PUBLICATIONS

List of publications

- **Manhar**, **A.K.,** et al. Cellulolytic potential of probiotic *Bacillus Subtilis* AMS6 isolated from traditional fermented soybean (Churpi): An in-vitro study with regards to application as an animal feed additive, *Microbiol. Res.* **186**, 62--70, 2016.
- **Manhar**, **A.K.,** et al. Assessment of goat milk-derived potential probiotic *L. lactis* AMD17 and its application for preparation of dahi using honey, *Ann. Microbiol.* DOI 10.1007/s13213-016-1210-x.
- **Manhar**, **A.K.,** et al. *In vitro* evaluation of celluloytic *Bacillus amyloliquefaciens* AMS1 isolated from traditional fermented soybean (Churpi) as an animal probiotic, *Res. Vet. Sci.* **99**, 149--156, 2015.

Manuscript under preparation

- Probiotic attributes of indigenous isolates *Lactobacillus plantarum* AMD6 and its cholesterol lowering effect in a hyperlipidaemic rat model.
- Identification and characterization of thermo active new cellulase from potential probiotic *Bacillus amyloliquefacies* AMS1 and its application on hydrolysis of agro-cellulosic waste material to be used as animal feed.

Other publications

- Purkayastha, M.D., Borah, A.K., Saha, S., **Manhar, A.K.**, Mandal, M., Mahanta, C.L. Effect of maleylation on physicochemical and functional properties of rapeseed protein isolate, *Journal of Food Science and Technology* 1-14, 2016.
- Roy, J.K., **Manhar, A.K**., Nath, D., Mandal, M., Mukherjee, A.K. Cloning and extracellular expression of a raw starch digesting α-amylase (Blamy-I) and its application in bioethanol production from a non-conventional source of starch, *J. Basic Microbiol.* **55**, 1287--1298, 2015.
- Nath, D., **Manhar, A.K**., Gupta, K., Saikia, D., Das, S.K., Mandal, M. Phytosynthesized iron nanoparticles: effects on fermentative hydrogen production by *Enterobacter cloacae* DH89, *Bull. Mater. Sci.* **38**, 1533--1538, 2015.

- Sharma, S., **Manhar, A.K**., Bora, P.J., Dolui, S.K., Mandal, M. Evaluation of antioxidant and antibacterial activity of various aspect ratio gold (Au) nanorods, *Adv. Mater. Lett.* **6**, 235--241, 2015.
- Purkayastha, M.D., **Manhar, A.K.,** Das, V.K., Borah, A., Mandal, M., Thakur, A.J., Mahanta, C.L. Antioxidative, hemocompatible, fluorescent carbon nanodots from an "end-of-pipe" agricultural waste: exploring its new horizon in the food-packaging domain, *J. Agric. Food Chem.* **62**, 4509--4520, 2014.
- Gupta, K., Barua, S., Hazarika, S.N., **Manhar, A.K**., Nath, D., Karak, N., Namsa, N.D., Mukhopadhyay, R., Kalia, V.C., Mandal, M. Green silver nanoparticles: enhanced antimicrobial and antibiofilm activity with effects on DNA replication and cell cytotoxicity, *RSC Advances* **4**, 52845--52855, 2014.
- Purkayastha, M.D., **Manhar, A.K**., Mandal, M., Mahanta, C.L. Industrial waste-derived nanoparticles and microspheres can be potent antimicrobial and functional ingredients, *Journal of Applied Chemistry*, 2014.
- Boruah, M., Gogoi, P., **Manhar, A.K**., Khannam, M., Mandal, M., Dolui, S.K. Biocompatible carboxymethylcellulose-g-poly(acrylic acid)/OMMT nanocomposite hydrogel for *in vitro* release of vitamin B 12, *RSC Advances* **4**, 43865--43873, 2014.
- Iman, M., **Manhar, A.K**., Mandal, M. Maji, T.K. Preparation and characterization of zinc oxide and nanoclay reinforced crosslinked starch/jute green nanocomposites, *RSC Advances* **4**, 33826--33839, 2014.
- Purkayastha, M.D., Das, S., **Manhar, A.K.**, Deka, D., Mandal, M., Mahanta, C.L. Removing antinutrients from rapeseed press-cake and their benevolent role in waste cooking oil-derived biodiesel: conjoining the valorization of two disparate industrial wastes, *J. Agric. Food Chem.* **61**, 10746--10756, 2013.

Poster presented in National and International conferences

1. **Manhar, A. K** and Mandal M. Microbial and biochemical characteristics of traditional fermented vegetables (Gundruk) from Tezpur. Poster presentation in national seminar on Biofoods 2011 organized by Tezpur University

2. **Manhar, A. K** and Mandal, M. Microbial and Nutraceuticals characterization of a traditional fermented food (Kharoli) from Assam**".** International conference on molecular medicine 2012 organized by VIT University.

3. **Manhar, A. K** and Mandal, M. In vitro evaluation of celluloytic *Bacillus* sp. isolated from traditional fermented soybean (Churpi) for Silage as Probiotic for Ruminants. Poster presentation in 2nd PAi conference and International Symposium 'Probiotics and Microbiome: Gut and Beyond' 2014 organized by Probiotic Association of India .

4. **Manhar, A.K** , Roy, R., Deka, B., Saikia, D., Namsa, N. D., Mandal, M. Molecular identification of indigenous probiotic isolates *Lactobacillus plantarum* AMD6 and *Saccharomyces cerevisiae* ARDMC1 and their cholesterol lowering activity in vitro. Poster presentation in National Seminar cum Workshop on Innovative Prospects in Food Processing IPFP 2015 organized by Tezpur University.

Research in Veterinary Science 99 (2015) 149–156

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

In vitro evaluation of celluloytic *Bacillus amyloliquefaciens* AMS1 isolated from traditional fermented soybean (Churpi) as an animal probiotic

Ajay K. Manhar, Devabrata Saikia, Yasir Bashir, Reba K. Mech, Dhrubajyoti Nath, Bolin K. Konwar, Manabendra Mandal *

Department of Molecular Biology and Biotechnology, Tezpur University, Tezpur 784028, Assam, India

ARTICLE INFO

Article history:
Received 29 May 2014
Accepted 16 January 2015

Keywords:
Probiotic
Bacillus amyloliquefaciens
Soybean
Cellulolytic
Animal feed

ABSTRACT

A microorganism showing probiotic attributes and hydrolyzing carboxymethylcellulose was isolated from traditional fermented soybean (Churpi) and identified as *Bacillus amyloliquefaciens* by analysis of 16S rRNA gene sequence and named as *B. amyloliquefaciens* AMS1. The potentiality of this isolate as probiotic was investigated *in vitro* and it showed gastrointestinal transit tolerance, cell surface hydrophobicity, cell aggregation and antimicrobial activity. The isolate was found to be non-hemolytic which further strengthens its candidature as a potential probiotic. The maize straw digestion was confirmed by scanning electron microscopy studies. The isolate was able to degrade filter paper within 96 hours of incubation. This study explores the possibility of combining the cellulase degrading ability of a microbe with its probiotic attributes to enhance gut health of animal and digestibility of the feed.

1. Introduction

Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host (FAO, 2001). Probiotics exert beneficial effects on the host by providing nutrients and enzymatic contribution to digestion, improving water quality, enhancing growth, inhibiting pathogenic microorganisms and enhancing immune responses (Balcazar et al., 2006; Verschuere et al., 2000). The supplementation of Lactic Acid Bacteria (LAB) as probiotics in ruminants has been extensively studied as compared with *Bacillus* probiotics (Brashears et al., 2003; Maragkoudakis et al., 2010; Peterson et al., 2007; Tabe et al., 2008; Younts-Dahl et al., 2004). However there are very few reports on cellulolytic activities of LAB. Since *Bacillus* is spore forming, it has an advantage over other non-spore formers such as *Lactobacillus* spp., of surviving the low pH of the stomach. *Bacillus amyloliquefaciens* is a potential probiotic strain (Das et al., 2013; Ji et al., 2013). *B. amyloliquefaciens* have also been found to have cellulolytic activity (Lee et al., 2008). The enzymes produced by *B. amyloliquefaciens* are expected to be able to transform complex molecules particularly lignocelluloses, which become the limiting factor in animal feed, into simpler molecules (Wizna et al., 2009). There is a demand for enhanced digestibility of animal feeds and it needs efficient cellulolytic microbes. It has been found that only a small subset of rumen microorganisms that include cellulolytic bacteria, fungi and protozoa, have the capacity to initiate degradation of plant cell walls. However, the most numerous group of rumen microorganisms are non-cellulolytic bacteria, and only actively cellulolytic rumen species have been found to cause extensive solubilization of plant cell wall material in pure culture (Morris and van Gylswyk, 1980). Cellulolytic bacteria play an important role in energy supply for forage animals. According to Varga and Kolver (1997), feed fibres were not completely converted to animal product in intensive animal farming and 20–70% undigested cellulose was carried out with feces. Keeping this in view, there is a possibility of combining the probiotic attributes of a *Bacillus* strain and its cellulose degrading capability to enhance the digestibility of animal feed and the productivity of animals.

This study discusses the potential probiotic attributes of a novel cellulolytic *B. amyloliquefaciens* strain. In the present study fermented soybean (Churpi) was collected from Bomdila, Arunachal Pradesh, India. Churpi is a fermented product that is prepared from local varieties of soybean seeds by *Monpa* tribe of Arunachal Pradesh, India. The topography is mostly mountainous and covered with the Himalayas which favor different habitats and microflora of beneficial use. In recent times, extensive research has been carried out on isolation and screening of microbes from traditional fermented foods due to their eco-friendly and genetically sturdy nature (Iyer et al., 2013). In addition, soybean meal is an important protein source in animal feed (Opazo et al., 2012).

* Corresponding author. Department of Molecular Biology and Biotechnology, Tezpur University, Tezpur-784028, Assam, India. Tel.: +91 986 4181445; fax: +91 03712 267005.
E-mail address: mandal@tezu.ernet.in, manavigib@yahoo.co.in (M. Mandal).

http://dx.doi.org/10.1016/j.rvsc.2015.01.008
0034-5288/

Ann Microbiol
DOI 10.1007/s13213-016-1210-x

ORIGINAL ARTICLE

Assessment of goat milk-derived potential probiotic *L. lactis* AMD17 and its application for preparation of dahi using honey

Ajay Kumar Manhar[1] · Devabrata Saikia[1] · Anjan Borah[2] · Anindhya S. Das[1] · Kuldeep Gupta[1] · Raju Roy[1] · Charu Lata Mahanta[2] · Rupak Mukhopadhyay[1] · Manabendra Mandal[1]

Received: 7 September 2015 / Accepted: 4 March 2016

Abstract *Lactococcus lactis* AMD17 isolated from free range goat milk was screened for potential probiotic attributes based on functional traits such as resistance to simulated gastric acid and bile salts, antimicrobial activity and inhibition of pathogen adhesion to intestinal epithelium cell line Caco-2. The isolate significantly reduced the adherence of foodborne pathogen *Listeria monocytogenes* AMDK2 (47.46 ± 0.17 %) to Caco-2 cells. Honey was used as an adjuvant of *L. lactis* AMD17 for preparation of dahi (curd) from buffalo milk and was found to support its survivability during storage ($P < 0.05$). Sensory evaluation studies revealed that dahi prepared with *L. lactis* AMD17 and the addition of 3 % honey exhibited the highest score in taste and color. The texture characteristics were found to be superior to dahi prepared with only *L. lactis* AMD17. Moreover, the Nisin gene was amplified and showed a similarity of 100 % to other NisR-producing *L. lactis* strains. The present study suggests that dahi prepared using honey-enriched milk with nisin-producing probiotic strain *L. lactis* AMD17 imparts health benefits and combats foodborne pathogens, possibly due to the antibacterial features of nisin peptide.

Keywords Goat milk · Probiotic · Sensory evaluation · Caco-2 · Nisin · Dahi

Introduction

Fermented foods play a significant role in diets since they contain enormous quantities of nutritious constituents with a wide diversity of aromas, flavors and textures (Shah and Prajapati 2014). Dahi is a popular fermented dairy product of South Asia. It has an appearance similar to that of yoghurt and plays an important role in the Indian diet. About 9 % of the total milk produced in India is converted into fermented milk products (Singh 2007). For dahi production, a small portion of previously fermented product containing live culture is added to lukewarm milk as a starter culture. However, production of dahi with an individual culture of *Lactococcus lactis* (Yadav et al. 2006) or a combination of cultures containing lactobacilli and lactococci (Yadav et al. 2007) have been reported. Strains belonging to *Lactococcus* species have been well documented in the dairy industry for contributing typical taste and flavor to a variety of fermented dairy products (Whetstine et al. 2006). Unlike yoghurt fermentation, which is carried out by a specific mixed culture of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, the starter culture of dahi is not well defined due to numerous species and strains of Lactic acid bacteria (LAB) also present in various traditional fermented milk products consumed by different ethnic communities of India.

Nisin, a natural antimicrobial peptide, is a broad-spectrum bacteriocin. It inhibits Gram-positive bacteria such as *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Lactobacillus plantarum*, *Micrococcus flavus*, and *Micrococcus luteus* (Tong et al. 2014). Nisin was permitted as a safe food additive in over 50 countries around

Electronic supplementary material The online version of this article (doi:10.1007/s13213-016-1210-x) contains supplementary material, which is available to authorized users.

✉ Manabendra Mandal
mandal@tezu.ernet.in

[1] Department of Molecular Biology and Biotechnology, Tezpur University, Tezpur 784028, Assam, India

[2] Department of Food Engineering and Technology, Tezpur University, Tezpur 784028, Assam, India

Published online: 16 March 2016

Microbiological Research 186 (2016) 62–70

Contents lists available at ScienceDirect

Microbiological Research

journal homepage: www.elsevier.com/locate/micres

Cellulolytic potential of probiotic *Bacillus Subtilis* AMS6 isolated from traditional fermented soybean (Churpi): An in-vitro study with regards to application as an animal feed additive

Ajay K. Manhar, Yasir Bashir, Devabrata Saikia, Dhrubajyoti Nath, Kuldeep Gupta, Bolin K. Konwar, Rahul Kumar, Nima D. Namsa, Manabendra Mandal*

Department of Molecular Biology and Biotechnology, Tezpur University, Tezpur 784028, Assam, India

ARTICLE INFO

Article history:
Received 9 December 2015
Received in revised form 23 February 2016
Accepted 22 March 2016
Available online 23 March 2016

Keywords:
Bacillus subtilis
Probiotics
Caco-2
Animal feed
Cellulolytic

ABSTRACT

The aim of the present study is to evaluate the probiotic attributes of *Bacillus subtilis* AMS6 isolated from fermented soybean (Churpi). This isolate exhibited tolerance to low pH (pH 2.0) and bile salt (0.3%), capability to autoaggregate and coaggregate. AMS6 also showed highest antibacterial activity against the pathogenic indicator strain *Salmonella enterica typhimurium* (MTCC 1252) and susceptibility towards different antibiotics tested. The isolate was effective in inhibiting the adherence of food borne pathogens to Caco-2 epithelial cell lines, and was also found to be non-hemolytic which further strengthen the candidature of the isolate as a potential probiotic. Further studies revealed *B. subtilis* AMS6 showed cellulolytic activity (0.54 ± 0.05 filter paper units mL^{-1}) at 37 °C. The isolate was found to hydrolyze carboxymethyl cellulose, filter paper and maize (*Zea mays*) straw. The maize straw digestion was confirmed by scanning electron microscopy studies. The isolate was able to degrade filter paper within 96 h of incubation. A full length cellulase gene of AMS6 was amplified using degenerate primers consisting of 1499 nucleotides. The ORF encoded for a protein of 499 amino acids residues with a predicted molecular mass of 55.04 kDa. The amino acids sequence consisted of a glycosyl hydrolase family 5 domain at N-terminal; Glycosyl hydrolase catalytic core and a CBM-3 cellulose binding domain at its C terminal. The study suggests potential probiotic *B. subtilis* AMS6 as a promising candidate envisaging its application as an animal feed additive for enhanced fiber digestion and gut health of animal.

1. Introduction

Probiotics are live microbes, which when administered in adequate amounts confer a health benefit to the host (Araya et al., 2002). The main probiotics include lactic acid bacteria such as *Lactobacillus, Bifidobacterium and Enterococcus*, which are inherent members in the gastrointestinal tract of humans and animals (Guo et al., 2015). To ensure proper functionality and promote health benefits by probiotics, the organisms must resist the harsh environment such as low pH and bile toxicity prevalent in the upper digestive tract (Kaushik et al., 2009). In addition, they should possess good surface hydrophobicity and aggregation properties for colonization in gut (Del Re et al., 2000). Lactic acid bacteria have been extensively studied as a potential probiotic for ruminants as compared with *Bacillus* probiotics (Brashears et al., 2003; Maragkoudakis et al., 2010; Peterson et al., 2007; Tabe et al., 2008; Younts-Dahl et al., 2004). However, *Bacillus* sp. also has potential probiotic as well as other attributes (Ji et al., 2013). Spore forming characteristics of *Bacillus* sp. has an advantage over other non-spore formers such as *Lactobacillus* sp. to withstand harsh environment such as low pH (Cutting, 2011) and high temperature. So there is a need to study *Bacillus* probiotics in order to explore the untapped potential they harbor. Further, there are few reports on the cellulolytic nature of the *Bacillus* probiotics. The usage of probiotics as animal feed additives demands these attributes and thus envisages a tremendous scope. Cellulase converts the highly recalcitrant cellulose to fermentable mono- and oligo-saccharides that can be easily assimilated in the body, thus improving utilization of dietary carbohydrate and enhancing digestion. The byproducts formed after action of enzymes is utilized as a prebiotic source by probiotics and thus enhancing digestion of dietary feed rich in cellulose. The most considerable effects of probiotics have been reported after incorporation of live beneficial microbes in the animal feed during stressful periods for the gut microbiota and the animal: at wean-

* Corresponding author.
E-mail addresses: mandal@tezu.ernet.in, manavigib@yahoo.co.in (M. Mandal).

http://dx.doi.org/10.1016/j.micres.2016.03.004
0944-5013/

Appendix

1. MRS media

Ingredients	**Concentration (gl^{-1})**
Proteose peptone	10
Beef extract	10
Yeast extract	5
Dextrose	20
Polysorbate 80	1
Ammonium citrate	2
Sodium acetate	5
Magnesium sulphate	0.1
Manganese sulphate	0.05
Dipotassium phosphate	2
Agar	12
Final pH (at 25°C)	6.5±0.2

2. Luria bertani broth

Ingredients	**Concentration (gl^{-1})**
Casein enzymic hydrolysate	10
Yeast extract	5
Sodium chloride	10
Final pH (at 25°C)	7.5±0.2

3. Minimal salt medium (MSM)

Ingredients	**Concentration (gl^{-1})**
Ammonium sulphate	2
Potassium dihydrogen phosphate	4.75
Magnesium sulphate	0.8
Calcium chloride	0.00005
Manganese sulphate	0.0001
Boric acid	0.00001
Zinc sulphate	0.00007
Ferrous sulphate	0.001
Copper sulphate	0.0001
Molybdenum trioxide	0.00005

4. Listeria Oxford Medium Base

Ingredients	**Concentration (gl^{-1})**
Peptone, special	23
Lithium chloride	15
Sodium chloride	5
Corn starch	1

Esculin	1
Ammonium ferric citrate	0.5
Agar	10.000
Final pH	7.0±0.2
Oxford Listeria Supplement (Added separately)	1 vial

5. Bismuth Sulphite Agar Modified

Ingredients	**Concentration (gl^{-1})**
Peptic digest of animal tissue	5
Beef extract	5
Dextrose	5
Disodium phosphate	4
Ferrous sulphate	0.3
Bismuth sulphite indicator	8
Brilliant green	0.016
Agar	12.7
Final pH (at 25°C)	7.6±0.2

6. Nutrient agar

Ingredients	**Concentration (gl^{-1})**
Peptic digest of animal tissue	5
Sodium chloride	5
Beef extract	1.5
Yeast extract	1.5
Agar	15

7. DNS regent:

Ingradients	**Concentration (g/100ml)**
3, 5 dinitrosallicylic acid	1
Sodium potassium tartarate	30

Reagent was prepared by dissolving 1g of 3,5 dinitrosallicylic acid in 20mL 2M NaOH, then slowly 30g sodium potassium tartrate was added and diluted to a final volume of 100 ml using distilled water.

8. PBS buffer (1X)

Ingredients	**Concentration (gl^{-1})**
Sodium chloride	8
Potassium choride	0.2
Disodium hydrogen phosphate	1.44
Potassium dihydrogern phosphate	0.24
pH	7.4

9. Lugol's iodine solution

Iodine crystals	1g
Potassium iodide	2g
Distilled water	300 ml

10. Minimal essential medium (MEM)

Ingredients	**Concentration mgl^{-1}**
Inorganic salts	
Calcium chloride dihydrate	265
Magnesium sulphate anhydrous	97.72
Potassium chloride	400
Sodium bicarbonate	2200
Sodium chloride	6800
Sodium dihydrogen phosphate anhydrous	122
Amino acids	
L-Arginine hydrochloride	126
L-Cystine dihydrochloride	31.3
L-Histidine hydrochloride monohydrate	42
L-Isoleucine	52
L-Leucine	52
L-Lysine hydrochloride	72.5
L-Methionine	15
L-Phenylalanine	32
L-Threonine	48
L-Tryptophan	10
L-Tyrosine disodium salt	51.9
L-Valine	46
Vitamins	
Choline chloride	1
D-Ca-Pantothenate	1
Folic acid	1
Nicotinamide	1
Pyridoxal hydrochloride	1

Riboflavin	0.1
Thiamine hydrochloride	1
i-Inositol	2
OTHERS	
D-Glucose	1000
Phenol red sodium salt	1
L-glutamine (added seperately)	73.07
pH	7.00 -7.60

100 U/mL penicillin and 100 g/mL streptomycin are added separately

11. Antibiotic discs: Hexa G- plus 6

Ampicillin	10 µg
Chloramphenicol	25 µg
Penicillin G	1 unit
Streptomycin	10 µg
Sulphatriad	300 µg
Tetracyclin	25 µg

12. CMCase Agar **1L**

Carboxymethylcellulose 10gm

Agar 17 gm

13. Primers used:

Name	**Sequence (5′-3′)**	**Specificity**
27 F	AGAGTTTGATCCTGGCTCAG	16s rRNA
1492R	GGTTACCTTGTTACGACTT	
nisRF	CTATGAAGTTGCGACGCATCA	Nisin gene
nisRR	CATGCCACTGATACCCAAGT	
CF	ACAGGATCCGATGAAACGGTCAATTTCTATTTT	Cellulase
CR	ACTCTCGAGATTGGGTTCTGTTCCCAAAT	
F	AGAAGAGGACAGTGGAAC	16 rRNA LAB genus specific
R	TTACAAACTCTCATGGTGTG	
BSHF	CGGCTGGATCCGATGTGCACTAGTCTAAC	Bile Salt hydrolase
BSHR	ATACTCGAGATGGGCCGCTGGCAAGGTG	

14. Preliminary Screening for probiotic attributes of isolated strains from goat milk of Tezpur, Assam, India

Table: Acid and bile tolerance of isolated strains grown in MRS medium

Strains	pH 2.0	0.3 % Oxgall
AMD1	+	–
AMD2	–	–
AMD3	+	–
AMD4	–	–
AMD5	–	–
AMD6	+	+
AMD7	–	–
AMD8	+	–
AMD9	+	–
AMD10	+	–
AMD11	–	+
AMD12	++	–
AMD13	+	–
AMD14	–	–
AMD15	+	–
AMD16	–	–
AMD17	**+++**	**+++**
AMD18	–	+
AMD19	+	–
AMD20	–	–
AMD21	++	–
AMD22	–	–
AMD23	+	–
AMD24	–	–
AMD25	–	+
AMD26	+	–
AMD27	–	–
AMD28	–	–
AMD29	++	–
AMD30	–	–
AMD31	–	+
AMD32	+	–
AMD33	–	–
AMD34	–	–
AMD35	–	–
AMD36	++	–
AMD37	–	–

AMD38	–	–
AMD39	–	–
AMD40	–	–
AMD41	+	++
AMD42	–	–
AMD43	–	–
AMD44	–	–
AMD45	++	++
AMD46	–	–
AMD47	–	–
AMD48	–	–
AMD49	–	–
AMD50	++	–
AMD51	–	–
AMD52	–	++
AMD53	–	–
AMD54	++	–
AMD55	–	–
AMD56	–	–
AMD57	–	–
AMD58	–	–
AMD59	+	++
AMD60	—	–
AMD61	–	–
AMD62	–	–
AMD63	+	–
AMD64	–	–
AMD65	–	–
AMD66	–	–
AMD67	–	++
AMD68	–	–
AMD69	–	–
AMD70	–	–
AMD71	+	–
AMD72	–	–
AMD73	–	–
AMD74	–	–
AMD75	–	+
AMD76	–	–
AMD77	–	–
AMD78	–	–
AMD79	–	–
AMD80	–	+

AMD81	–	–
AMD82	+	–
AMD83	–	–
AMD84	–	–
AMD85	–	–
AMD86	–	–
AMD87	–	–
AMD88	–	–
AMD89	–	++
AMD90	–	–
AMD91	–	–
AMD92	–	–
AMD93	–	–
AMD94	–	–
AMD95	–	–
AMD96	+	–
AMD97	–	–
AMD98	–	+
AMD99	–	–
AMD100	–	–
AMD101	–	–
AMD102	–	–
AMD103	++	–
AMD104	–	+
AMD105	–	–
AMD106	++	–
AMD107	–	–
AMD108	–	++
AMD109	–	–
AMD110	++	–
AMD111	–	–
AMD112	–	–
AMD113	–	–
AMD114	–	++

Symbols; '–': No growth; '+' :Viability <50%; '++': Viability >50%; '+++': Viability>75%

15. Preliminary Screening for probiotic attributes of isolated strains from fermented milk (Doi) of Dibrugarh, Assam, India

Table: Table: Acid and bile tolerance of isolated strains grown in MRS medium

Strains	pH 2.0	0.3 % Oxgall
AD1	–	–
AD2	+	+
AD3	–	++
AD4	+	–
AD5	–	+
AD6	**+ + +**	**+ + +**
AD7	–	+
AD8	–	–
AD9	+	–
AD10	–	+
AD11	–	–
AD12	–	+
AD13	+	++
AD1	–	–
AD14	++	++
AD15	–	++
AD16	–	–
AD17	–	+
AD18	+	+
AD19	–	++
AD20	+	+
AD21	–	–
AD22	–	–
AD23	–	+
AD24	–	+
AD25	–	+
AD26	–	+

Symbols; '–': No growth; '+' :Viability <50%; '++': Viability >50%; '+++': Viability>75%

16. Preliminary Screening for probiotic attributes of isolated strains from traditional fermented soybean (Churpi, prepared from local varieties of brown soybean seeds), Arunachal Pradesh, India.

Table: Acid and bile tolerance of isolated strains grown in Luria Bertani medium

Strains	pH 2.0	0.3 % Oxgall
AMS1	**+++**	**+++**
AMS2	+	++
AMS3	–	+
AMS4	–	–
AMS5	–	+
AMS6	++	–
AMS7	–	–
AMS8	+	+
AMS9	–	+
AMS10	–	–

AMS11	+	++
AMS12	–	–
AMS13	+	+
AMS14	–	–
AMS15	–	–
AMS16	++	++
AMS17	–	–
AMS18	–	–
AMS19	–	–
AMS20	–	–
AMS21	+	+
AMS22	–	–
AMS23	–	–
AMS24	+	++
AMS25	–	–
AMS26	+	++
AMS27	–	–
AMS28	++	+
AMS29	–	–
AMS30	–	–
AMS31	++	++
AMS32	–	–
AMS33	–	–
AMS34	–	+
AMS35	–	–
AMS36	–	–
AMS37	++	+
AMS38	–	–
AMS39	–	–
AMS40	–	–
AMS41	–	–
AMS42	+	–
AMS43	+	–
AMS44	–	+
AMS45	–	+
AMS46	–	–
AMS47	–	–
AMS48	–	–
AMS49	–	+
AMS50	–	–
AMS51	–	–
AMS52	+	–

Symbols; '–': No growth; '+' :Viability <50%; '++': Viability >50%; '+++': Viability>75%

17. Preliminary Screening for probiotic attributes of isolated strains from traditional fermented soybean (Churpi, prepared from local varieties of yellow soybean seeds), Arunachal Pradesh, India.

Table: Acid and bile tolerance of isolated strains grown in Luria Bertani medium

Strains	pH 2.0	0.3 % Oxgall
AS1	–	–
AS2	+	–
AS3	–	+
AS4	–	–
AS5	–	+
AS6	**+++**	**+++**
AS7	–	–
AS8	–	–
AS9	+	++
AS10	–	–
AS11	+	–
AS12	–	–
AS13	–	–
AS14	+	++
AS15	+	–
AS16	–	–
AS17	–	++
AS18	–	–
AS19	+	–
AS20	–	–
AS21	–	++
AS22	+	–
AS23	–	–
AS24	–	+
AS25	+	–
AS26	–	–
AS27	–	+
AS28	+	–
AS29	++	+
AS30	++	–
AS31	+	–
AS32	–	+
AS33	++	–
AS34	–	–
AS35	+	–
AS36	–	+
AS37	+	–

AS38	+	–
AS39	++	+
AS40	+	–
AS41	–	–
AS42	–	+
AS43	–	–
AS44	–	–
AS45	–	++
AS46	–	–
AS47	+	–
AS48	–	+
AS49	–	–
AS50	–	+
AS51	–	–
AS52	–	–
AS53	++	–
AS54	–	–
AS55	–	+
AS56	–	–
AS57	–	–
AS58	+	–
AS59	–	+
AS60	–	–
AS61	++	–
AS62	–	++
AS63	+	–
AS64	–	–

Symbols; '–': No growth; '+' :Viability <50%; '++': Viability >50%; '+++': Viability>75%