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Original Article

Antibacterial activity of soil bacteria isolated from Kochi, India and their molecular identification

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ABSTRACT

The present study, deal about the antibiosis activity of soil bacteria, isolated from 10 different locations of rhizosphere and diverse cultivation at Kochi, Kerala, India. The bacteria were isolated by standard serial dilution plate techniques. Morphological characterization of the isolate was done by Gram's staining and found that all of them gram positive. Isolated bacteria were tested against 6 human pathogens *viz.*, *Escherichia coli*, *Enterococcus* sp., *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Acinetobacter* sp. Primary screening was carried out by perpendicular streaking and seed overlay method. Based on the result of primary screening most potential isolates of S1A1 and S7A3 were selected for secondary screening. Both the isolates showed positive results against *Enterococcus* sp. and *S.aureus*. The maximum antagonistic activity of 20.98 and 27.08 mm zone of inhibition was recorded at S1A1 against *Enterococcus* sp. and *S. aureus* respectively, at 180 μl concentration. Molecular identification was carried out by 16S rRNA sequence. The 16S rRNA was amplified from the DNA samples by using PCR. The amplified 16S rRNA PCR products were purified and sequenced. The sequences were subjected to NCBI BLAST. The isolates S1A1 and S7A3 BLAST results showed 99% and 95% respectively, similarity with the available database sequence of *Bacillus amyloliquefaciens*. The sequences were deposited in GenBank and the accession numbers KY864390 (S1A1) and KY880975 (S7A3) were obtained.

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1. Introduction

Eight decades ago the antibiotics was discovered, they have revolutionised the treatment of infections, transforming once deadly diseases into manageable health problems [1]. Availability of effective antibiotics has revolutionized public health and has been responsible for enabling countless advancements in medical care; enabled by effective antibiotic therapies have, in turn, created a crisis where many antibiotics are no longer effective against the simple infections. Such infections often result in an increased number of hospitalizations, more treatment failures and the persistence of drug-resistant to pathogens [2].

From the 1980 to the early 2000s, there was a 90% decline in the approval of new antibiotics. Many companies have shifted away from drug development due to scientific, regulatory, and economic hurdles that proved antibiotic development to be less attractive compared with more profitable therapeutic areas [3]. The microbes are continuing to become more resistant, the antibiotic pipeline continues to diminish, and the majority of the public remains unaware of this critical situation. This has led to a current situation where, infectious diseases alone kill roughly 13 million people worldwide, annually, a toll that continues to rise, aided and assisted by resistance genes.

The crude infectious disease mortality rate is 416.75 per 100,000 persons (calculations based on World Bank data and the Global Burden of Disease, 1990) and is twice the rate prevailing in the United States when antibiotics were introduced (roughly 200 per 100,000 persons). About 2 million infections and 23,000 deaths are caused by antibiotic resistance pathogens per year in United States. In Europe 25,000 people die every year due to antibiotic resistant bacteria. About 2.2 million deaths in diarrheal disease [4]. The prevalence of antibiotic-resistant *Streptococcus pneumonia* has increased over the last decade in the United States. The proportion of pneumococci non susceptible to penicillin has reached 35% in some areas [5].

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The major drive of resistance is due to continuous uses of antibiotics. It is predictable in any environment where antibiotics are released. Bacteria acquire resistance to antibiotics, due to the wide availability of antibiotics, and their improper usage and disposal. In 2010, India was the world's largest consumer of antibiotics for human health at 12.9×10^9 units (10.7 units per person). The next largest consumers was China at 10.0×10^9 units (7.5 units per person) followed by US at 6.8×10^9 units (22.0 units per person). Seventy-six percent of the overall increase in global antibiotic consumption between the years 2000 to 2010, due to BRICS countries, (Brazil, Russia, India, China, and South Africa).

In BRICS countries, 23% of the increase in the retail antibiotic sales in India, and up to 57% of the increase in the hospital sector in China [6]. Antimicrobial drugs used for prophylactic or therapeutic purposes in human, veterinary and agricultural purposes also favours the survival and spread of resistant organisms [7]. Agricultural use accounts for at least half of the antibiotics produced in the United States [8].

The improper disposal of animal waste and its excessive application as fertilizers also leads to the spread of resistance in Soil bacteria (potentially by lateral gene transfer), which then serve as persistent reservoir of antibiotic resistance. Besides, poultry, cattle, and swine raised with antibiotics harbour significant populations of antibiotic-resistant bacteria, which are transmitted to humans through direct contact with the animals and through their meat, eggs, and milk [9].

Growing antimicrobial resistance and a diminishing antibiotic pipeline have resulted in an emerging post-antibiotic era, as patients are now dying from bacterial infections that were once treatable. Now it is important to research deliberately for the development of new, safe and effective antibiotics to combat the menace of concomitant MDR (Multi drug resistant pathogens [10].

As natural products have a novel structure, they remain to be the major promising source of secondary metabolites [11] and also served as antibacterial activity against pathogenic bacteria [12]. The natural products obtained from microorganisms still appears as the most auspicious source of the future antibiotics [13]. Among the different unexplored habitats, soil is considered as one of the most suitable environments for microbial growth [14], the microorganisms which have been isolated from the soil is leading in the source of antibiotic discovery. Soil and plant-associated environments harbour numerous bacteria that produce antibiotic metabolites with specific or broad-spectrum activities against coexisting microorganisms depending on the pH, nutrient availability and humus content.The activity and diversity of soil organisms are regulated by a hierarchy of abiotic and biotic factors. The main abiotic factors are climate, including temperature, moisture, soil texture, soil structure, salinity and pH. The climatic conditions also influences the physiology of soil organisms as it differs across the globe and also, in the same places, between seasons. Even though the soil is naturally rich in microbes capable of antibiotic synthesis, the frequency with which synthesis occurs at ecologically significant levels has been much less clear. Even though traditional approach of random screening which has been done for the past 50 years to produce new antibiotics which are in favor human beings. Keeping in this mind, in the present study was aimed to isolate and characterize antibiotic producing bacteria from rhizosphere soil of different locations in Kochi, India.

2. Materials and methods

2.1. Study area

The samples were collected from Kochi, district of Ernakulum in the state of Kerala. It is located on the southwest coast of India at 9°58′N 76°13′E. In this area soil consists of sediments such as alluvium, laterites, brown sands, etc. Hydromorphic saline soils are also found in the areas and predominantly, rock types soil.

2.2. Soil sample collection

The rhizosphere soil samples were collected from 10 different locations in differ crops field at Kochi, Kerala, India. The debris from soil samples were removed before collection. The site was digged into 5–15 cm and approximately 10 g of the rhizosphere soil was collected in a sterile tube and transported into laboratory and stored at 4 °C.

2.3. Isolation and maintenance of soil bacteria

Soil bacteria were isolated by the standard serial dilution plate technique. At 1 g of each soil sample was weighed and soaked in 10 ml of sterile physiological saline. The samples were then serially diluted. Out of the 4 dilutions, 100 μl from the each dilution (10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}) of each sample were used to prepare nutrient agar spread plates. The plates were incubated up to 7 days to find out antagonistic bacterial colony. The colonies which showed antagonism were picked up and streaked on nutrient agar plate separately in order to obtain pure isolated colonies. Pure culture was stored at 4 °C for subsequent studies.

2.4. Morphological characterization of isolated bacteria

Morphological characterizations of the isolated antagonistic bacteria were carried out using Gram's Staining method. Twenty four hours old nutrient broth cultures of each isolates were used for Gram's staining.

2.5. Primary screening

Primary screening of the antagonistic bacteria was done *in vitro* against 6 human pathogenic bacteria. The screening was done by both perpendicular streaking and seed overlay method. The test pathogens include *Escherichia coli*, *Enterococcus* sp., *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Acinetobacter* sp.

2.6. Perpendicular streaking

The antagonistic bacteria was isolated from the sample by streaked as a single straight line through the center of the nutrient agar plate. After 48 h of incubation of all the 6 tested pathogens were streaked perpendicular to the antagonistic bacteria. Immediately after streaking, the length of the streak was marked on the plate using marker pen. The observations were made after 24 h. After incubation, any substances produced by the soil bacteria were expected to lyse/inhibit the tested human pathogen [15].

2.7. Seed overlay method

The antagonistic bacteria isolated from the sample were spot inoculated on to a nutrient agar plate using a sterile tooth pick. After 48 h of incubation, 2 ml of chloroform was added into the lid; the plate was kept inverted and sealed, so that the fumes of chloroform would kill the inoculated bacteria by preventing its growth. This ensures that only the secondary metabolites of the inoculum diffused into the nutrient agar media remains active. After 1 h, the plates were opened and the fumes were allowed to evaporate for 20 min. Then, 100 μl of each test human pathogen culture was mixed with 5 ml of sterile and cooled (40 °C) nutrient broth with 0.6% agar. After thorough mixing, the medium was

overlaid on to the nutrient agar plate and were incubated for 24 h. The antagonistic activity was measured by determining the zone of inhibition.

2.8. Secondary screening

The bacteria which showed positive results in primary screening, selected for secondary screening by well diffusion method. In this method, initially all the 6 test pathogens were swabbed separately on to 6 different Muller Hinton Agar plates using sterile cotton swab. Immediately after swabbing, two (10 mm) wells were made on each plate with the help of sterile cork borer. The wells were loaded with 90 μl and 180 μl culture supernatant of antagonistic bacterium. The antibiotic disc amoxyclav (*Enterococcus* sp.) and streptomycin (for other test human pathogen) was used as positive control. After 24 h of incubation, the plates were checked for the presence of zone of inhibition. The length of zones produced by both the antagonistic bacteria and the antibiotic disc were measured accurately using a Vernier calliper.

2.9. Isolation of genomic DNA from bacteria

At 1.5 ml of overnight grown bacterial isolates maintained in nutrient broth were transferred to 2 ml of micro centrifuge tube and centrifuged at 10,000×*g* for 2 min and pellet was collected. The same was repeated for another 1.5 ml of culture to harvest enough quantity of cells (100 mg). The pellet was washed with 0.9% saline and suspended in 1 ml of digestion buffer and was incubated at 50 °C with occasional shaking in tightly capped micro centrifuge tubes for 60 min. The sample was then extracted with equal volume of phenol-chloroform-isoamyl alcohol (25:24:1) and centrifuged for 10 min at 10,000×*g*. The top aqueous layer was transferred to a new tube. At 0.5 ml of 7.5 M ammonium acetate was mixed gently and 2 ml of 100% ice cold ethanol was added. It was centrifuged for 5 min at 5000×*g*. The DNA pellet was washed with 70% ethanol. The pellet was air dried and re suspended in 25 μl of TE buffer (pH 8.0) and stored at 4 °C.

The isolated genomic DNA was quantified using spectrophotometer at wavelengths 260 and 280 nm. The purity of genomic DNA was checked by run in 0.8% agarose gel. After run, the gel was stained with ethidium bromide and photographed with GELSTAN gel documentation system.

2.10. Molecular identification of bacteria by 16S rRNA amplification and sequencing

The universal primers (Forward primer 5′- AGAGTTTGATCCTGGCTCAG -3′ and reverse primer 5′- GGTTACCTT GTTACGACT-3′) were used for the amplification of the 16S rRNA gene fragment. The PCR cycling conditions were as follows: an initial denaturation for 5 min at 94 °C, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 2 min and then a final extension for 5 min at 72 °C.

The rRNA amplification reaction mixture (30 μl) consists of 2X Amplicon Red master mixes (amplicon®) with 10 ng of total genome of each isolate, 10 pmol of each forward and reverse primer. The amplified PCR products were electrophoresed on 1% agarose gel. The gel was stained in ethidium bromide and photographed with GELSTAN gel documentation system.

2.11. Sequencing and analysis of 16S rRNA

The amplified 16S rRNA gene fragment was purified and sequenced using DNA sequencing services (Eurofins Scientific, Bangalore) employing the same primer used for PCR amplification. 16S rRNA gene sequences were exported into "Basic Local Alignment Search Tool" (BLAST) available from the website of National Center for Biotechnology Information (NCBI-http://www.ncbi.nlm.nih.gov) to identify matches with existing characterized reference sequences.

3. Results

3.1. Collection of soil samples

The soil samples were collected from different rhizosphere crops such as Papaya, Ginger, Coconut, Plantain, Nutmeg, Brinjal and Tapioca (Table 1).

3.2. Isolation and maintenance of microbial isolates

The colony forming units (CFU) of each soil sample was diverse. The suitable dilution was selected based on the plate having countable number of colonies. Maximum number of CFU was recorded in soil sample (S6) and minimum number of CFU was noticed in soil sample (S2). Out of 10 soil samples screened 5 soil samples (S1, S2, S4, S7 and S10) showed antagonistic property at dilutions either 10^{-2} or 10^{-3}. S7 possessed three colonies with antagonistic activity. Followed by S1, which had two colonies. The rest of the three samples *i.e.* sample S2, S4 and S10 had single colonies exhibiting antagonism (Table 2). Hence, these 8 (S1A1, S1A2, S2A2, S4A1, S7A1, S7A2, S7A3, S10A1) antagonistic bacteria was selected for further screening.

3.3. Morphological characterization

Morphological characterization of the microbial isolates was done by Grams staining which revealed all the 8 microbial isolates were gram positive. Among them the different gram positive bacteria like bacilli, long chain bacilli and cocci were observed (Table 3).

3.4. Primary screening

In primary screening, 8 bacterial isolates was chosen to check their antibacterial activity against human pathogens *S. aureus, K. pneumoniae, Enterococcus* sp., *P. aeruginosa, E. coli, Acinetobacter* sp. Among 8 different isolates, 4 of them showed antagonistic activity in perpendicular streaking method.

Both the isolates S1A1 and S7A3 revealed antibiotic producing ability by showing zone of inhibition against *E. coli*, *Enterococcus* sp., *S. aureus*and *K. pneumoniae* while the isolates S7A1 showed inhibitory zones against *E. coli, Enterococcus* sp. and *S. aureus*. The isolate S1A2 produced zone of inhibition against *K. pneumonia* and *Enterococcus* sp. and *S. aureus*. The remaining isolate S2A2, S4A1, S7A2 and S10A1 produced no zone of inhibition (Table 4).

In seed overlay method, both the isolates S1A1 and S7A3 produced zone of inhibition against *S. aureus*, *K. pneumoniae*, and *Enterococcus* sp. while the isolates S1A2 produced zone of inhibition against *Enterococcus* sp. and *S. aureus*. On the other hand S7A1 produced zone of inhibition against only a single test pathogen *i.e.*, against *S. aureus* (Table 5).

3.5. Secondary screening

After perpendicular streaking and seed overlay method, the most potential isolates S1A1 and S7A3 having significant antagonistic activity against 4 test human pathogens viz., *Enterococcus* sp., *S. aureus, K. pneumonia* and *P. aeruginosa* (Table 6). Both the

Table 1
Locations of soil sample collection and Crops.

Soil sample	Location	Crop	Latitude	Longitude
S1	Kariyad	Papaya	11.683478	75.56697
S2	Ankamaly	Ginger	10.184909	76.375305
S3	Karukutty	Coconut	10.226975	76.375022
S4	Elavoor	Plantain	10.206397	76.333336
S5	Edakkunnu	Nutmeg	10.254458	76.401347
S6	Alangadu	Brinjal	10.102357	76.291499
S7	Puthenpally	Tapioca	10.083500	76.272531
S8	Kalady	Nutmeg	10.167286	76.439884
S9	Kothamangalam	Plantain	10.060190	76.635083
S10	Muvattupuzha	Plantain	9.989423	76.578975

Table 2
Number of colony forming units and the colonies showing antagonism.

Soil sample	Number of colonies	Dilution	Number of CFU/ ml	Number of colonies showing antagonism
S1	33	10^{-3}	3.3×10^6	A1, A2
S2	86	10^{-2}	8.6×10^5	A2
S3	62	10^{-3}	6.2×10^6	–
S4	95	10^{-2}	9.5×10^5	A1
S5	37	10^{-3}	3.7×10^6	–
S6	72	10^{-3}	7.2×10^6	–
S7	282	10^{-2}	2.8×10^5	A1, A2, A3
S8	63	10^{-3}	6.3×10^6	–
S9	39	10^{-3}	3.9×10^6	–
S10	260	10^{-2}	26×10^5	A1

Table 3
Gram staining results of various antagonistic bacteria.

Screened isolates	Reactivity to the stain	Shape
S1A1	+	Long chain Bacilli
S1A2	+	Filamentous
S2A2	+	Bacilli
S4A1	+	Cocci
S7A1	+	Bacilli
S7A2	+	Cocci
S7A3	+	Long chain Bacilli
S10 A1	+	Bacilli

isolates showed positive results for *Enterococcus* sp. and *S. aureus*. At 90 μl and 180 μl of the isolate S1A1 showed 20.68 mm and 20.98 mm zone of inhibition respectively against *Enterococcus* sp. The zone of inhibition of antibiotic disc AMC 30 (Amoxyclav) was 22.98 mm against *Enterococcus* sp.

Similarly 18.93 mm and 20.66 mm zone of inhibition was produced by 90 and 180 μl of isolate S7A3 respectively against *Enterococcus* sp. while the standard disc produced zone of inhibition was 24.58 mm. During the screening against *S. aureus*, both the isolates S1A1 and S7A3 produced increased zones of inhibition than the zone produced by standard disc streptomycin. The zone of inhibition produced by the isolate was greater against *S. aureus* when compared with *Enterococcus* sp.

At concentration of 90 μl, S1A1 showed maximum zone of 23.82 mm against *Staphylococcus*, while the concentration of 180 μl inhibit 27.08 mm. The zone produced by standard disc streptomycin was 18.79 mm. Similarly 90 μl of S7A3 produced 21.58 mm and 180 μl of the same produced 26.68 mm against *Staphylococcus*. The standard disc showed a zone of 18.25 mm.

3.6. Isolation of genomic DNA from bacteria

The genomic DNA extraction was carried out as described earlier in methodology section, in order to obtain high molecular weight and quality genomic DNA from the two unidentified antagonistic bacteria (S1A1 and S7A3). Genomic DNA was isolated successfully from two unidentified antagonistic bacterial cultures. The genomic DNA extraction method involves breakdown of the cell wall, centrifugation to remove the cell fragments and debris, precipitation of nucleic acid from the pelleted cells and purification. The 16S rRNA was amplified from DNA samples of isolated antagonistic bacteria by using PCR. The amplified product was verified on 1.2% agarose gel that showed a single fragment of 1.5 kb in size.

Table 4
Antagonistic activity of isolated soil bacteria against test human pathogens by perpendicular streak.

Isolates	*E.coli*	*Enterococcus* sp.	*P. aeruginosa*	*S. aureus*	*K. pneumoniae*	*Acinetobacter* sp.
S1 A1	+	+	–	+	+	–
S1 A2	–	+	–	+	+	–
S2 A2	–	–	–	–	–	–
S4 A1	–	–	–	–	–	–
S7 A1	+	+	–	+	–	–
S7 A2	–	––	–	–	–	–
S7 A3	+	+	–	+	+	–
S10 A1	–	–	–	–	–	–

+Indicate zone of inhibition.
-Indicate absence of zone of inhibition.

Table 5
Antagonistic activity of soil isolated bacteria against human pathogens by Seed Overlay method.

Isolates	*E.coli*	*Enterococcussp.*	*P. aeruginosa*	*S. aureus*	*K. pneumoniae*	*Acinetobacter* sp.
S1 A1	–	+	–	+	+	–
S1 A2	–	+	–	+	–	–
S2 A2	–	–	–	–	–	–
S4 A1	–	–	–	–	–	–
S7 A1	–	–	–	+	–	–
S7 A2	–	–	–	–	–	–
S7 A3	–	+	–	+	+	-
S10 A1	–	–	–	–	–	–

+Indicate zone of inhibition.
–Indicate absence of zone of inhibition.

Table 6
Antagonistic activity of soil bacteria culture supernatant against test human pathogens.

Isolates	Pathogen	Concentrations		Standard Antibiotic Disc
		90 µl	180 µl	
		Zone of inhibition (mm)		
S1 A1	*Enterococcus*	20.68	20.98	22.98 *
	Staphylococcus	23.82	27.08	18.79 #
S7 A3	*Enterococcus*	18.93	20.66	24.58 *
	Staphylococcus	21.58	26.68	18.25 #

* Amoxyclav (AMC 30).
# Streptomycin (S10).

3.7. Molecular identification of bacteria by 16s r RNA amplification and sequencing

The amplified 16s rRNA PCR products of two antagonistic bacteria were purified and sequenced (Table 7). The sequence of bacterial isolates S1A1 and S7A3 were subjected to NCBI blast. The isolates S1A1 and S7A3 BLAST results showed 99% and 95% respectively, similarity with the available database sequence of *Bacillus amyloliquefaciens*. The sequences were deposited in GenBank and the accession numbers KY864390 (S1A1) and KY880975 (S7A3) were obtained.

4. Discussion

The aim of the study was to isolate antibiotic producing microorganisms from the rhizosphere regions of variety of crops in Kochi. Rhizosphere soil has been selected for sampling since microbial community exceeds in soil than any other environment. The rhizosphere microbes exhibit high level of antagonistic activity [16]. Many scientists have chosen soil for the isolation of novel antibiotics as it is a source of many antibiotic producing bacteria including Actinomycetes [13,15,17–19], Constancias et al. [20] also reveals that the heterogeneity of soil results in a wide variety of ecological niches and a high diversity of soil microorganisms. This outcome correlated with our sample collection methodology implemented where the samples collected from different locations and diverse cultivations. Random sampling described by Williams and Vickers [21] was a conventional method of sample collection; this method was supports to in this present study.

Morphological characterization was done by Grams staining method, which is a conventional method of characterization followed by many scientists [19,22,23]. The Gram's staining indicates that all the bacterial isolates as gram positive. These results are in confirmatory with those of Wadetwar and Patil [18] who obtained most of the soil isolates as gram positive.

The isolates were screened for the production of antibiotics through primary screening (perpendicular streaking and seed overlay method) followed by secondary screening (agar well diffusion method). This is an agreement with some previous literatures which used the same methods for the screening of isolates [15,17–19,24]. Bacterial culture filtrate was used in agar well diffusion method for secondary screening. This same method has been followed by many scientists [25,26].

The secondary screening of the isolates S1A1 and S7A3 was done against the following human pathogens, *E. coli, Enterococcus* sp., *K. pneumoniae, S. aureus, P. aeruginosa* and *Acinetobacter* sp. and maximum zone of inhibition was shown by both the isolates S1A1 and S7A3 against *S. aureus*. These results corroborated with the results of Saadoun and Gharaibeh [27] and Wadetwar and Patil [18] reported that the soil isolates showed maximum zone of inhibition against *S. aureus*. Similarly, Oskay et al. [17] who reported that isolate showed maximum zone of inhibition against *S. aureus*. But on controversy to the above result, at concentrations of 12.5 µg/ml (Minimum Inhibitory Concentration) the maximum zone of inhibition was produced for *K. pneumonia*, followed by *P. aeruginosa* and then *E. coli* [24].

The molecular identification of bacteria was carried out by 16S rRNA amplification and sequencing. A significant advantage of this protocol is that a bacterial isolate can be identified within 2–3 days than conventional biochemical test, which generally take several weeks. Several previous reports supported that 16S rRNA gene sequence analysis was improved method for identification of bacteria compared to conventional phenotypic methods [28–30]. The subsequent sequencing and BLAST analysis proved both the isolates to be *Bacillus amyloliquefaciens*. This result provided strong support to earlier studies which have already proved *Bacillus* species as the most predominant bacteria present in soil [13,31,32]. This also provides a strong sustenance to the previous studies which have proved *B. amyloliquefaciens* as a potent source for antibiotic [33,34]. Boottanun et al. [35] has proved *B. amyloliquefaciens* has the capacity to produce various antimicrobial peptides and secondary metabolites, which supports our present result. Results are also in confirmatory with Vijayalkshmi et al. [36] who reported that *B. amyloliquefaciens* produces maximum antimicrobial proteins and this was maximum against gram positive *S. aureus*. Gram positive bacteria are more susceptible to antibiotics because it carries only outer peptidoglycan layer which is not an effective barrier. This may be the reason for both S1A1 and S7A3

Table 7
Antagonistic bacteria 16S rRNA gene sequences.

Bacteria	Nucleotide sequences of 16S rRNA gene
Bacillus amyloliquefaciens	CTTCCACGCCCGTTATCTCAGGCGGAGTGCTTATGCGTTACCTGCAGCACTAAGGGGCGGAAACCCCCTAACACTTAGC ACTCATCGTTTACGGC GTGGACTACCAGGGTATCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGTCAGTTACAGACCAGAGAGTCGCCTTCGCCA CTGGTGTTCCTCCACATCTCTACGCATTTCACCGCTACACGT GGAATTCCACTCTCCTCTTCTGCACTCAAGTTCCCCAGTTTCCAATGACCCTCCCCGGTTGAGCCGGGGGCT TTCACATCAGACTTAAGAAACCGCCTGCGAGCCCTTTACGCCCAATAATTCCGGACAA CGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTA GTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTGCCGCCCTATTTGAACGGCACTTGTTCTTCCCTAACAACAGAGCTTTACGA TCCGAAAACCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAG TCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTC GCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCC GCGGGTCCATCTGTAAGTGGTAGCCGAAGCCACCTTTTATGTCTGAACCATGCGGTTCAAACAAGCA TCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTACTCACCCGTCCGCCGCTAACATCAGGGAGCAAGCT CCCATCTGTCCGCTCGACTTGCATGTATTAGCACGCCGCCAGCGTTCGTCC TGAGCAGAAACACAAAACTCATTAGAGGGGGGTGGAGGTGGGGGGTGGAAAAAGAGAA
Bacillus amyloliquefaciens	ATTTTCCTCTCCAGGGCGGTGTGCTTAATGCGTTAGCTGCAGCACTAAGGGGCGGAAACCCCCTAACACTTACCACTCATCGTTTA CGGCGTGGACTACCAGGGTATCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCA GCGTCAGTTACAGACCAGAGAGTCGCCTTCGCCACTGGTGTTCCTCCACATCTCTACGCATTTCACCGCTACACGTGGAATTC CACTCTCCTCTTCTGCACTCAAGTTCCCCAGTTTCCAATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTA AGAAACCGCCTGCGAGCCCTTTACGCCCAAT AATTCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAG GTGCCGCCCTATTTGAACGGCACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTT CATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTACGAGTCTGGGCCGT GTCTCAGTCCCAGTAGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTCGCTCTTGGTGAGCCGTTTCCTCACCAACTAGCTA ATGCGCCGCGCGTTCCATCTGTAAGTGGTACCCGAAGCCACCGTTTATGTCTGAACCCTGCATTTCAGACAACAACTCTGGCATTA CGCCTCGGTTTCCCGGAGTAACCCAGTCTTACGGACGGGTACCCCACCTGTTTCTCCCACGTCCGCCGCTATCATCAGGGACAAGCTCCATCT GATAGTTTGACATTGCATGCATCTATGCCAACTGCCATCGTTCGTCCCTGTAGTTATGTAATAAACTATAACTGTTGTTCCTTGGCC

to produce comparatively larger zones of inhibition against *S. aureus* which is a gram positive bacterium.

B. amyloliquefaciens have proved to be potent source of antibiotics against plant pathogens [37–41] and is mostly associated with plant rhizosphere [42] and it also provide strong support for our study. Besides, Bacillaene produced by *B. amyloliquefaciens* is found to be effective against human pathogens such as *Serratiam arcescens, K. pneumonia* and *S.* aureus [33]. In our study in which *B. amyloliquefaciens* have produced maximum zone of inhibition against *S. aureus*. Similarly, *B. amyloliquefaciens* also produces Iturin A which is effective against fungal pathogens like *Rhizoctonia solani* [43]. Difficidin and Bacilysin from the same have antibacterial activity against *Xanthomonas oryzae* rice pathogens [44]. *B. amyloliquefaciens* has been used as antibacterial agent against pathogenic bacteria of dairy and veterinary animals [45]. All these findings provide strong support and underlie our result that, *B. amyloliquefaciens* serve as the potent source of antibiotics.

5. Conclusion

The continuous use of antibiotics has resulted in a situation where the pathogens have acquired resistance against the antibiotics. So, now there is an urgent need for the development of novel, safe and effective antibiotics. Since, natural products have a novel structure; it remains as a major source of secondary metabolites. The present study, which was aimed to isolate soil microbes exhibiting antibiosis activity, yielded *B. amyloliquefaciens* as the potent antibiotic source. The screening of isolates from both the soil samples S1 and S7 obtained *B. amyloliquefaciens* which produced maximum zone of inhibition against the test human pathogens *S. aureus* and *Enterococcus sp*. These results advocate that the microbial isolates from this particular strain can be used commercially for the production of antibiotics after purification and proper standardization.

Conflict of interest statement

We declare that we have no conflict of interest.

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