academicJournals

Vol. 11(14), pp. 553-559, 14 April, 2017
DOI: 10.5897/AJMR2017.8469
Article Number: 1737F0463729
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Probiotic potential *Streptomyces* species from the grains of pearl millet (*Pennisetum glaucum*)

Rajyalakshmi Kunchala, Priyanka Durgalla, Roopa Banerjee, Saikat Datta Mazumdar, Vadlamudi Srinivas and Subramaniam Gopalakrishnan*

International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502 324, Telangana, India.

Received 3 February, 2017; Accepted 27 March, 2017

Actinomycetes have been demonstrated for plant growth-promotion, antagonistic against plant pathogens and insect pests and biofortification traits in many agriculturally important crops. The present investigation was conducted to characterize probiotic properties of actinomycete(s) isolated from pearl millet flour and batter samples. A media selective and specific were used for isolation, actinomycetes isolation agar (AIA), and the most prominent actinomycete (found abundantly in the AIA plate) was isolated and maintained on AIA slants at 4°C for further investigation. The most prominent actinomycete was characterized for traits including Gram staining, morphology (such as color, margin, size, shape, elevation, form and surface), biochemistry (such as urease, catalase, oxidase, hydrogen sulphide, nitrogen reduction, gelatin liquefaction, starch hydrolysis and carbohydrate utilization), IMViC tests (such as indole, methyl red, Voges Proskauer and citrate utilization), probiotic potentials (such as acid [pH 2, 3], bile [0.5%], NaCl [6 and 9%], phenol tolerance [0.4%]), antibiotic tolerance (such as tetracycline, streptomycin, kanamycin, chloramphenicol, ciprofloxacin, ampicillin, penicillin, erythromycin and vancomycin) and antimicrobial activities against human pathogens (such as *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi*). It was possible to isolate only one probiotic actinomycete based on the properties. The sequences of 16s rDNA gene of the actinomycete was matched with *Streptomyces* species in BLAST analysis. The sequences of the *Streptomyces* spp. were submitted to NCBI and accession number obtained. This study indicated that the selected *Streptomyces* spp. could be used to develop new probiotic foods.

Key words: Probiotics, pearl millet, *Streptomyces* species, product development.

INTRODUCTION

Nutritional quality of food is important not only for maintaining human health but also for physical well-being and this is attained mainly by eating cereals. Wheat and rice are the important staple food for people across the world that lead not only to an array of emerging life style diseases but also challenging nutrition and human health. Hence, there is an urgent need for recommending diversity in diets through inclusion of other

*Corresponding author. E-mail: s.gopalakrishnan@cgiar.org. Tel: +91 40 30713610. Fax: +9140- 30713074.

cereals such as millets in order to enhance the nutritional status and address malnutrition across the world. Millets are not consumed widely as a staple, except by people inhabiting the semi-arid tropics in Asia and Africa, though these are widely used as animal or bird feed. Pearl millet (*Pennisetum glaucum*) is one of the important foods for people living in Asia and Africa, being a major source of calories and a vital component of food security (Amadou et al., 2013). Pearl millet is well-adapted to cultivation systems characterized by high temperature, low rainfall (below 600 mm), low soil fertility and resistance to pests and diseases and thus is suited to the semi-arid tropics. It is often ground into flour and consumed as porridge, roti and beverages (Obilana and Manyasa, 2002; Amadou et al., 2011). It also serves as the source of prebiotics (such as total oligosaccharides, resistant starch, total dietary fiber and β-glucan) for functional food (Awika and Rooney, 2004; Ige, 2013). Any food or food ingredients in association with probiotic microbes either influence beneficial effect on the host or reduce the risk of chronic diseases are referred as functional foods (Huggett and Schliter, 1996; Charalampopoulos et al., 2002; Hassan et al., 2014). Probiotic foods normally contain a single or mixture of probiotic microorganisms that improve the health of the host by improving intestinal microbial balance (Fuller, 1989). Probiotic microbes are being used for preparation of dairy food for thousands of years. Recently, non-dairy based probiotic drinks utilizing pearl millet are also reported (Syal and Vohra, 2014; Mridula and Sharma, 2015). Probiotic microbes associated with cereals such as wheat, rice and sorghum are reported widely but not much information is available for pearl millet cultivars (Badau, 2006).

Actinomycetes are a group of Gram-positive bacteria, with high G + C content belonging to the order Actinomycetales, found commonly in compost and rhizospheric soil. They play an important role, not only on plant growth-promotion (PGP), antagonistic action against pathogens and insect pests and biofortification traits in many agriculturally important crops, but also on decomposition of organic materials and production of secondary metabolites (Glick, 2010). Plant growth-promoting properties of actinomycetes was reported on cereals (such as wheat (Sadeghi et al., 2012), rice (Gopalakrishnan et al., 2014) and sorghum (Gopalakrishnan et al., 2013)) as well as legumes (such as bean (Nassar et al., 2003), chickpea (Gopalakrishnan et al., 2015, 2016a), pigeonpea (Gopalakrishnan et al., 2016b) and pea (Tokala et al., 2002). Actinomycetes have also been demonstrated for biocontrol of soil-borne pathogens (Mahadevan and Crawford, 1997; Trejo-Estrada et al., 1998; Macagnan et al., 2008; Gopalakrishnan et al., 2011) and insect pests (Gopalakrishnan et al., 2016c; Sathya et al., 2016 a, b). However, not much information on the usefulness of actinomycetes on prebiotics is available. Therefore, in the present investigation, an effort has been made to characterize probiotic properties of actinomycete(s) from flour and batter samples of pearl millet cultivars.

MATERIALS AND METHODS

Reference bacteria used

Reference human pathogens used in this study, *Salmonella typhi* (ATCC 14028), *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 25922), were acquired from ATCC, USA. Reference plant pathogens used in this study, *Fusarium oxysporum* f. sp. *ciceri* (FOC), *Rhizoctonia bataticola* (RB-6, RB-24 and RB-115), *Sclerotium rolfsii*, *Botrytis cinerea*, *Macrophomina phaseolina*, *Fusarium proliferatum* -242 and *Fusarium andiyazi* were acquired from Plant Pathology unit at ICRISAT-Patancheru, India.

Collection and preparation of pearl millet grain samples

A total of 2 pearl millet grain varieties including dual purpose hybrid (DPH) and high Fe hybrid were grown at ICRISAT-Patancheru and used for this study. The grain samples were dried at 30 to 32°C for 72 h and milled in Cyclotech^{TM}- Mill. The flour were sieved through 0.2 mm sieve and mixed (5 g) in sterilized water (5 ml) and further incubated at 28°C for 12 h. At the end of incubation, the batter samples were used for isolating the actinomycetes.

Isolation of actinomycete(s)

Ten grams of flour/batter sample was separately suspended in 90 ml of sterilized physiological saline (0.85% of NaCl in distilled water) in a flask and placed on an orbital shaker (at 120 rpm) for 45 min. At the end of shaking, the samples were serially diluted (up to 10^6 dilutions) with physiological saline. Dilutions 10^4 to 10^6 were spread plated (0.1 ml) on actinomycetes isolation agar and incubated at 28°C for 72 h. The most prominent actinomycete colonies, were found abundantly in the AIA plate, isolated and maintained on AIA slants at 4°C for further studies.

Morphological and biochemical properties of the actinomycetes

The actinomycete isolates were streaked on AIA plate and incubated at 28°C for 72 h. At the end of incubation, the colonies were observed for its morphological traits including form, surface, texture, color, elevation and margin. Gram staining of the isolates was done as per the protocols of Pelczar et al. (2008). The isolates were further characterized for their biochemical properties including hydrogen sulphide, urease, catalase, oxidase, nitrate reduction, gelatin liquefaction, starch hydrolysis and IMViC (Indole, Methyl red, Voges Proskauer and Simmons citrate) tests as per the methods of Holt (1984). Utilization of carbohydrates including lactose and sucrose were determined as per Forouhandeh et al. (2010).

Antibiotic resistance pattern of the isolates was conducted by disc diffusion method. The resistance or susceptibility to antibiotics of actinomycete isolates to ampicillin (10 µg), chloramphenicol (30 µg), ciprofloxacin (10 µg), erythromycin (15 µg), kanamycin (30 µg), penicillin (10 µg), streptomycin (10 µg), tetracycline (30 µg) and vancomycin (10 µg) (HiMedia, Mumbai, India) were determined as per the guidelines of Clinical and Laboratory Standards Institute (Wilker, 2006). In brief, antibiotic discs were placed on the AIA plates immediately after swab with actively grown actinomycete isolates. The plates were incubated at 28°C for 72 h. At the end of incubation, zone of inhibition was measured.

Antimicrobial activity of the actinomycetes

The antimicrobial activity of the actinomycete isolates against human pathogens such as *E. coli*, *S. aureus* and *S. typhi*, was done by the ditch assay method (Aswathy et al., 2008) with slight modifications. In brief, the isolates were grown in Muller-Hinton broth at 28°C for 72 h. At the end of incubation, the culture filtrates were collected by centrifuging at 10,000 *g* for 20 min, concentrated on a rotary evaporator until one fifth of the original volume and filter-sterilized through 0.2 µm membrane filter. The pathogens grown separately on nutrient broth at 37°C for 24 h were amended with sterilized nutrient agar (2%) at 45°C and poured on Petri plates. Upon solidification, a ditch (6 mm) was cut in the Petri plate and filled with filter sterilized culture filtrate of actinomycete isolates (0.2 ml). The plates were initially placed at 4C for 1 h, for diffusion of metabolites present in the culture filtrate, and further incubated at 37°C for 18 h. At the end of incubation, zone of inhibition was measured.

Probiotic traits of the actinomycetes

The isolates were characterized for their probiotic traits including tolerance to acid (low pH), bile salt, phenol and NaCl. Acid tolerance was done, for identifying the actinomycetes which could tolerate simulated gut acidic conditions, as per the protocols of Liu et al. (2007) and Kunchala et al. (2016). Bile tolerance test was done as per the methods of Aswathy et al. (2008) and Kunchala et al. (2016) by checking the growth of actinomycetes in brain heart infusion (BHI) broth amended with various concentrations (0.3, 0.5 and 0.8%) of bile salt. NaCl tolerance was done as per the methods of Graciela and Maria (2001) and Kunchala et al. (2016) in Muller-Hinton broth (MHB) adjusted with different concentration of NaCl (3, 6, 9, and 12%). The phenol tolerance of the actinomycete isolates was assessed using the protocols of Teply (1984) and Kunchala et al. (2016) in MHB containing 0.2 and 0.4% of phenol.

Plant growth-promoting (PGP) and biocontrol traits of the actinomycetes

The actinomycete isolates were evaluated for their PGP and biocontrol traits including indole acetic acid (IAA), siderophore, lipase, cellulase, chitinase and hydrocyanic acid (HCN) production. Estimation of IAA and siderophore productions was done as per the protocols of Patten and Glick (2002) and Schwyn and Neilands (1987), respectively. The lipase and cellulase productions were estimated as per the standard protocols of Bhattacharya et al. (2009) and Hendricks et al. (1995), respectively. Chitinase production was estimated by amending agar plates with colloidal chitin suspension and mineral salts according to the protocols of Hsu and Lockwood (1975). HCN was qualitatively assessed by the method described by Lorck (1948). The rating scales for lipase, cellulase and chitinase were as follows: 0 = no halo zone; 1 = halo zone of 1-10 mm; 2 = halo zone of 11-20 mm; 3 = halo zone of 21-30 mm; 4 = halo zone of 31-40 mm; and 5 = 41-50 mm. The following rating scale was used for HCN production: 0 = no color change, 1 = light reddish brown, 2 = medium reddish brown, and 3 = dark reddish brown.

The actinomycete isolates were also screened for their antagonistic potential against selected plant pathogens of chickpea (including *R. bataticola* [three strains, viz., RB-6, RB-24 and RB-115], *S. rolfsii*, *B. cinerea* and *F. oxysporum* f. sp. *ciceri* [FOC]) and sorghum (*M. phaseolina*, *F. proliferatum*-242 and *F. andiyazi*) by dual culture assay (Gopalakrishnan et al., 2011) and the zone of inhibition measured.

Molecular identification of the actinomycetes

Pure cultures of probiotic potential actinomycete isolates were grown in starch casein broth until log phase and genomic DNA isolated as per the standardized protocols of Bazzicalupo and Fani (1995). The amplification of 16S rDNA gene was done using universal bacterial primer 1492R (5'-TACGGYTACCTTGTTACGACTT-3') and 27F (5'-AGAGTTTGATCMTGGCTC AG-3') (Pandey et al., 2005). The PCR product was sequenced at Macrogen Inc., Seoul, South Korea. The sequences were compared with those from GenBank using the BLAST program (Alschul et al., 1990), aligned using the ClustalW software (Thompson et al., 1997) and phylogenetic trees inferred using the neighbor-joining method (Saitou and Nei, 1987). The sequences of potential probiotic actinomycete were submitted to NCBI and the GenBank accession number was obtained.

RESULTS

Isolation, morphological and biochemical characterization of the actinomycete

The most prominent isolate, PDPF-21 (which was found abundantly in the actinomycetes isolation agar (AIA); selective and specific media for isolation of actinomycete), was isolated from the fermented batter samples of pearl millet and maintained on AIA slants at 4°C for further studies. PDPF-21 was found to be circular in form, punctiform in size, rough in surface, dry in texture, white in color, umbonate in elevation and entire in margin in the morphological studies. It was found to be Gram positive in the Gram staining test. When characterized for biochemical traits, it was found positive for urease, catalase, Vogues Proskauer and sucrose utilization tests but negative for hydrogen sulfide, oxidase, nitrate reduction, gelatin liquefaction, starch hydrolysis, indole, methyl red, citrate utilization and lactose utilization tests (Table 1).

Antibiotic resistance pattern and antimicrobial activity of the actinomycete PDPF-21

The selected actinomycete PDPF-21 was found resistant to tetracycline (at 30 µg), streptomycin (at 10 µg), kanamycin (at 30 µg), chloramphenicol (at 30 µg), ciprofloxacin (at 10 µg), ampicillin (at 10 µg), penicillin (at 10 µg), erythromycin (at 15 µg) and vancomycin (at 10 µg). PDPF-21 was also found to have antagonistic properties against human pathogens including *E. coli*, *S. aureus* and *S. typhi*, as it significantly inhibited all three pathogens (more than 10 mm inhibition zone), of which the strongest antagonistic activity was noted against *E. coli* (Table 2).

Probiotic traits and molecular identification of the actinomycete PDPF-21

When PDPF-21 was tested for its probiotic properties, it

Table 1. Morphological and biochemical characterizations of the actinomycete isolate PDPF-21.

Morphological traits		Biochemical traits		Carbohydrate utilization traits	
Form	Circular	Hydrogen sulfide test	-	Lactose test	-
Size	Punctiform	Urease test	+	Lactose gas production test	-
Surface	Rough	Catalase test	+	Sucrose test	+
Texture	Dry	Oxidase test	-	Sucrose gas production test	=
Color	White	Nitrate reduction test	-		
Elevation	Umbonate	Gelatin liquefaction test	-		
Margin	Entire	Starch hydrolysis test	-		
Gram staining	+	Indole test	-	-	
		Methyl red test	-		
-		Vogues Proskauer test	+		
		Citrate utilization test	-		

Table 2. Antibiotic resistance pattern and antimicrobial activity of the actinomycete isolate PDPF-21.

Antibiotic resistance pattern (zone of inhibition in mm)		Antimicrobial activity (zone of inhibition in mm)	
Tetracycline (30 µg)	34	*Escherichia coli*	18
Streptomycin (10 µg)	30	*Staphylococcus aureus*	11
Kanamycin (30 µg)	31	*Salmonella typhi*	14
Chloramphenicol (30 µg)	31		
Ciprofloxacin (10 µg)	32		
Ampicillin (10 µg)	14	-	
Penicillin (10 µg)	17		
Erythromycin (15 µg)	22		
Vancomycin (10 µg)	29		

Table 3. Probiotic properties, identity and NCBI accession number of the actinomycete isolate PDPF-21.

Probiotic properties		Identification by 16S rDNA analysis	NCBI accession number
Acid tolerance (pH)	2	*Streptomyces* spp.	KP326565
Bile tolerance (%)	0.5		
Phenol tolerance (%)	0		
NaCl tolerance (%)	6		

was found to tolerate acidic pH (pH 2), bile (0.5%) and NaCl (6%) but not phenol (Table 3 and Figure 1). A neighbor-joining dendrogram was prepared using the sequences of PDPF-21 (1400 bp) and representative sequences from the databases. Phylogenetic analysis of sequences of the PDPF-21 matched with *Streptomyces* species (Table 3 and Figure 1).

Plant growth-promoting (PGP) and biocontrol properties of PDPF-21

The actinomycete PDPF-21 produced PGP and biocontrol traits including IAA, siderophore, lipase, cellulase, chitinase and HCN. In the dual culture assay, PDPF-21 inhibited plant pathogens of chickpea including three strains of *R. bataticola* (RB-6, RB-24 and RB-115), *S. rolfsii*, *B. cinerea* and FOC and in sorghum, *M. phaseolina*, *F. proliferatum*-242 and *F. andiyazi*-943 (Table 4).

DISCUSSION

Probiotic foods help the existing microflora to stabilize or repopulate the microflora in the colon, lost due to disease, antibiotics and/or chemotherapy. Pearl millet is one of the important staple food for millions of poor

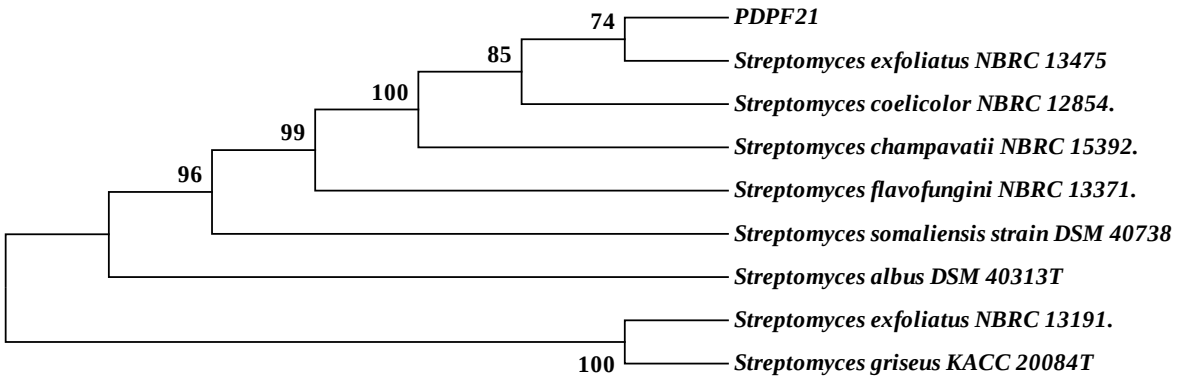

Figure 1. Phylogenetic relationship between PDPF-21 and representative species based on full length 16S rDNA sequences constructed using the neighbor-joining method.

Table 4. Plant growth-promoting (PGP) and biocontrol traits and antagonistic (against important pathogens) properties of the actinomycete isolate PDPF-21

PGP and biocontrol traits		Antagonistic properties (zone of inhibition in mm)	
Indole acetic acid (µg/ml)	0.06	*Rhizoctonia bataticola* -6	4
Siderophore (% units)	0.50	*Rhizoctonia bataticola* -24	4
Lipase (rating)	4	*Rhizoctonia bataticola* -115	1
Cellulase (rating)	2	*Macrophomina phaseolina*	4
Chitinase (rating)	3	*Sclerotium rolfsii*	3
Hydro cyanic acid (rating)	2	*Botrytis cinerea*	3
		Fusarium oxysporum f. sp. *ciceri*	4
-		*Fusarium proliferatum* -242	4
		Fusarium andiyazi -943	4

The rating scale for lipase, cellulase and chitinase were as follows: 0 = no halo zone, 1 = halo zone of 1-10 mm, 2 = halo zone of 11-20 mm, 3 = halo zone of 21-30 mm, 4 = halo zone of 31-40 mm, 5 = 41-50 mm. For hydro cyanic acid production, the following rating scale was used: 0 = no color change, 1 = light reddish brown, 2 = medium reddish brown, 3 = dark reddish brown.

people in Africa and Asia as it contains high carbohydrate energy and nutrition, thus making it useful component of dietary and nutritional balance in human food and animal feed. Foods from pearl millet are reported to be rich in phytochemicals (such as phytic acid and phytates) and generate vitamins, fatty acids and other vital nutrients that improve body's resistance against pathogens, lower cholesterol and reduce the risk of cancer (Coulibaly et al., 2011; El-Salam et al., 2012). In the present study, pearl millet flour and batter samples were explored for specifically isolating and characterizing probiotic actinomycetes.

It is widely accepted that one of the important criteria for isolating and/or selecting probiotic potential bacteria should be its ability to inhibit human pathogens and resistance to broad range of antibiotics. In the present investigation, the selected actinomycete PDPF-21 was found to inhibit all the tested pathogens, including *E. coli*, *S. aureus* and *S. typhi* and exhibited resistance to tetracycline, streptomycin, kanamycin, chloramphenicol, ciprofloxacin, ampicillin, penicillin, erythromycin and vancomycin in the antibiotics resistance pattern (Table 2). The main aim of using probiotic strains should be to beneficially affect the gut microbial composition and functionality. Oluwajoba et al. (2013) reported similar results on lactic acid bacteria (LAB). The same authors noted that LAB, isolated from millet grains and fermented products, inhibited human pathogens such as *S. aureus* 25923, *E. coli* 25922, *Pseudomonas aeruginosa* 27853 and *Enterococcus faecalis* 29212. Probiotic potential bacteria such as *Lactobacillus fermentum*, *Bifidobacterium* species and *Weissella confuse* were also found to help in preventing and treating acute diarrhea (Lei and Jakobsen, 2004). It is concluded that the selected actinomycete PDPF-21 has good antagonistic potential against important human pathogens and antibiotic resistance patterns.

Perhaps, the most important selection criteria for

isolating and identifying suitable probiotic microbe should be their ability to survive in acidic environment of the final fermented product and the adverse conditions of the gastrointestinal tract. Hence, in the present investigation, the actinomycete PDPF-21 was characterized for its probiotic properties including acid tolerance, bile tolerance, phenol tolerance and NaCl tolerance and found to tolerate acidic pH (pH 2), bile (up to 0.5%) and NaCl (up to 6%) but not phenol (Table 3). Therefore, it is concluded that the selected actinomycete has the desirable properties in order to qualify as a probiotic.

In order to determine the identity of the selected actinomycete, its 16S rDNA was sequenced and analyzed. A neighbor joining dendrogram generated using the sequence from the selected actinomycete (1400 bp) and representative sequences from the databases revealed that it belonged to *Streptomyces* spp. Pearl millet flour has been used for isolation of LAB including *Lactobacillus plantarum*, *Lactobacillus cellobiosus*, *Lactobacillus pentosus*, *Leuconostoc mesenteroids*, *Bacillus subtilis*, *Pedicoccus pentosaceus*, *Streptococcus lactis* and *Torulopsis glabrata* (Badau, 2006; Kamgar et al., 2013; Okoronkwo, 2014). Microbes such as *Lactobacillus*, *Bifidobacterium* and *Weissella* spp. are reported widely as probiotic microbes. Mridula and Sharma (2015) reported a non-dairy probiotic drink from the sprouted cereals, legumes and soy milk using LAB. *Lactobacillus acidophilus* was used to ferment a food mixture containing sorghum flour, whey powder and tomato pulp (Jood et al., 2012). However, to our knowledge, there are no reports till date on any actinomycete, *Streptomyces* spp., showing probiotic potential. Therefore, the selected actinomycete *Streptomyces* spp. can be exploited for development of functional foods. *Streptomyces* spp. have been widely reported and demonstrated under field conditions as plant growth-promoters and biocontrol agents in both cereals and legumes (Tokala et al., 2002; Nassar et al., 2003; Sadeghi et al., 2012; Gopalakrishnan et al., 2013, 2014, 2015, 2016a, b, c). In the present investigation, when the *Streptomyces* spp. PDPF-21 was evaluated for its PGP and biocontrol traits, it was found to produce IAA, siderophore, lipase, cellulase, chitinase and HCN and inhibit many plant pathogens of chickpea and sorghum (Table 4). Hence, it is concluded that the selected *Streptomyces* spp. PDPF-21 is having not only probiotic properties but also plant growth-promoting and biocontrol properties.

Conclusion

Pearl millet is not only a valuable source of prebiotics and bioactive compounds (such as resistant starch, total oligosaccharides, total dietary fiber and β-glucan), useful for development of functional foods, but also source of probiotic microbial cultures, which should be exploited for the production of new and innovative functional foods. The multiple beneficial effects of pearl millet can be used in association with probiotic microbes isolated from pearl-millet in designing novel cereal-based functional foods targeting different consumer segments having specific health requirements. The present work has identified a probiotic actinomycete strain from pearl millet that can be exploited for designing novel cereal based functional foods for addressing food and nutritional security for millions of malnourished people living in the poor countries of Africa and Asia. Probiotic foods obtained using a multiple microbial strains provide the opportunity to develop innovative food products with enhanced flavor, taste and texture based on consumer preference, unlike single strain based products that are usually sour and acidic in taste (Saarela et al., 2000). This study, thus, gives an opportunity to further explore the possibility of amending the selected actinomycetes with other known probiotic strains and/or exploring more probiotic actinomycetes towards development of functional foods.

CONFLICT OF INTERESTS

The authors have not declared any conflicts of interests.

REFERENCES

Alschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990). Basic local alignment search tool. J. Mol. Biol. 215:403-410.

Amadou I, Gounga ME, Guo-Wei L (2013). Millets: Nutritional composition, some health benefits and processing – A Review. Emir. J. Food Agric. 25:501-508.

Amadou L, Gbadamois OS, Guo-Wei L (2011). Millet-based traditional processed foods and beverages- A review. Cereal Food World 56:115-121.

Aswathy RG, Ismail B, John RP, Nampoothiri KM (2008). Evaluation of the probiotic characteristics of newly isolated lactic acid bacteria. Appl. Biochem. Biotechnol. 151:244-255.

Awika JM, Rooney LW (2004). Sorghum phytochemicals and their potential impact on human health. Phytochemistry 65:1199-1221.

Badau MH (2006). Microorganisms associated with pearl millet cultivars at various malting stages. Internet J. Food Safe 8:66-72.

Bazzicalupo M, Fani R (1995). The use of RAPD for generating specific DNA probes for microorganisms. In. Clap JP (ed.) Methods in molecular biology, species diagnostic protocols: PCR and other nucleic acid methods. Humana Press Inc, Totowa NJ, pp. 112-124.

Bhattacharya A, Chandra S, Barik S (2009). Lipase and protease producing microbes from the environment of sugar beet field. Ind. J. Agric. Biochem. 22:26-30.

Charalampopoulos D, Wang R, Pandiella SS, Webb C (2002). Application of cereals and cereal components in functional foods: a review. Int. J. Food Microbiol. 79:131-141.

Coulibaly A, Kouakou B, Chen J (2011). Phytic acid in cereal grains: structure, healthy or harmful ways to reduce phytic acid in cereal grains and their effects on nutritional quality. Am. J. Plant Nutr. Fertil. Technol. 1:1-22.

El-Salam AMH, Hippen AR, Salem MM, Assem FM, El-Aassar M (2012). Survival of probiotic *Lactobacillus casei* and *Enterococcus facium* in Domiati cheese of high conjugated linoleic acid content. Emir. J. Food Agric. 24:98-104.

Forouhandeh H, Zununi, VS, Hejazi MS, Nahaei MR, Akbari DM (2010). Isolation and phenotypic characterization of *Lactobacillus* species from various dairy products. Curr. Res. Bacteriol. 3(2):84-88.

Fuller R (1989). Probiotics in man and animals. J. Appl. Bacteriol. 66:365-378.
Glick BR (2010). Using soil bacteria to facilitate phytoremediation. Biotechnol. Adv. 28:367-374.
Gopalakrishnan S, Pande S, Sharma M, Humayun P, Kiran BK, Sandeep D, Vidya MS, Deepthi K, Rupela O (2011). Evaluation of actinomycete isolates obtained from herbal vermicompost for biological control of *Fusarium* wilt of chickpea. Crop Prot. 30:1070-1078.
Gopalakrishnan S, Sathya A, Vijayabharathi R (2016a). A book entitled "Plant Growth-Promoting Actinobacteria: A New Avenue for Enhancing the Productivity & Soil Fertility of Grain Legumes" published by Springer Singapore ISBN 978-981-10-0705-7.
Gopalakrishnan S, Srinivas V, Alekhya G, Prakash B (2015). Effect of plant growth-promoting *Streptomyces* spp. on growth promotion and grain yield in chickpea (*Cicer arietinum* L). 3 Biotech 5:799-806.
Gopalakrishnan S, Srinivas V, Prakash B, Sathya A, Vijayabharathi R, Rupela O, Himabindu K, Krishnamohan K, Rajeev KV (2014). Evaluation of *Streptomyces* strains isolated from herbal vermicompost for their plant growth-promotion traits in rice. Microbiol. Res. 169:40-48.
Gopalakrishnan S, Srinivas V, Srinivasan S, Kumar CVS (2016b). Plant growth-promotion and biofortification of chickpea and pigeonpea through inoculation of biocontrol potential bacteria, isolated from organic soils. SpringerPlus 5:1882.
Gopalakrishnan S, Srinivas V, Vidya MS, Rathore A (2013). Plant growth-promoting activities of *Streptomyces* spp. in sorghum and rice. SpringerPlus 2:574.
Gopalakrishnan S, Vijayabharathi R, Sathya A, Sharma HC, Srinivas V, Bhimineni RK, Gonzalez SV, Melø TM, Simic N (2016c). Insecticidal activity of a novel fatty acid amide derivative from *Streptomyces* species against *Helicoverpa armigera*. Nat. Prod. Res. 30:2760-2769.
Graciela FDV, Maria PT (2001). Food Microbiology Protocols. (Spencer JFT and Spencer ALRD, Ed.), Totowa, New Jersey: Humana Press Inc., Chap. 21 (Probiotic properties of *Lactobacilli*), Pp. 173-181.
Hassan A, Ch MN, Rasco B (2014). Microencapsulation, survival and adherence studies of indigenous probiotics. Afr. J. Microbiol. Res. 8(8):766-775.
Hendricks CW, Doyle JD, Hugley B (1995). A new solid medium for enumerating cellulose-utilizing bacteria in soil. Appl. Environ. Microbiol. 61:2016-2019.
Holt JG (1984). Bergey's Manual of Systematic Bacteriology. Williams and Wilkins, Baltimore.
Hsu SC, Lockwood JL (1975). Powdered chitin agar as a selective medium for enumeration of actinomycetes in water and soil. J. Appl. Microbiol. 29:422-426.
Huggett AC, Schliter B (1996). Research needs for establishing the safety of functional foods. Nutr. Rev. 54:S143-S148.
Ige BA (2013). Probiotics use in intensive fish farming. Afr. J. Microbiol. Res. 7(22):2701-2711.
Jood S, Khetarpaul N, Goyal R (2012). Effect of germination and probiotic fermentation on pH, titratable acidity, dietary fibre, β-glucan and vitamin content of sorghum based food mixtures. J. Nutr. Food Sci. 2(164):1-4.
Kamgar M, Pougholam R, Ghiasi M, Ghane M (2013). Studies on *Bacillus subtilis*, as potential probiotics on the biochemical parameters of rainbow trout, *Oncorhynchus mykiss* (Walbaum) to challenge infections. Adv. Stud. Biol. 5:37-50.
Kunchala R, Banerjee R, Mazumdar SD, Durgalla P, Srinivas V, Gopalakrishnan S (2016). Characterization of potential probiotic bacteria isolated from sorghum and pearl millet of the Semi-Arid Tropics. Afr. J. Biotechnol. 15:613-621.
Lei V, Jakobsen M (2004). Microbiological characterization and probiotic potential of koko and koko sour water, African spontaneously fermented millet porridge and drink. J. Appl. Microbiol. 96:384-397.
Liu K, Peterson KL, Raboy V (2007). Comparison of the phosphorus and mineral concentrations in bran and abraded kernel fractions of a normal barley (*Hordeum vulgare*) cultivar versus four low phytic acid iso-lines. J. Agric. Food Chem. 55:4453-4460.
Lorck H (1948). Production of hydrocyanic acid by bacteria. Plant Physiol. 1:142-146.
Macagnan D, Romeiro RDA, Pomella AMV, deSouza JT (2008). Production of lytic enzymes and siderophores, and inhibition of germination of basidiospores of *Moniliophthora* (ex *Crinipellis*) *perniciosa* by phylloplane actinomycetes. Biol. Control 47:309-314.
Mahadevan B, Crawford DL (1997). Properties of the chitinase of the antifungal biocontrol agent *Streptomyces lydicus* WYEC108. Enzyme Microb. Technol. 20:489-493.
Mridula D, Sharma M (2015). Development of non-dairy probiotic drink utilizing sprouted cereals, legumes and soymilk. LWT Food Sci. Technol. 62:482-487.
Nassar AH, El-Tarabily KA, Sivasithamparam K (2003). Growth promotion of bean (*Phaseolus vulgaris* L.) by a polyamine producing isolate of *Streptomyces griseoluteus*. Plant Growth Regul. 40:97-106.
Obilana AB, Manyasa E (2002). Millets. In. Belton PS and Taylor JRN (Eds.). Pseudo cereals and less common cereals: Grain properties and utilization potential. Springer-Verlag: New York. Pp. 177-217.
Okoronkwo CU (2014). Isolation and characterization of lactic acid bacteria involved in the fermentation of millet and sorghum sold in Nkwo-Achara Market, Abia state. IOSR J. Environ. Sci. Food Technol. 8:42-45.
Oluwajoba SO, Akinyosoye FA, Oyetayo VO (2013). *In vitro* screening and selection of probiotic lactic acid bacteria isolated from spontaneously fermenting kunu-zaki. Adv. Microbiol. 3:309-316.
Pandey P, Kang SC, Maheswari DK (2005). Isolation of endophytic plant growth-promoting *Burkholderia* sp. MSSP from root nodules of *Mimosa pudica*. Curr. Sci. 89:177-180.
Patten C, Glick BR (2002). Role of *Pseudomonas putida* in indole acetic acid in development of host plant root system. Appl. Environ. Microbiol. 68:3795-3801.
Pelczar MJ, Chan ECS, Krieg NR (2008). Microbiology. Fifth Edition, Tata McGraw-Hill Publishing Company Limited, New Delhi.
Saarela M, Mogensen G, Fonden R, Matto J, Mattila-Sandholm T (2000). Probiotic bacteria: Safety functional and technological properties. J. Biotechnol. 84:197-205.
Sadeghi A, Karimi E, Dahazi PA, Javid MG, Dalvand Y, Askari H (2012). Plant growth promoting activity of an auxin and siderophore producing isolate of *Streptomyces* under saline soil condition. World J. Microbiol. Biotechnol. 28:1503-1509.
Saitou N, Nei M (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4:406-425.
Sathya A, Vijayabharathi R, Srinivas V, Gopalakrishnan S (2016a). Plant growth-promoting actinobacteria on chickpea seed mineral density: An upcoming complementary tool for sustainable biofortification strategy. 3 Biotech 6:138.
Sathya A, Vijayabharathi R, Vadlamudi S, Sharma HC, Gopalakrishnan S (2016b). Assessment of tryptophan based diketopiperazine, cyclo (L-Trp-L-Phe) from *Streptomyces griseoplanus* SAI-25 against *Helicoverpa armigera* (Hübner). J. Appl. Entomol. Zool. 51:11-20.
Schwyn B, Neilands JB (1987), Universal chemical assay for the detection and determination of siderophore. Annu. Biochem. 160:47-56.
Syal P, Vohra A (2014). Probiotic attributes of yeast-like fungus *Geotrichum klebahnii*. Afr. J. Microbiol. Res. 8(20):2037-2043.
Teply M (1984). Ciste mlekarske kultury. Phara. SNTL Nakladatelstvi. Technicke Litertury. In. Starters for Fermented Milks, Kurmann JA (Ed.), IDF Bulletin 227; pp. 41-55.
Thompson JD, Gibsom TJ, Plewnaik F, Jeanmougin F, Higgins DG (1997). The clustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 24:4876-4882.
Tokala RK, Strap JL, Jung CM, Crawford DL, Salove MH, Deobald LA, Bailey JF, Morra MJ (2002). Novel plant-microbe rhizosphere interaction involving *Streptomyces lydicus* WYEC108 and the pea plant (*Pisum sativum*). Appl. Environ. Microbiol. 68:2161-2171.
Trejo-Estrada SR, Paszczynski A, Crawford DL (1998). Antibiotics and enzymes produced by the biocontrol agent *Streptomyces violaceusniger* YCED-9. J. Ind. Microbiol. Biotechnol. 21:81-90.
Wilker MA (2006). Performance standards for antimicrobial susceptibility testing: Sixteenth informational supplement. Clinical and Laboratory Standards Institute.