP2 and TP3 and their characterization. Morphologically TP1, TP2 and TP3 colonies appeared cream, white and cream coloured respectively with entire margin, raised elevation and smooth texture on MRS (Table 1). Biochemical tests viz., MRVP test, casein hydrolysis, indole production and H_2S production were observed negative while fermentation of carbohydrates, citrate utilization and motility test were found positive for TP1, TP2 and TP3 and identified according to Bergey's Manual of Determinative Bacteriology as *Lactococcus* sp (Table 2). Out of all the isolates, TP2 was identified by using 16S rRNA technique and sequence similarity search for the isolate TP2 was found 99% similar with

Table 1 — Isolation of potential lactic acid bacteria from turmeric pickle and their morphological characteristics

Sr. No.	Isolates	Food source	Color	Form	Margin	Elevation	Texture
1.	TP1	Turmeric pickle	Cream	Circular	Entire	Raised	Smooth
2.	TP2	Turmeric pickle	White	Circular	Entire	Raised	Smooth
3.	TP3	Turmeric pickle	Cream	Circular	Entire	Raised	Smooth

Table 2 — Biochemical characteristics of isolated bacteria and their tentative identification

Sr No.	Isolates	Gram's Staining	Shape	Catalase	Mode of growth	Tentative identification
1.	TP1	+ve	Cocci	-ve	Facultative anaerobe	*Lactococcus*
2.	TP2	+ve	Cocci	-ve	Facultative anaerobe	*Lactococcus*
3.	TP3	+ve	Cocci	+ve	Facultative anaerobe	*Lactococcus*

the sequence of *Enterococcus durans.* So the isolateTP2 identified as *Enterococcus durans* TP2 and had assigned with the accession no [MH916769] by NCBI, US.

All these isolates were having microbial load > 10^6 CFU/g of sample, thus fulfilling the criteria of WHO that recommends a product with 10^6 CFU/g of load adequate to establish and colonize in a healthy gut for beneficial effects. Being fermenting microorganisms, lactic acid bacteria as probiotics are considered good for health with high therapeutic potential. Since most of them are recognized probiotics, but their health potential may vary from strain to strain and that's why characterization to enlist their efficacy and viability is very important. Therefore, in the present study, all of three isolated lactic acid bacteria- TP1, TP2 and TP3 were further evaluated to screen and characterize these fermenting LAB as robust probiotics and to assess their health potential.

Antagonistic spectrum of isolated bacterial isolates

Isolated LAB was further confirmed for their antagonistic spectrum against selected challenging pathogens viz., *Listeria monocytogene*, *Bacillus cereus* CRI and *Staphylococcus aureus.* Those isolates showing clear zones less than 9mm against the test strain indicated poor antimicrobial activity, while the strains which depicted halo zones of greater than 12 mm and 20 mm were shown to have appreciable and strong antimicrobial activity against the indicators (Fig. 1). Though all 3 fermenting isolated lactic acid bacteria had shown high antimicrobial potential but further their activity was compared to find out the best one amongst them to be used as a probiotic reference strain for further study. Out of 3 isolates, TP2 isolate was selected for its further identification on the molecular level. In a similar study, new *Lactobacilli* strains were isolated and identified as well as screened for their probiotic potential activities[13].

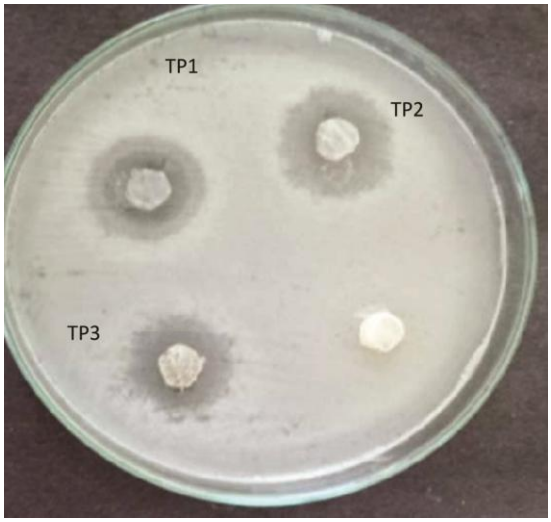


Fig. 1 — Antagonistic activities shown by TP1, TP2 and TP3

Bacteriocin production

In this study, three isolates TP1, TP2 and TP3 after testing their antagonistic spectrum were further subjected to check their bacteriocin production potential. Only one lactic acid bacteria i.e., TP2 was found positive for bacteriocin production where as TP1 and TP3 were observed negative (Table 3). A maximum zone size of 29 mm and 25 mm was depicted during the growth cycle of the isolate TP2, against *S. aureus* and *L. monocytogenes* respectively, after 44h of incubation at stationary phase (Fig. 2). Bacteriocin production is additional desirable trait of probiotics because of its antimicrobial nature and thus it is capable of inhibiting/ suppressing the growth of many challenging pathogens in the system[14] (Fig. 3).

Molecular identification of potential lactic acid bacteria TP2

On the basis of highest antimicrobial activity and bacteriocin production, the best selected isolate TP2 (Fig. 4) was studied on its molecular level by using 16S rRNA approach. 16S rRNA sequences analysis revealed that TP2 strain isolated from turmeric pickle displayed 99% homology with *Enterococcus durans*

Table 3 — Bacteriocin production by potential of lactic acid bacteria

Sr No.	Isolates	Bacteriocin production	Zone size (mm)		Average zone size (mm)
			S. aureus (mm)	*L. monocytogens* (mm)	
1.	TP1	-	-	-	-
2.	TP2	+	29	25	27
3.	TP3	-	-	-	-

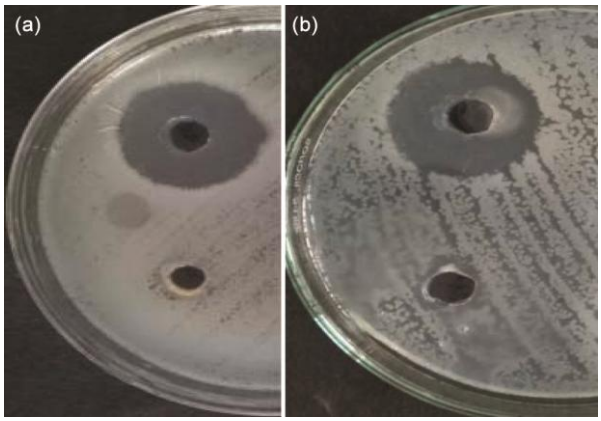


Fig. 2 — Bacteriocin production by isolate lactic acid bacteriaTP2 against (a) Indicator: *S. aureus (b)* Indicator: *L. monocytogenes*

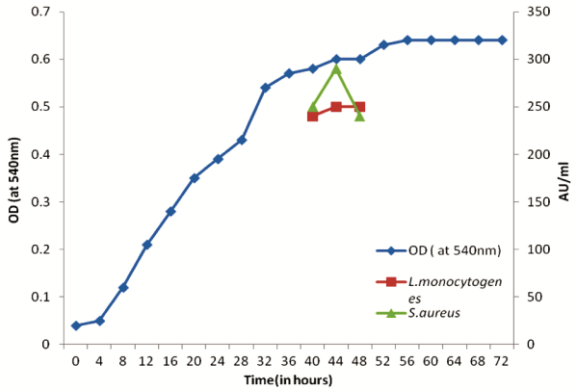


Fig. 3 — Bacteriocin production by *E. durans* TP2 during its growth phase against *S. aureus* and *L. monocytogenes*

and indexed with accession number MH916769 in NCBI, US and its phylogenetic tree is presented in (Fig. 5). In literature identification of probiotic lactic acid bacteria on species level is being done on the basis of their biochemical and physiological characteristics according to Bergey's manual of determinative bacteriology while confirmed authenticated identification is mediated only using 16S rRNA gene technique.

Probiotic potential of E. durans TP2

***A. Evaluation of safety assessment of E. durans* TP2 [MH916769]**

(i) DNase activity

E. durans TP2 isolated in the present investigation was found to be negative for the production of DNase

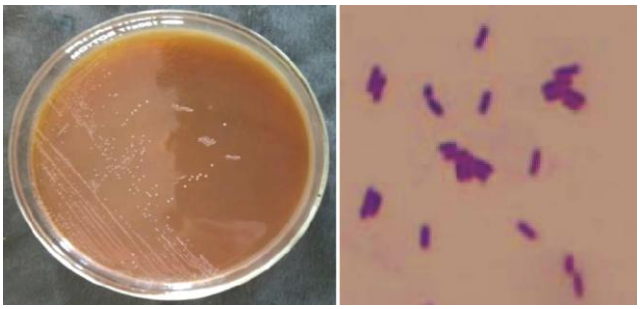


Fig. 4 — Morphology of TP2 isolate

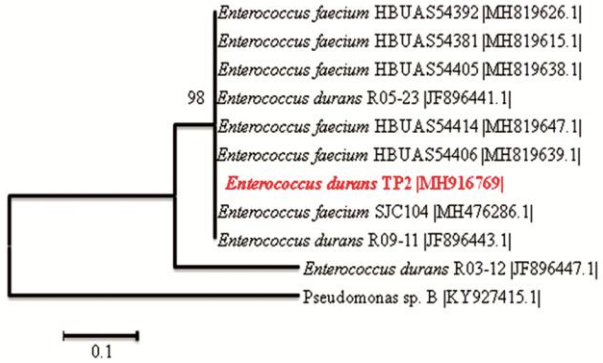


Fig. 5 — Phylogenetic relationship of *Enterococcus durans* TP2 (MH916769) based on a neighbor joining analysis of 16S rRNA sequences by MEGA 6.0 software

enzyme assigning a complete safe status to this isolate for further use (Fig. 6). Microorganism producing DNase enzyme cannot be used as a probiotic in food and feed industry. DNase is an extracellular enzyme which helps in the growth of pathogen by amplifying the pool of available nucleotides through DNA hydrolysis process and helps in dispersal of the pathogens. DNase also escapes the innate immune response by degrading neutrophils extracellular traps (NETs)[15].

(ii) Gelatinase activity

E. durans TP2 was assayed for gelatinase activity and there was absence of clear zone which indicated nil gelatinase activity, thus revealing its safe nature (Fig. 7). Gelatinases are the metalloproteinases produced by pathogenic bacteria which are capable of degrading extracellular matrix and other membrane bound components[16].

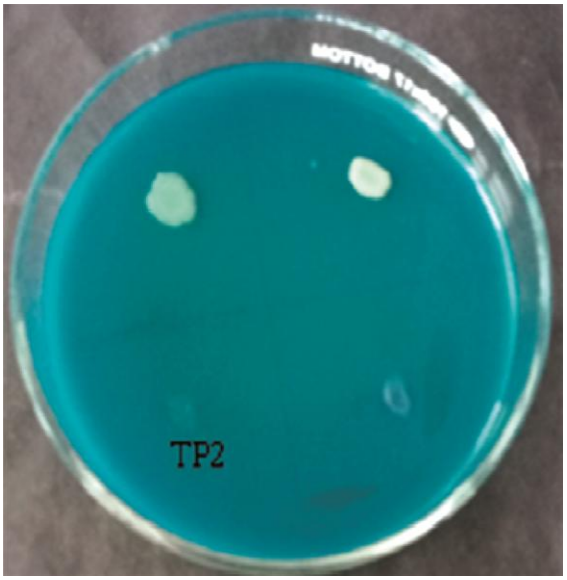


Fig. 6 — DNase activity: negative shown by *E. durans* TP2

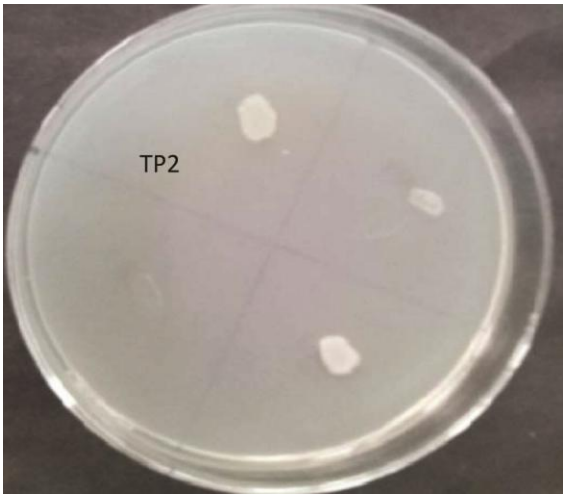


Fig. 7 — Gelatinase activity: negative shown by *E. durans* TP2

(iii) Haemolytic activity

E. durans TP2 was found negative for haemolysis on blood agar plates. Absence of clear zones (α-haemolysis) and green-hued zones (β-haemolysis) around colonies, thereby prove its safe and non-virulent nature (Fig. 8). A safe probiotic should be nonpathogenic as well as non-invasive hence elimination of pathogenic strains is essential for the safe and healthy probiotics[17].

B. *Primary characterization of Enterococcus durans* TP2 [MH916769]

(i) Acid and bile tolerance

E. durans TP2 had tolerated pH 3 maximum upto 77.53% after 180 min and shown minimum cell survival of 39.24% after 180min at pH 2.0. *E. durans*

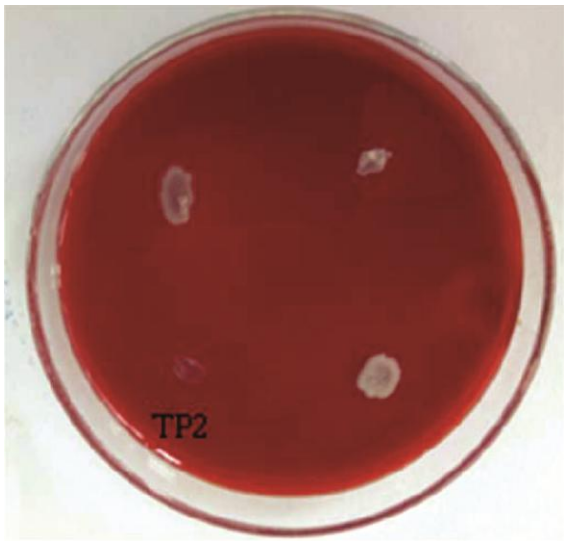


Fig. 8 — Haemolytic activity: negative shown by *E. durans* TP2

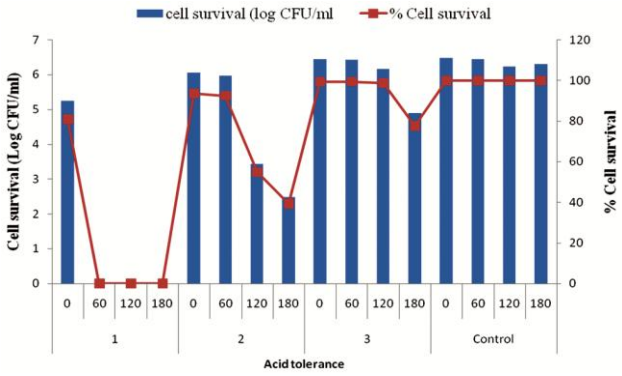


Fig. 9 — Acid tolerance of *E. durans* TP2

TP2 showed maximum log CFU/mL of 6.37 at pH 6.5 and minimum log CFU/mL of 1.31 at pH 1.0 (Fig. 9). The exposure to pH 2.0 violently decreased the counts of LAB after 1 or 2 h of incubation period. When isolates were exposed to pH 5.0 and to bile salts (0.15, 0.30 and 1.00%), there is nil decrease in the counts of the *Lactobacillus* strains.

(ii) Antibiotic sensitivity

E. durans TP2 was found to be sensitive for the most of antibiotics used in the present study (Table 4), thereby presenting its inability to show resistance in the presence of antibiotics and thus proving its safe status (Fig. 10). Mostly, LAB are sensitive to inhibitors of protein synthesis such as Erythromycin, Tetracycline, Chloramphenicol and Clindamycin and resistant to glycopeptides like Gentamycin, Kanamycin, Streptomycin, etc. The antibiotic susceptibility confirmed all these isolates as safe and thus proposes their use as potential probiotics successfully. According to world Health Organization bacteria used

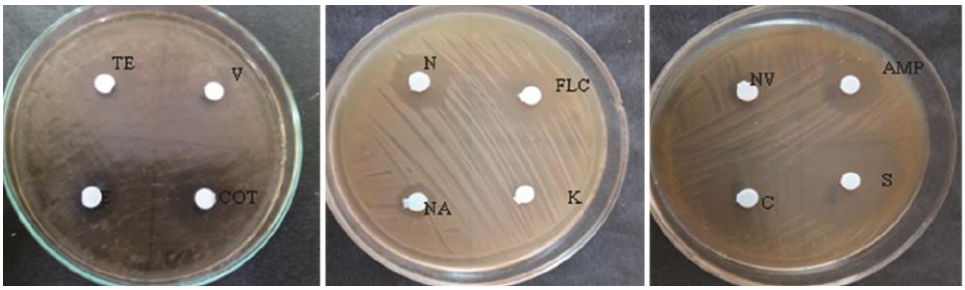


Fig. 10 — Antibiotic sensitivity of *E. durans* TP2

Table 4 — Antibiotic sensitivity of *E. durans* TP2

Antibiotics	*E. durans* TP2
Gentamycin (GEN)	S
Ofloxacin (OF)	S
Kanamycin (K)	R
Amoxicillin (AMX)	S
Augmentin (A)	S
Novobiocin (Nv)	S
Ampcillin (AMP)	S
Chloramphenicol(C)	S
Sulphatriad (S)	R
Tetracycline (TE)	S
Vancomycin (V)	S
Erythromycin (E)	S
Co-trimoxazole(COT)	S
Nystatin (N)	S
Fluconazole (FLC)	S
Nalidixic acid (NA)	S
Sensitivity (%)	88.23

as probiotics should not carry any transferable antibiotic resistance gene[17]. The presence of large number of transferable resistance genes within the intestinal microflora is unacceptable due to the risk caused by pathogens present in GI tract and following further failure of antibiotic treatment. Therefore, it is necessary to confirm that probiotic strains lack acquired antibiotic resistance characteristics to consider them safe for human and animal consumption[18]. Thus the sensitivity of these lactic acid bacteria minimizes the chances of dispersing the resistance genes to pathogens in the food matrix as well as in the GI tract.

(iii) Auto aggregation and co aggregation

E. durans TP2 exhibited highest auto-aggregation percentage **(**97.45) at 5 h of incubation period (Fig. 11). Bacterial aggregation between cells of the same strain (auto-aggregation) or between different species and strains (co-aggregation) is of significant importance and is one of the basic factors to check the

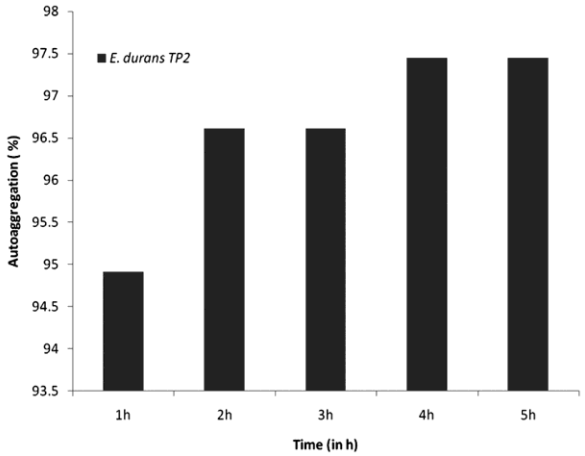


Fig. 11 — Autoaggregation of *E. durans* TP2 at different incubation period.

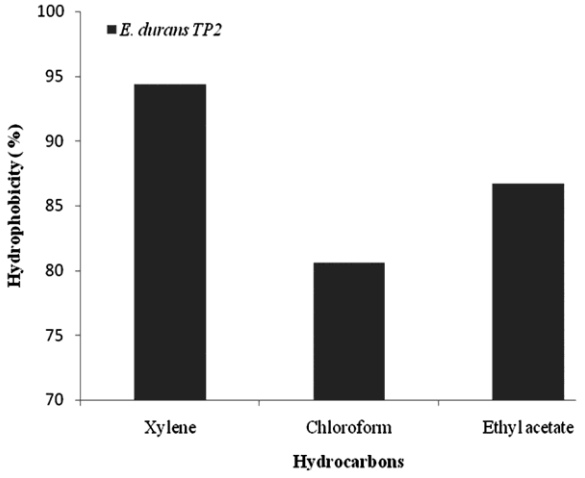


Fig. 12 — Coaggregation of *E. durans* TP2 showed by different indicators

potential of the probiotic strains to bind to the GI tract, where probiotics are to be vital and employ their favourable effects[19]. In the present study, *E. durans* TP2 showed highest coaggregation with *B. cereus* (16.6%) and lowest with *L. monocytogenes* (14.6%) (Fig. 12).

C. Therapeutic potential of E. durans TP2 [MH916769]

Cell surface hydrophobicity

Cell surface hydrophobicity is a nonspecific interaction between microbial cells and host. *E. durans* TP2 exhibited maximum adhesion i.e., 94.39% towards xylene, 80.61% towards chloroform and 86.73% towards ethyl acetate (Fig. 13). Bacterial adhesion to xylene, chloroform and ethyl acetate is tested to assess the Lewis acid-base characteristics of the bacterial cell surfaces[20]. The capability to bind with mucus and epithelial cells is an important criteria for a potential probiotic strain which make smooth adhesion to intestinal epithelium.

Antioxidant activity

Probiotic microorganisms express antioxidant potential by producing enzymes like superoxide dismutase (SOD) which encourage the production of non-enzymatic antioxidant and free radical scavenger glutathione (GSH) and certain antioxidant biomolecules. Antioxidants increase the body's immune system and shield our cells from deleterious effects of free radicals. The antioxidant activity of the screened lactic acid bacteria i.e., *E. durans* TP2 was investigated through the DPPH scavenging ability of the cell free extracts had been recorded significantly high i.e., 63.78% proving it a very strong immunity booster. The antioxidant activity was observed with *E. durans* TP2 was 63.78%.

Cholesterol lowering property

High cholesterol level in the blood and diet is a crucial factor for cardiac disease and colon cancer**.** In this study, isolate *E. durans* TP2 was studied for cholesterol assimilation and found 68.56% cholesterol lowering property. Recently lactic acid bacteria have great attention towards worldwide as potential agent for cholesterol–lowering property. Utilization of fermented food products viz. dairy products having probiotics has been proposed as a mean to lower serum cholesterol. The depletion of serum cholesterol could be a prime health benefit of LAB for humans as well as in animals[21].

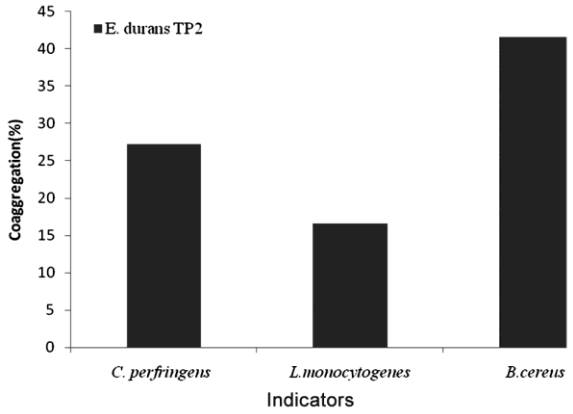


Fig. 13 — Hydrophobicity showed by *E. durans* TP2 strain with different hydrocarbons

Siderophore production

Iron deficiency is more common in Indian population, which causes birth defects, anemia, cancer, etc. Hence, probiotic microorganisms also help to combat the deficiencies of iron. When the selected *E. durans* TP2 was subjected to siderophore production, it was found that it was able to produce zones on the plates supplemented with CAS (Chrome Azurol Sulfonate) dye depicting its ability for siderophore production. Panda *et al*., specified the *Enterococcus* strain isolated from dairy products as their potential for probiotic and siderophore production[22].

Sodium nitrite depletion test

Nitrite is an important N-nitrosamines precursor, which has ability to cause methemoglobinemia and carcinogenesis. The control of Sodium nitrite concentration is important from the food safety point of view. LAB has been found to deplete nitrite in many fermented foods. *E. durans* TP2 strain showed good sodium nitrite depletion (73.58 %).

Bile Salt hydrolase activity of E. durans TP2

BSH activity is a desirable attribute of probiotics. LAB isolates are streaked on MRS plates containing taurine conjugated bile acid BSH, they produce bile salt hydrolase. The deconjugation activity of LAB isolate is displayed in plates and copious amounts of deoxycholic acid get precipitated around active colonies and diffused into the surrounding medium. In the present study *E. durans* TP2 showed white precipitation around the colonies marking it positive for bile salt hydrolase activity.

The overall probiotic potential of bacterial strains is based upon their cumulative probiotic score achieved by it depending upon the different parameters evaluated in the present study. In this study probiotic potential of *E. durans* TP2 has exhibited an impressive probiotic score of 95.66% proving its worth as an outstanding probiotic for improving health and immunity. This prepared turmeric pickle had enhanced therapeutic effects due to the presence of probiotic which are already established immunity enhancers. In the wake of the Covid-19 pandemic outbreak, entire humanity across

the world is suffering. Thus, increasing the body's immune system becomes a major goal of all of us in maintaining good health. The Ministry of AYUSH, Govt. of India issued some self-care guidelines as preventive health measures for boosting immunity with special reference to respiratory health amidst the coronavirus outbreak. According a traditional Indian system of treatment AYUSH recommended Golden Milk- (milk + turmeric), an Ayurveda medicine to promote immunity[23]. Curcumin is the active ingredient in turmeric; it has powerful biological properties as immunity boosters. So keeping in view the therapeutic importance of turmeric itself turmeric pickle – a concentrated product with the source of immunity booster probiotics is highly recommended for daily use to promote health of the consumers.

Conclusions

The present investigation was carried out to prepare a traditional fermented product- turmeric pickle followed by isolation of efficient potential probiotic microorganisms from turmeric pickle, their screening, identification, safety assessment as well as evaluation of probiotic attributes. Three lactic acid bacteria were isolated from fermented turmeric pickle and characterize as good probiotics and present in adequate load ($>10^6$ CFU/g) as per WHO recommendation. Upon screening, a strain TP2 identified as *Enterococcus durans* using 16S r RNA gene technique had shown high grade of 95.66% of probiotic score along with therapeutic potential. The potential lactic acid *E. durans* TP2 [MH916769] had good antioxidant activity led to depletion of sodium nitrite and cholesterol reduction. Probiotic when tested had shown high immunity boosting potential with antioxidant activity of ~ 64%. Turmeric itself established as a good immunity enhancer further coupled with health promoting probiotics in fermented pickle have turned this traditional pickle as an excellent health product amidst covid-19 pandemic threat.

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Conflict of interest

Authors declare that they have no conflict of interest.

Author's contribution statement

Chandel M: Performed experimental part of the research; Sharma N: Conceptualization, writing, editing and review of the manuscript; Sharma N: Preparation of draft and writing.

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