Probiotic potential of *Lactobacillus* strains isolated from different food sources

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Abstract: Probiotics are gaining popularity worldwide because people are getting aware of the health benefits provided by them. This review explains the major properties that a bacterial strain should possess to be used in the production of probiotic products. The review focuses mainly on the strains of *Lactobacillus* species with potential probiotic properties that have been isolated from the foods of different origins. These strains with potential probiotic properties can be evaluated further and can be used as starter cultures in the production of probiotic foods

Keywords: Probiotics, Lactobacillus, potential probiotic properties, starter cultures

INTRODUCTION

The term 'probiotic' is derived from a Greek word in which 'pro' means 'promoting' and 'biotic' means 'life'. The term probiotic generally refers to a few specific types of beneficial microbes contained in a probiotic supplement or added to food. Probiotics include friendly microbes that live normally in our digestive tract and are naturally present in fermented foods such as yogurt, pickles, etc. Probiotics compete successfully with the harmful microbes in our digestive tract by lowering their population they contribute towards gut health (Gary Huffnagle & Sarah Wernick, 2007). Lactobacillus species that are used as probiotics are *L. acidophilus*, *L. casei*, *L. gasser*, *L. johnsonii*, *L. paracasei*, *L. plantarum*, *L. reuteri*, *L. rhamnosus*, L. lactis. The strains that are used as probiotics should exhibit some special properties like acid and bile tolerance which is important for oral administration of probiotics, adhesion to mucosal and epithelial cells, bile salt hydrolase activity, antimicrobial activity against pathogenic bacteria (Kechagia et al. 2013). Lactobacillus belongs to the group of Lactic Acid Bacteria that can degrade carbohydrates. Some common characteristics of Lactic Acid Bacteria are gram-positive, cocci, coccobacilli, or rods that are non-spore-forming and most genera have a DNA base composition of less than 50% G+C, catalase-negative, prefers to grow under micro-aerophilic or anaerobic conditions, and typically ferment glucose mainly to lactic acid (homo-fermentative), but can also have lactic acid, CO2, and ethanol/acetic acid as end products (hetero-fermentative). It is found naturally in the gastrointestinal tract of mammals and fermented food products (George et al. 2018).

Probiotic evaluation of lactobacillus strains is essential to confer the required health benefits to the consumers the bacteria should arrive alive in the intestinal tract. It is also essential for the bacteria, that it must survive during food processing, product maturation, and storage. It is necessary that after consumption the bacteria should be resistant to the acidic conditions of the stomach and bile salts of the small intestine. The approach to protecting the microbe from processing and gastro-intestinal damage is micro-encapsulation (Liu et al. 2019). There is a huge demand for probiotics in the world as people are getting concerned about their health each day and they are aware of the health benefits that are related to probiotic consumption. "Guidelines for evaluations of probiotics in Food" were published by the Food and Agricultural Organization of the United Nations/ World Health Organization in 2002. These guidelines suggest the various criteria that can be used in probiotic selection (Araya et al. 2002).

Lactic acid bacteria may have a negative impact on the growth of *Helicobacter pylori* which is responsible for numerous gastrointestinal disorders from type B gastritis to gastric cancer. *H pylori* secrets urease enzyme to strive in the acidic environment of the stomach, LAB decreases the growth of *H pylori* in the stomach by decreasing the urease enzyme activity. *L. fermentum* SR 2 and *L. salivarius* SR16 are known to have a significant negative impact on the growth of *H. pylori* (Ryan et al. 2008). *Lactobacillus acidophilus* is known to have good antimicrobial activity against gastrointestinal pathogens and Lactobacillus bulgaricus is known to have the lowest antimicrobial activity against gastrointestinal pathogens (Philip et al. 2017). This antimicrobial activity of lactobacillus is linked to bacteriocin production as an antagonistic activity (Gibson et al. 2000). Twelve strains of lactobacillus that are known to have good antimicrobial activity are *Lactobacillus casei*, *Lactobacillus acidophilus Lactobacillus fermentum*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus pentosus* (Neha et al. 2017). The production of bacteriocins by lactobacillus strains can be used in biopreservation (Sarika et al. 2019). Bacteriocins produced by the strains of lactobacillus can be used as natural preservative agents in foods the activity of bacteriocins is heat stable and not affected by

alterations in ph from 1-4 (Tzu-Hsing et al. 2019). Commonly used bacteriocins in the dairy industry are nicin and pediocin AcH. Nicin and Pediocin PA-1/AcH are used in the meat industry for bio-preservation. Vegetable product industries make use of nisin in canned vegetables and fruits, pediocin PA-1/AcH in salad and fruit juices, and enterocin AS-48 is effective in preventing spoilage against alicyclobacillus (Singh, 2018).

To confer the desired benefits the probiotic strain must survive the acidic environment of the stomach. The acid tolerance of lactobacilli is due to a gradient between the pH of extracellular space and cytoplasm. Gram-positive organisms use the F-F1 ATPase mechanism to survive the acidic environment. The F-F1 ATPase creates a high intracellular pH at a low extracellular pH (Corcoran et al. 2005). Resistance to bile salts varies among Lactobacillus also among different strains. This property is due to the presence of BSH (bile salt hydrolase) an enzyme that reduces the toxic effect by conjugating bile. This activity is mostly found in those bacterial species which are isolated from the feces of animals. Among the various strains of lactobacilli L. casei ASCC 292 and L. acidophilus 4962 have good acid and bile tolerance as concluded by M.T. Liong and N.P. Shah (2005) from their experimental study on the acid and bile tolerance of the various strains of lactobacilli. L. fermentum SR2 and *L. gasseri* SR1 are also acid-tolerant (Ryan et al. 2008).

The properties of aggregation and adhesion are essential for probiotics as it leads to the formation of biofilms which protects the hosts from the invasions by pathogens. It was concluded from the study conducted by Grigoriyan et. al (2017) that L. helveticus INRA-2010-H11 and L. acidophilus JM-2012 show auto and co aggregation as well as adhesion to mice gut. Therefore, these strains have the potential to be used as probiotics. L. acidophilus M92 is known to have a good ability to establish itself in the human gastrointestinal tract due to its good autoaggregation and adhesion ability (Kos et al. 2003).

Probiotic bacteria may have the potential to lower down cholesterol levels by the process of cholesterol assimilation. The study conducted by Duchesneau et. al (2014) demonstrates the ability of various strains of Lactobacillus to assimilate cholesterol among the various strains studied L. plantarum ATCC 14917 was found to be the best cholesterol assimilator grown in MRS Broth containing 100ug/ml of cholesterol-PEG 600 and L. reuteri NCIMB 701089 assimilated 67% of cholesterol under-stimulated intestinal conditions that are by using intestinal fluid containing Cholesterol-PEG 600 as the growth medium.

This review throws light on the Lactobacillus strains that are isolated from foods of different origins and screened for their probiotic potential so that they can be used as starter cultures in the manufacture of probiotic products. The strains that were found superior in their probiotic properties to other isolated strains and are of industrial importance to be used as starter cultures in probiotic product manufacture are stated in this review.

INDIAN FERMENTED FOODS

Fermentation is one of the traditional methods used worldwide to develop aroma, flavor, and texture in food products using microbes. It is also used to preserve and biologically enrich food products. Indian fermented foods include fermented vegetables like 'gundruk', 'sinki', fermented cereals, and pulses like 'kinema', 'tungrimbai', 'dosa' (Rawat et al. 2018). In the study conducted by Khagwal et al. (2019) samples of 102 traditional Indian fermented foods like Bhatura dough, idli batter, dosa batter, etc were collected aseptically and 200 microbial cultures were isolated using MRS media out of which 120 isolates tested catalase and oxidase negative. As they belonged to the Lactobacillus genus. Among 120 Lactobacillus isolates 7 isolates were found to be acid, bile, and pancreatic tolerant. These 7 isolates were further evaluated for other probiotic properties including anti-biotic resistance, hemolysis, auto-aggregation, and carbohydrate fermentation. The results obtained showed that the strains of Lactobacillus plantarum and Lactobacillus fermentum have excellent probiotic potential. These two strains were also found to be superior to Lactobacillus casei Shirota (LcS) the commercial probiotic strain that was used as control.

A traditional fermented beverage consumed by the tribal folks of Lahaul and Spiti of Himachal Pradesh is produced from the spontaneous fermentation of rice by the addition of traditional inoculum called 'Phab' is 'chhang'. In the study conducted by Handa et al. (2016) lactic acid bacteria were isolated from chhang. These lactic acid bacteria were identified and evaluated for antagonistic activity against serious foodborne/spoilage-causing bacteria- Staphylococcus aureus IGMC, Listeria Monocytogenes MTCC 839, Enterococcus faecalis MTCC 2729, Clostridium perfringens MTCC 1739, Leuconostoc mesenteroids MTCC 107, and Bacillus cereus CRI. The highest metabolic activity was observed in Lactobacillus plantarum F22. Therefore, this strain was subjected to probiotic screening and was found to be acid and bile tolerant, anti-biotic sensitive, had strong autoaggregation and hydrophobicity, and was found suitable in the production of health foods.

Dosa is a south Indian fermented food produced by fermenting rice and black gram. In the study conducted by Gupta et al. (2014) lactic acid bacteria were isolated from various traditional Indian foods and Lactobacillus plantarum LD1 isolated from Dosa was selected for probiotic evaluation based on the anti-microbial property against Micrococcus leuteus MTCC106. It was concluded from this study that this strain can be used in probiotic production as it was found acid and bile tolerant, possessed bile hydrolase activity, antibiotic sensitive except for Kanmicin as well as showed survival under stimulated conditions with antacids such as Eno.

Raabadi is a fermented beverage consumed in summers in the regions of Haryana and Rajasthan. The main ingredients of this are barley flour, pearl millet flour, buttermilk, salt, black pepper, cumin seed powder, and dry green coriander. In the study conducted on Raabadi samples, 24 lactobacillus isolates were subjected to in vitro probiotic evaluation, *L. plantarum* RYPR1 showed good probiotic potential out of the 24 strains. Therefore, this strain can be used in the production of probiotics (Yadav et al. 2016). The strain was found to be acid, bile, and lysozyme tolerant.

MEAT PRODUCTS

Using probiotic cultures for producing meat products is gaining popularity worldwide. Microbes isolated from traditional indigenous foods are widely used for this purpose. Isolating microbes with probiotic potential from meat products will be beneficial in the formation of probiotic meat products as these microbes are capable of adapting to the meat environment. The strains selected as starter cultures in probiotic meat product manufacture must possess the essential probiotic properties as well as the property of bacteriocin production to be able to survive among the well-adapted microbiota of the product (Ojha et al. 2015, Favaro et al. 2017).

In the study conducted by Moreno et al. (2018) five strains were isolated from meat products and tested for their probiotic properties. These five strains were L. lactis subsp. hordinae CTC 484, Lactobacillus plantarum CTC 368 and CTC 469, and Lactococcus lactis subsp. cremoris CTC 204 and CTC 483. All the isolated strains were found to be acid tolerant (pH 2), showed growth at a temperature of the gastrointestinal tract and the ability to form biofilms. The highest intensity of biofilm was produced by CTC 204. All the strains were amoxicillin sensitive and erythromycin-resistant. Also, CTC 204 was resistant to chloramphenicol, CTC 368, and CTC 469 to chloramphenicol and vancomycin, CTC 483 to tetracycline and vancomycin, and CTC 484 to clindamycin and chloramphenicol. CTC 469 was also found to be bile tolerant and it was concluded from the study that *L. lactis* subsp. cremoris CTC 204 and *Lactobacillus plantarum* CTC 368 strains had the greatest potential to be used as starter cultures in probiotic meat production.

Fermented sausages are considered to be a good carrier for probiotic bacteria because they are very mildly heated during processing but on the other hand, bacterial viability may be reduced due to high salt content, low water activity, and low pH. Therefore, the results of the studies to testing the probiotic potential of the strains isolated from fermented sausages will be strain dependent. Candidate probiotic strains have been isolated through probiotic screening of all the isolates obtained from fermented sausages that may be naturally present in the product. These isolates can then be used as starter cultures in the production of probiotic meat products (Vuyst et al. 2008).

PICKLED VEGETABLES

Paocai is a pickle made by a combination of acidified cabbage, carrot, cucumber, radish, and bamboo shoots and is a popular side dish in the homes of Taiwan. Paocai fermentation starts with the various microbes that are naturally present in the raw materials and at last lactic acid bacteria dominate. Taiwanese paocai is crunchy in texture and has a tangy taste. It is made by a combination of vegetables, spices, and other ingredients. The mode of fermentation in the production of paocai is anaerobic. In the study conducted by Chang et al. (2013) 33 strains were isolated from paocai and out of these 11 strains were found acid and bile-tolerant. These 11 strains were *Lactobacillus plantarum* (E1,E38,E46,E51,E55), *Lactobacillus casei* (E7,E15,E30,E33,E40) and *Lactobacillus rhamnosus* (E8). Adhesion to the monolayer of Caco 2 cells was highest in E51 and moderate in E33, E38. All isolated strains were found to inhibit food-borne pathogens *S. aureus*, *B. cereus*, *Shigella fexneri and S. typhimurium*.

Sauerkraut is prepared by fermenting shredded cabbage by lactic acid bacteria in the presence of 2-3% salt. In the study conducted by Yu et al. (2012) 7 strains of *Lactobacillus plantarum* were isolated from Chinese sauerkraut and these strains were subjected to probiotic evaluation. These 7 strains were S2-5, S2-6, S4-1, S52, S38, S56, and S72 among these 7 strains S2-5 and S4-1 can be used in the production of probiotic foods as they were found superior to other strains in probiotic evaluation. These strains were found to be acid and bile-tolerant, had high beta-galactosidase activity, showed adherence to Caco-2-cells, showed anti-microbial activity against *Escherichia coli* 0157, and *Shigella flexneri* CMCC(B), these strains were found resistant to kanamycin, vancomycin, polymyxin B, streptomycin and gentamycin. The highest cholesterol assimilation activity was observed in S4-1 in vitro study and was chosen for in vivo study. Other lactic acid bacteria that can be isolated from sauerkraut include *Lactobacillus brevis* and *Leuconostoc mesenteroids*. The probiotic potential of *Lactobacillus brevis* was studied by Singh et al. (2020) and *Lactobacillus brevis* ATCC 14869 was isolated from the sample of sauerkraut. This strain was found to be acid and bile tolerant, resistant to a broad range of antibiotics, and showed antagonistic activity against E. coli, K. pneumoniae, S. aureus, and P. aeruginosa.

L. brevis is a heterofermentative, gram-positive organism that can be isolated from foods such as sauerkraut, cheese, milk, mouth, and human intestinal tract.

A traditional fermented Korean vegetable mix made from Chinese cabbage, radish, red pepper powder, green onion, garlic, ginger, and fermented seafood. This dish is prepared at home and served as a side dish with meals. *Lactobacillus sakei* and *Lactobacillus plantarum* strains isolated from Kimchi were subjected to cholesterol assimilation tests. In the results, it was found that the majority of *L. sakei* strains reduced cholesterol from 50% to 60%, and two strains of *L. plantarum* isolated reduced cholesterol to about 48% (Lee et al. 2011).

Table 1- % cholesterol assimilation from Lactobacillus isolated from kimchi (Lee et al. 2011).

Strains	%cholesterol
	Assimilation
L. sakei	
B6a4	40-60%
I8	
NR11	
NR24	
NR25	Page 1
NR26	
NR27	
NR28	
NR29	4
NR65	16
1 st M3	1
L. plantarum	About 48%
NR74	15
L. Plantarum	About 48%
GC	M. 6
. plantarum NR74 . Plantarum	Y C

OLIVES

Olives are commonly consumed in the following processed forms- Spanish style which is alkali-treated green olives, the Californian style that ripe olives by alkaline oxidation, and natural olives that directly brined olives. Lactobacillus pentosus and Lactobacillus plantarum species, are the most important lactic acid bacteria present in table olives (Hurtado et al. 2012). In the work conducted by Cabello et al. (2020) 16 strains of *lactobacillus* were isolated from table olives and tested for immunomodulatory and anti-proliferative properties on epithelial human cellular lines. It was concluded from the results of the following tests that Lactobacillus pentosus LPG1, showed the best anti-inflammatory and immunomodulatory properties. Therefore, it has the potential to be used as a probiotic. The complex microbiota of table olives to identify probiotic organisms is not much investigated to date but this food matrix is a very good source of beneficial lactic acid bacteria (Botta et al. 2014). Probiotic lactic acid bacteria can be isolated from table olives and they can be incorporated in the formation of other fermented foods with enhanced nutritional and organoleptic properties. In the study conducted by Argyri et al. (2012) 71 lactic acid bacteria were isolated from table olives out of which 13 strains were of Lactobacillus plantarum, 37 were of Lactobacillus pentosus, 1 was of Lactobacillus paraplantarum and 2 were Lactobacillus paracasei. The reference strains used for the study were Lactobacillus rhamnosus GG and Lactobaccillus casei Shirota. The isolated strains were subjected to in vitro probiotic screening and the strains that were found superior to other isolated strains were Lactobacillus pentosus B281, Lactobacillus pentosus E97, Lactobacillus pentosus E104, Lactobacillus pentosus E108, Lactobacillus plantarum B282, Lactobacillus plantarum E10, Lactobacillus plantarum E69, Lactobacillus paracasei sub sp. paracasei E93 and Lactobacillus paracasei sub sp. paracasei E94. These strains were found acid and bile-tolerant. Two strains B282 and E10 showed partial hydrolysis to bile salts. All the strains were found to be Kanamycin resistant and maximum adherence to Caco 2 cells was shown by Lactobacillus paracasei sub sp. paracasei E94. These strains are good candidates to be used as starter cultures in the development of fermented probiotic products.

The traditional variety of table olives from the Apulian region in Southern Italy are Bella Di Cerignola. Special features of these olives are their big size and their green/black color of their skin. The method of production of these olives involves- Manual harvesting, transportation, grading, treatment with alkaline lye (1.3%- 2.6% NaOH) for 12-15 hours, washing, brining, and fermentation for 30-60 days in NaCl (Bevilacqua et. al 2010). Dominant lactic acid bacteria species found in Bella di Cerignola olives are *Lactobacillus plantarum*, *Lactobacillus brevis*, and *Leuconostoc mesenteroides* (Campaniello et al. 2005).

DAIRY PRODUCTS

Milk and its products are a very good source of lactobacilli. Lactobacillus species from milk have been isolated and identified in numerous studies. Lactobacilli that are naturally present or are intentionally added in milk and milk products provide flavor, nutritional value, and texture to these foods and provide additional health benefits to the consumers. Some species of lactobacilli that can be commonly isolated from milk samples of cows and buffalo are *L.fermentum*, *L.acidophilus*, *L.viridescens*, *L.brevis*, and *L.gasseri* (Mithun et al. 2015). *Lactobacillus alimentareus*, Lactobacillus sake, Lactobacillus colloids isolated from cheese, yogurt, buffalo milk, and cow milk showed anti-microbial activity against *S. aureus* (ATCC-6538), *B. subtilis* (ATCC-12711), and *P. aeruginosa* (ATCC-835). Therefore, these strains can be raised and incorporated in the preparation of functional foods (Karami et al. 2017).

Sheep milk is used to produce a traditional Iranian dairy product Koome it is produced in sheepskin bags in areas of rural Iran. Koome is the best traditional dairy product with a long shelf life. In the study conducted by Shemshad et al. (2021) fifteen isolates from Koome were subjected to probiotic evaluation. The results obtained from this study showed that Lactobacillus crustorum LMG 23699 and 20314 and Lactobacillus brevis ATCC 14869 were acid and bile-tolerant, anti-biotic resistant, cholesterol-reducing, bile salt hydrolyzing and Lactobacillus crustorum LMG 23699 showed anti-microbial activity against Pseudomonas aeruginosa. In the study conducted by Bao et. al (2010) Ninety strains of *Lactobacillus fermentum* were isolated from traditional dairy products and were screened for desirable probiotic traits such as acid and bile tolerance, aggregation activity, and antibacterial activity. In the in vitro probiotic screening 11 strains of Lactobacillus fermentum were found to contain the desirable probiotic properties. It was concluded from this study these 11 strains of Lactobacillus fermentum-IMAU60151, IMAU60092, IMAU60120, IMAU20084, IMAU60083, IMAU60071, F6, IMAU20044, IMAU20080, IMAU20081, IMAU60145 were found effective in vitro studies of probiotic evaluation and can be further evaluated in vivo for their probiotic potential. The results obtained from this study showed that Lactobacillus fermentum F6 showed the highest tolerance to acid and bile salts, broad antimicrobial activity, and good aggregation properties. Maragkaudakis et al. (2006) isolated twenty-nine Lactobacillus strains from dairy products and the isolates were screened for probiotic properties. Among these twenty-nine strains, three strains were the best based on pepsin, pancreatin and bile salts resistance, bile salt hydrolase activity, non-hemolytic behavior, anti-biotic resistant, adhesion to caco 2 cells, and inhibit the adhesion of Escherichia coli and Salmonella typhimurium to Caco-2 cells. These three strains that were found to possess the desired probiotic properties were L. casei Shirota ACA-DC 6002, L. plantarum ACA-DC 146, and L. paracasei subsp. tolerans ACA-DC 4037.

Tufail et al. (2011) isolated *Lactobacillus bulgaricus* strains from yogurt, isolated bacteriocin produced by *Lactobacillus bulgaricus*, and evaluated the anti-microbial activity of the bacteriocin against pathogenic and spoilage causing bacteria by agar well diffusion method. The results obtained from this study showed that *Lactobacillus bulgaricus* strains isolated from yogurts showed anti-bacterial activity against V. cholera and E. coli because a significant amount of bacteriocin was produced by these strains. The maximum bacteriocin was produced within an incubation period of 48 hours.

FLOWERS

A wide range of flowers is consumed by adding to foods and drinks to enhance their flavor and color to foods. In India, they are widely used in the preparation of sweet dishes. Fructose lactic acid bacteria are a group of lactic acid bacteria that use D-fructose over D glucose as a carbon source. These bacteria can be isolated from fruits and flowers because they are high in fructose content (Endo et al. 2009). The probiotic potential of fructose lactic acid bacteria has not been explored much. Fructose is a precipitation factor in irritable bowel syndrome and this syndrome can be prevented by fermenting cereals with fructose lactic acid bacteria. In the study conducted by Sakandar et al. (2019) three fructose lactic acid bacteria strains of *Lactobacillus kunkeei* (JNGBKS6, JNGBKS7, JNGBKS8) were isolated from Narcissus, yellow rose and pink rose from China. These strains when evaluated for their probiotic potential were found to be resistant to low pH, bile tolerant, cholesterol assimilatory, and antibiotic-resistance. These strains also showed anti-microbial activity against E. coli 25922, S. aureus NCTC 8325, and S. typhimurium CMCC5011.

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TABLE OF CONCLUSIONS:

Table 2- Conclusions

Table 2- Conclusions				
Food	Isolated probiotic strain	Reference		
1. Traditional Indian foods	L. plantarum, L. fermentum	Khagwal et al. (2019)		
• Chhag	L. plantarum F22	Handa et al. (2016)		
• Dosa	L. plantarum LD1	Gupta et al. (2014)		
 Raabdi 	L. plantarum RYPR1	Yadav et al. (2016)		
2. Meat products	L. lactis subsp. cremoris CTC 204	Moreno et al. 2018		
2 8 11 1	L. Plantarum CTC 368			
3. Pickled vegetables	(E1 F0 F46 F61 F65)	GI (2012)		
• Paocai	L. plantarum (E1, E8, E46. E51, E55),	Chag et al. (2013)		
	L. casei (E7, E15, E30, E33,E40), L.			
	rhamnosus (E8)			
		** (2012)		
 Sauerkraut 	L. plantarum (S4-1)	Yu et al. (2012)		
	L. brevis ATCC14869	Singh et al. (2020)		
Kimchi	L. plantarum NR74, L. Plantarum GC	Lee et al. (2011)		
4. Olives				
 Table olives 	L. pentosus LPG 1	Cabello et al. (2020)		
	L. paracasei sub sp. paracasei E94, L.	Argyri et al. (2012)		
	plantarum B282, L. plantarum E10			
	(
	L. casei Shirota ACA-DC 6002, L.	N		
Dairy products	plantarum ACA-DC 146 and L.	Maragkaudakis et al. (2006)		
	paracasei subsp. tolerans ACA-DC	-38 A		
	4037	-30' I		
		~30 , N		
	Lactobacillus crustorum LMG 23699,			
	Lactobacillus c <mark>rustorum 2</mark> 0314,			
• Koome	Lactobacillus brevis ATCC 14869	Shemshad et. al (2021)		
	Lactobacillus kunkeei (JNGBKS6,			
6. Flowers	JNGBKS7, JNGBKS8)	ASSET		
		Sakandar et. al (2019)		
 Narcissus, yellow rose, 				
and pink rose				

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