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PHYTOCHEMISTRY OF THE HOST PLANT *ALSTONIA VENENATA* R.BR (APOCYNACEAE) AND TRANSFORMATION OF ENDOPHYTIC MICROBES TO *E. COLI*

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Abstract

The medicinally active substance were isolated from dried bark of *Alstonia venenata* R. Br and identified by photochemical tests. The extraction in powdered form was performed using butanol and water. The result indicated that alkaloids and flavanoids were present in butanol and water extracts. Saponins, carbohydrates and proteins were present in water extract. Tannins, glycosides and phenols were absent in any of the extract. The endophytes were isolated from the bark of Alstonia venenata. The endophytic bacteria were *Nocardia callitridis, Sporolactobacillus spathodeae, Bacillus subtilis, Mycobacterium platani* and *Paenibacillus* cathormi. The transformation of the plasmid of these endophytes to *E. coli* was performed to check their effect on host cells and the transformed E coli shows variation in their biochemical activities.

Key words: *Alstonia venenata*, medically active substance, phytochemicals, endophytes, transformation.

Introduction

A medicinal plant is a plant that is used for maintaining health in traditional and modern medicine (Smith-Hall *et al.,* 2012). Medicinal plants have been used for the medical purpose since prehistoric times and human beings utilize their environment to recover from a disease, the use of plants was their only choice of treatment (Halberstein, 2005). Human is mainly dependent on raw plant materials to meet medical needs to maintain health and cure diseases (Jack, 1997). Single plants contain diverse phytochemicals, hence the whole plant is used as medicine and used to develop drug synthesis. Treatment with the medicinal plant is considered very safe with no or minimal side effects. The medicinal uses of the plants were developed through observations of wild animals by trial and error. Awareness and knowledge of medicinal plants can play a key role in the exploitation and discovery of natural plant resources (Jamshidi-Kia *et al.,* 2018). The forests of India are the principal source of medicinal plants from ancient times (Khan, 2016). The different ethnic communities in India have used different species of Alstonia in the treatment of various human ailments (Pant and Pandey, 1995; Prasad *et al.,* 1987). Alstonia species are used to treat snakebite (Prakasha *et al.,* 2010). The plant species are threatened with extinction from overharvesting and natural anthropogenic habitat destruction (Chen *et al.,* 2016). The bioactive compounds are produced at a very low level and accumulated in native plants. Endophytes present in the medicinal plants are capable to produce secondary metabolites similar to those produced by their host plants (Kusari *et al.,* 2012; Venieraki *et al.,* 2017). The exploitation of the medicinal plants can be reduced by using these endophytes for the production of bioactive compounds. In the present study, we isolated endophytic bacteria from *Alstonia venaneta* and transformed the endophytic plasmid to *E. coli* cells to study its effects on the host. This study reveals the effects of endophytes in the host and further studies can be conducted to identify the effects of these endophytes in *A. venanata*. The selection of medicinal plants is based on their traditional uses in India.

Materials and Methods

Collection of sample

The dried bark of the plant *A. venenata* was collected from the Njallur forest area near Konni, Pathanamthitta district. Due to the geographical diversity, there is a chance of variation in constituents and efficiency of the bark.

Phytochemical analysis

Plants contain many chemical constituents which are therapeutically active or inactive. Tannin, phenol, carbohydrate, protein, saponins, glycosides, alkaloids and flavanoids were qualitatively analyzed.

Isolation of endophytes

The barks were homogenized by using motor and pestle. The sample were serially diluted and 10^{-1}, 10^{-2}, 10^{-3} dilutions are taken for pour plate method. Four different media such as nutrient agar, urea agar, MacConky agar and EMB agar medium were used. The isolated colonies after 24 hour incubation were sub cultured into fresh growth medium.

Optimization of growth

The microorganisms were incubated at different temperature such as, 30^0, 32^0, 34^0, 36^0 and 38^0C for examine the optimum temperature required for the microbe to live. The microorganisms were incubated at 30°C in different pH such as 1, 3, 7, 8 and 9.

Identification of endophytes

Gram staining and motility test

Gram's staining was performed for all the isolated bacterial colonies. Mobility is the ability of the organism to move independently, using metabolic energy. Hanging drop preparation is a special type of wet mount, often used to observe the motility of bacteria.

Biochemical analysis

Biochemical tests are the tests used for the identification of bacteria species based on the differences in the biochemical activities of different bacteria. Biochemical testing necessitates the determination of different parameters, and the identification of the main biological chemical compounds. Sugar fermentation test, nitrate reduction test, citrate utilization test, MR test, VP test, urease test and starch hydrolysis test were performed.

Bacterial DNA Isolation

DNA isolation is the process of purification of DNA from sample using a combination of physical and chemical methods.

1. Bacterial culture (2ml) were transferred into a micro centrifuge tube and centrifuged for 10minutes at 8000rpm.

2. The supernatant were discarded and the pellets were resuspended in 875µL of Tris- EDTA buffer. Lysis buffer (100µL) was added and mixed well.

3. The tube was incubated it at 37°C for 1hour.

4. Equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) mixture were added to the tubes and mixed properly by inverting the tubes.

5. The tube was centrifuged at 10000rpm for 10 minutes.

6. Upper aqueous layer was collected in a fresh tube.

7. Repeated the process twice.

8. Equal volume of chloroform was added and spinned for 5 minute at 10000rpm.

9. Upper layer was transferred into a fresh tube.

10. 3M sodium acetate (0.1 ML) and double volume isopropyl alcohol were added to the tube.

11. Centrifuged at 10000rpm for 10minutes.

12. Decanted the supernatant.

13. The supernatant were discarded and 1ml 70% ethanol were added and mixed well. The pellets were air

Table 1: PCR reaction mixture.

Sl No	Components	Volume(µl)
1	10 X reaction mixture	5
2	10mM dNTP	5
3	Taq polymerase	0.5
4	Forward primer	1
5	Reverse primer	1
6	Template DNA	2
7	Nuclease free water	25.5
	Total	40

Table 2: PCR Programme for the amplification of the DNA.

Reaction	Duration & Temperature
Initial denaturation	94^0C for 3 min
Denaturation	94^0C for 1 min
Primer annealing	52^0C for 35 sec
Extension	72^0C for 45 sec
34 cycles repeats	
Final extension	72^0C for 10 min
4^0C for storage	

Table 3: Preliminary phytochemical screening of bark extracts of *Alstonia venenata* R. Br.

Test	Water	Butanol
Tannins - Ferric chloride Test	-	-
Saponins - Foam Test	+	-
Flavanoids - Alkaline test	+	+
Glycosides - Keller-killiani test	-	-
Alkaloids - Hager's Test	+	+
Carbohydrate - Benedict's test	+	-
Proteins - Xanthoprotein Test	+	-
Phenols - Ferric chloride Test	-	-

Note: + Present; - Absent of photochemical compound.

dried.

14. The pellet was dissolved in minimum volume of TE buffer (100µL) and store at -20°C.

15. The quality and quantity of the isolated DNA was determined by using agarose gel electrophoresis (1%).

PCR

PCR is a technique that takes specific sequence of DNA of small amount and amplifies it to be used for further testing. PCR was performed by preparing reaction mixture table 1 and optimum conditions were provided table 2. Agarose gel electrophoresis is performed to confirm the amplification of the DNA fragments.

Sequence Analysis and Sequence Similarity Search

Methodologies used include sequence alignment, searches against biological databases, and others. Comparing these new sequences to those with known functions is a key way of understanding the biology of an organism from which the new sequence comes. FASTA and BLAST were used for sequence similarity search.

Plasmid isolation

Plasmid is an extra chromosomal double stranded self-replicating DNA molecule. The genes carried in plasmids provide bacteria with genetic advantages, such as antibiotic resistance. The procedure for isolation of plasmid DNA as follows:

1. Sterile nutrient broth was inoculated with single bacterial colony and incubated overnight at 30^0 C.

2. The broth (1.5ml) were transferred to centrifugation tube and centrifuged at 10,000 rpm for 1 minute.

Table 4: Optimization of temperature.

	30°C	32°C	34°C	36°C	38°C
C1	TNTC	80 colony	Nil	Nil	Nil
C2	TNTC	70 colony	Nil	Nil	Nil
C3	TNTC	80 colony	Nil	Nil	Nil
C4	TNTC	TNTC	Nil	Nil	Nil
C5	TNTC	TNTC	Nil	Nil	Nil

Note: TNTC - To numerous to count.

Table 5: Optimization of pH.

	1	3	7	8	9
C1	Nil	Nil	TNTC	50 colonies	70 colonies
C2	Nil	Nil	TNTC	83 colonies	TNTC
C6	Nil	Nil	TNTC	TNTC	TNTC
C9	Nil	Nil	TNTC	60 colonies	85 colonies
C12	Nil	Nil	TNTC	TNTC	50 colonies

Table 6: Gram staining and motility test.

Colony	Gram staining	Shape	Motility
C1	Gram positive	Ovoid	Non motile
C2	Gram positive	Rod	Motile
C3	Gram positive	Rod	Motile
C4	Gram negative	Rod	Motile
C5	Gram positive	Rod	Motile

Table 7: Biochemical test.

Test	C1	C2	C3	C4	C5	*E. coli*	RC1	RC2	RC4	RC5
Sugar fermentation	-	+	+	+	+	+	-	-	-	+
Nitrate reduction	-	+	+	-	+	+	-	-	-	+
Citrate utilization	+	+	+	+	+	-	+	+	+	+
VP test	-	-	-	-	-	-	-	-	-	-
MR test	+	+	+	+	+	+	+	+	+	-
Urease test	-	-	-	-	-	-	-	-	-	-
Starch hydrolysis	+	+	+	+	+	+	+	-	-	+

Table 8: *Nocardia callitridis.*

Phylum	Actinobacteria
Class	Actinobacteria
Order	Actinomycetales
Family	Nocardiaceae
Gram staining	Positive
Cell length	0.8im
Cell width	0.7im
Cell shape	Ovoid
Motility	No
Catalase enzyme	Positive
Oxygen tolerance	Aerobic
Spore formation	Positive
Optimum temperature	28°C
Optimum pH	5-10
GC content	68.7 mol %

Table 9: *Sporolactobacillus spathodeae.*

Phylum	Firmicutes
Class	Bacilli
Order	Bacillales
Family	Sporolactobacillaceae
Gram staining	Positive
Cell length	1.6-5.1im
Cell width	0.3-0.4im
Cell shape	Straight rod
Motility	Yes
Acid production	Positive
Oxygen tolerance	Facultative anaerobic
Spore formation	Positive
Optimum temperature	30°C
Optimum pH	5-8
Salt tolerance	9%

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Nocardia callitridis strain CAP 290 16S ribosomal RNA, partial sequence	2560	2560	100%	0.0	100.00%	NR_116818.1
Nocardia alboflava partial 16S rRNA gene, type strain YIM 37293T = CCTCC AA 205004T	2449	2449	100%	0.0	98.56%	AM295159.1
Nocardia endophytica strain KLBMP 1256 16S ribosomal RNA, partial sequence	2435	2435	100%	0.0	98.34%	NR_108990.1
Nocardia sp. X1654 16S ribosomal RNA gene, partial sequence	2368	2368	100%	0.0	97.48%	KM233637.1
Nocardia nova gene for 16S ribosomal RNA, partial sequence, strain: 6-17	2361	2361	100%	0.0	97.40%	AB546312.1
Nocardia nova strain S556 16S ribosomal RNA gene, complete sequence	2361	2361	100%	0.0	97.40%	AF430029.1
Nocardia sp. SPNT22 16S ribosomal RNA gene, partial sequence	2355	2355	100%	0.0	97.33%	KU382351.1
Nocardia sp. NTSP21 16S ribosomal RNA gene, partial sequence	2355	2355	100%	0.0	97.33%	KU382341.1
Nocardia nova strain MFB025 16S ribosomal RNA gene, partial sequence	2355	2355	100%	0.0	97.33%	KU356872.1
Nocardia nova strain APN00023 16S ribosomal RNA gene, partial sequence	2355	2355	100%	0.0	97.33%	KC262097.1
Nocardia nova gene for 16S rRNA, partial sequence, strain: IFM 11287	2355	2355	100%	0.0	97.33%	AB636656.1
Nocardia nova gene for 16S rRNA, partial sequence, strain: IFM 11125	2355	2355	100%	0.0	97.33%	AB632401.1
Nocardia nova gene for 16S rRNA, partial sequence, strain: IFM 11104	2355	2355	100%	0.0	97.33%	AB630968.1
Nocardia sp. D27 16S ribosomal RNA gene, partial sequence	2355	2355	100%	0.0	97.33%	HQ241933.1
Nocardia sp. YIM 65653 16S ribosomal RNA gene, partial sequence	2355	2355	100%	0.0	97.33%	GU367176.1
Nocardia nova strain ATCC 33726 16S ribosomal RNA, partial sequence	2355	2355	100%	0.0	97.33%	NR_117343.1
Nocardia nova gene for 16S rRNA, partial sequence, strain:IFM 10265	2355	2355	100%	0.0	97.33%	AB162790.1
Nocardia nova gene for 16S rRNA, partial sequence, strain:IFM 0272	2355	2355	100%	0.0	97.33%	AB162784.1
Nocardia nova strain JCM 6044 16S ribosomal RNA, partial sequence	2355	2355	100%	0.0	97.33%	NR_041858.1
N.nova gene for 16S ribosomal RNA	2355	2355	100%	0.0	97.33%	Z36930.1

Fig. 1: Sequence similarity search result of C1.

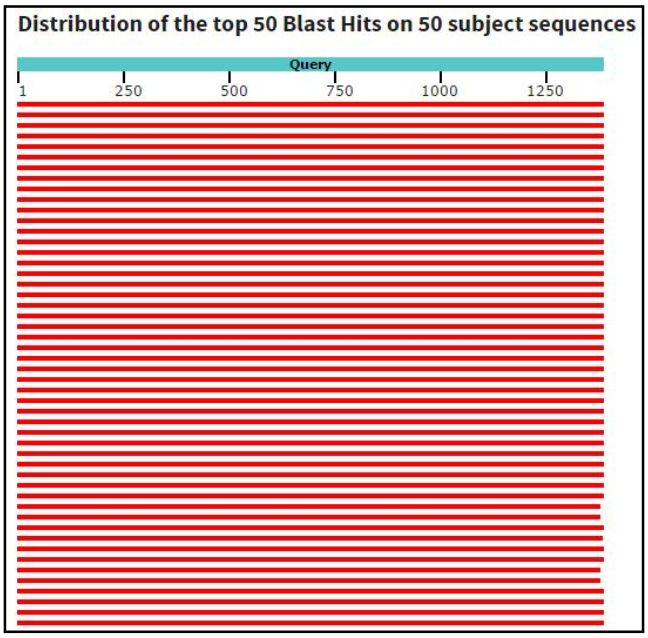

Fig. 2. BLAST hits of C1.

3. The supernatant were discarded and the pellets were resuspended in 100 µl ice cold suspension buffer. The tubes were incubated on ice for 15 minutes.

4. Freshly prepared lysis buffer (20 µl) was added and mixed by inverting the tubes and incubated on ice for 5 minutes.

Table 10: *Bacillus subtilis.*

Phylum	Firmicutes
Class	Bacilli
Order	Bacillales
Family	Bacillaceae
Gram staining	Positive
Cell length	4-10ìm
Cell width	0.25-1ìm
Cell shape	Rod
Motility	Yes
Acid production	Positive
Oxygen tolerance	Obligate aerobic
Spore formation	Positive
Optimum temperature	30℃
Optimum pH	5-8
Salt tolerance	9%

5. Ice cold 5 M potassium acetate solution (150µl) was added and centrifuged at 10,000 rpm for 5 minutes.

6. The supernatants were transferred into fresh centrifugation tubes.

7. Mixture of chloroform isoamyl alcohol (2:1) (0.5 ml) was added to the supernatant and mixed well by inverting the tubes and centrifuges at 12, 000 rpm for 5 minutes.

8. Twice volume of ice cold absolute isopropanol was

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Sporolactobacillus spathodeae strain BK117-1 16S ribosomal RNA, partial sequence	2495	2495	100%	0.0	100.00%	NR_134816.1
Sporolactobacillus spathodeae gene for 16S ribosomal RNA, partial sequence, strain: NN3-1	2490	2490	100%	0.0	99.93%	LC062597.1
Sporolactobacillus spathodeae gene for 16S ribosomal RNA, partial sequence, strain: NN3-2	2484	2484	100%	0.0	99.85%	LC062598.1
Sporolactobacillus sp. 22-1-A gene for 16S ribosomal RNA, partial sequence	2383	2383	99%	0.0	98.45%	AB549327.1
Sporolactobacillus sp. 2-1-A gene for 16S ribosomal RNA, partial sequence	2381	2381	99%	0.0	98.59%	AB549328.1
Uncultured bacterium clone DB30 16S ribosomal RNA gene, partial sequence	2366	2366	99%	0.0	98.30%	KT337627.1
Sporolactobacillus sp. 30-1 gene for 16S ribosomal RNA, partial sequence	2362	2362	100%	0.0	98.16%	AB549329.1
Uncultured bacterium clone DB25 16S ribosomal RNA gene, partial sequence	2361	2361	99%	0.0	98.22%	KT337626.1
Sporolactobacillus sp. P3J 16S ribosomal RNA gene, partial sequence	2302	2302	100%	0.0	97.41%	HQ285998.1
Sporolactobacillus putidus strain QC81-06 16S ribosomal RNA, partial sequence	2279	2279	99%	0.0	97.11%	NR_112774.1
Sporolactobacillus shoreae strain BK92 16S ribosomal RNA, partial sequence	2278	2278	99%	0.0	97.11%	NR_134815.1
Sporolactobacillus sp. MB-051 gene for 16S ribosomal RNA, partial sequence	2278	2278	99%	0.0	97.11%	AB548940.1
Sporolactobacillus sp. MB-025 gene for 16S ribosomal RNA, partial sequence	2270	2270	99%	0.0	97.04%	AB548941.1
Sporolactobacillus nakayamae subsp. racemicus gene for 16S rRNA, partial sequence, strain: NRIC 0349 (= ATCC 700381, = JCM 3417)	2270	2270	99%	0.0	97.04%	AB362636.1
Sporolactobacillus terrae strain M-116 16S ribosomal RNA, partial sequence	2270	2270	99%	0.0	97.10%	NR_115531.1
Sporolactobacillus sp. strain JBARES-F-2 16S ribosomal RNA gene, partial sequence	2268	2268	100%	0.0	96.97%	KX622696.1
Sporolactobacillus terrae strain JCM 3516 16S ribosomal RNA, partial sequence	2266	2266	99%	0.0	96.96%	NR_112772.1
Sporolactobacillus terrae strain NBRC 101527 16S ribosomal RNA, partial sequence	2266	2266	99%	0.0	96.96%	NR_112762.1
Sporolactobacillus terrae gene for 16S ribosomal RNA, partial sequence, strain: BK70-2	2265	2265	99%	0.0	96.96%	LC150755.1
Sporolactobacillus terrae gene for 16S ribosomal RNA, partial sequence, strain: BK70-1	2265	2265	99%	0.0	96.96%	LC150754.1
Sporolactobacillus nakayamae subsp. racemicus gene for 16S rRNA, partial sequence, strain: NRIC 0356	2265	2265	99%	0.0	96.96%	AB362639.1

Fig. 3: Sequence similarity search result of C2.

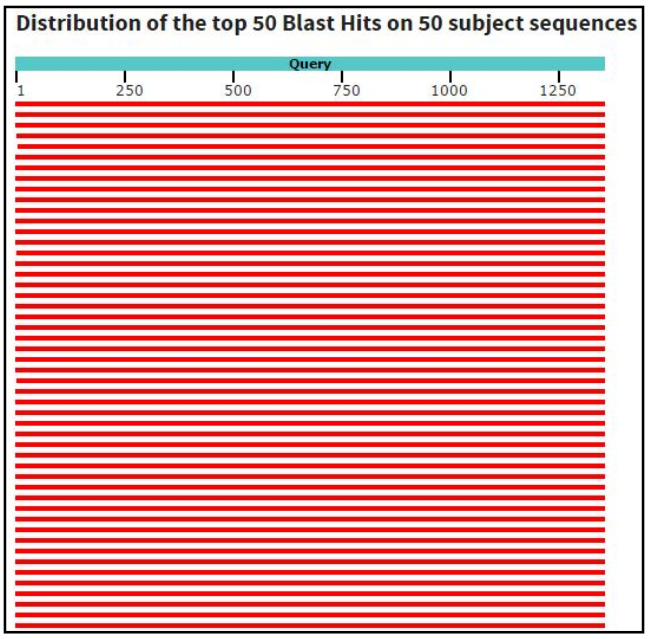

Fig. 4. BLAST hits of C2.

added to the supernatant and mixed well. the tubes were centrifuges at 12,000 rpm for 5 minutes.

9. The supernatant was discarded and pellets were resuspended in 1 ml of isopropanol and centrifuged the mixture at 12,000 rpm for 5 minutes.

10. The supernatant were discarded and the pellets were air dried.

Transformation

Transformation is the process by which foreign DNA is introduced into a cell. Transformation is carried out by

Table 11: *Methylobacterium platani.*

Phylum	Proteobacteria
Class	Alphaproteobacteria
Order	Rhizobials
Family	Methylobacteriaceae
Gram staining	Negative
Cell length	1.6-5.1ìm
Cell width	0.2-1.5ìm
Cell shape	Straight rod
Motility	Yes
Catalase activity	Positive
Oxygen tolerance	Aerobic
Spore formation	Positive
Optimum temperature	20-30℃
Optimum pH	6-8
Salt tolerance	Less than 1%

inserting plasmid DNA of endophytes into *E. coli* cells. *E. coli* is serves as the recipient and endophytes are donors of the plasmid.

1. The competent cells were prepared by using calcium chloride.

2. Plasmid DNA (5µl) was added to 2 ml of competent cells and mixed well. The tubes were incubated on ice for 20 minutes.

3. Heat shock was provided by placing the vials in water bath at 28^0 C for 2 minutes.

4. The cells were incubated in ice for 5 minutes.

5. LB broth (1ml) was added to the cells and incubated at 28^0 C for half an hour.

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Bacillus sp. K2(2014) 16S ribosomal RNA gene, partial sequence	2693	2693	100%	0.0	100.00%	KJ130059.1
Bacillus sp. (in: Bacteria) strain QS15-17 16S ribosomal RNA gene, partial sequence	2639	2639	98%	0.0	99.79%	MH769188.1
Bacillus subtilis strain SR3-30 16S ribosomal RNA gene, partial sequence	2638	2638	98%	0.0	99.86%	MN421487.1
Bacillus sp. WPCB092 16S ribosomal RNA gene, partial sequence	2638	2638	98%	0.0	99.86%	FJ006890.1
Bacillus sp. WPCB033 16S ribosomal RNA gene, partial sequence	2638	2638	98%	0.0	99.86%	FJ006875.1
Bacillus tequilensis strain SH41 16S ribosomal RNA gene, partial sequence	2634	2634	98%	0.0	99.72%	KC172031.1
Bacillus subtilis strain DL3 16S ribosomal RNA gene, partial sequence	2632	2632	98%	0.0	99.79%	MT043898.1
Bacillus subtilis strain Cpl4 16S ribosomal RNA gene, partial sequence	2632	2632	98%	0.0	99.79%	MN960273.1
Bacillus subtilis strain 2014-3557 chromosome, complete genome	2632	26292	98%	0.0	99.79%	CP045672.1
Bacillus subtilis strain SRCM101393 chromosome, complete genome	2632	23621	98%	0.0	99.79%	CP031693.1
Bacillus subtilis strain SRCM102756 chromosome, complete genome	2632	26265	98%	0.0	99.79%	CP028218.1
Bacillus subtilis strain SRCM102751 chromosome, complete genome	2632	26265	98%	0.0	99.79%	CP028217.1
Bacillus subtilis strain SRCM102750 chromosome, complete genome	2632	26281	98%	0.0	99.79%	CP028215.1
Bacillus subtilis strain SRCM102749 chromosome, complete genome	2632	26274	98%	0.0	99.79%	CP028213.1
Bacillus subtilis strain SRCM102748 chromosome, complete genome	2632	23626	98%	0.0	99.79%	CP028212.1
Bacillus subtilis strain SRCM102745 chromosome, complete genome	2632	26287	98%	0.0	99.79%	CP028209.1
Bacillus subtilis strain SRCM102754 chromosome, complete genome	2632	26265	98%	0.0	99.79%	CP028202.1
Bacillus subtilis strain SRCM102753 chromosome, complete genome	2632	26242	98%	0.0	99.79%	CP028201.1
Bacillus subtilis strain 1-17 16S ribosomal RNA gene, partial sequence	2632	2632	98%	0.0	99.86%	MN938175.1
Bacillus subtilis strain BJQ0005 chromosome, complete genome	2632	26325	98%	0.0	99.79%	CP047485.1
Bacillus subtilis strain 7PJ-16 chromosome, complete genome	2632	26292	98%	0.0	99.79%	CP023409.1

Fig. 5: Sequence similarity search result of C3.

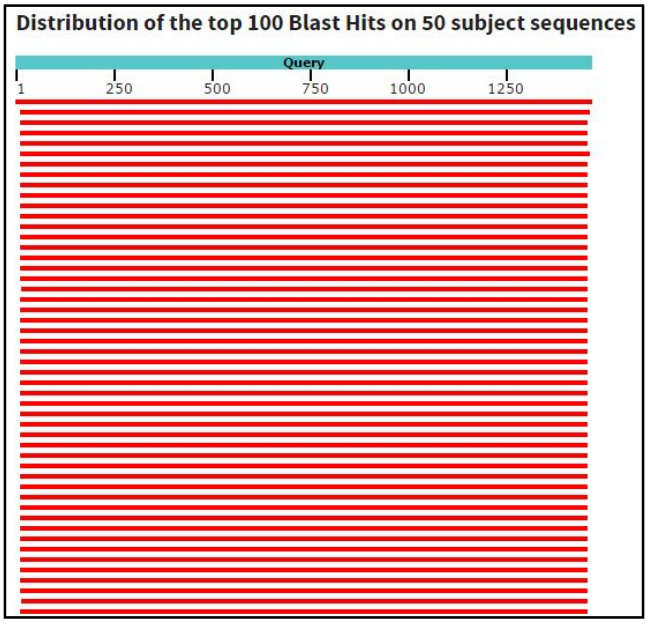

Fig. 6. BLAST hits of C3.

The transformation was confirmed by antibiotic sensitivity test. *E. coli* cells used for this study are sensitive to chloramphenicol, where all the five endophytes are resistant to chloramphenicol. Hence chloramphenicol is used to detect the presence of transformed *E. coli* cells. Sugar fermentation test, nitrate reduction test, citrate utilization test, MR test, VP test, urease test and starch hydrolysis test were performed in recombinant cells.

Table 12: C5 *Paenibacillus cathormi.*

Phylum	Firmicutes
Class	Bacilli
Order	Bacillales
Family	Paenibacillaceae
Gram staining	Positive
Cell shape	Rod
Motility	Motile
Acid production	Positive
Oxygen tolerance	Aerobic
Spore formation	Yes
Optimum temperature	20-30°C
Optimum pH	7-9

Results

Phytochemical Analysis

Saponins, proteins and carbohydrates were present in water and negative in butanol. Flavanoids and alkaloids were present in both water and butanol. Tannins, glycosides and phenols were show negative results in

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Methylobacterium platani strain PMB02 16S ribosomal RNA, partial sequence	2641	2641	100%	0.0	100.00%	NR_044211.1
Methylobacterium sp. 20 16S ribosomal RNA gene, partial sequence	2501	2501	99%	0.0	98.32%	JF905619.1
Methylobacterium sp. RB978 gene for 16S rRNA, partial sequence, isolate: RB978	2497	2497	99%	0.0	97.97%	AB252210.1
Uncultured alpha proteobacterium clone Vsin1-23 16S ribosomal RNA gene, partial sequence	2490	2490	99%	0.0	98.11%	KM978266.1
Methylobacterium aquaticum DNA, complete genome, strain: MA-22A	2488	24630	99%	0.0	98.11%	AP014704.1
Methylobacterium sp. Hojyo1 gene for 16S rRNA, partial sequence, isolate: Hojyo1	2488	2488	99%	0.0	98.04%	AB252204.1
Methylobacterium platani gene for 16S ribosomal RNA, partial sequence, strain: KB2	2486	2486	99%	0.0	98.11%	LC025992.1
Uncultured alpha proteobacterium clone CIIWT6-24 16S ribosomal RNA gene, partial sequence	2484	2484	99%	0.0	98.04%	KM978303.1
Methylobacterium platani gene for 16S ribosomal RNA, partial sequence, strain: KK13	2484	2484	99%	0.0	98.11%	LC025998.1
Methylobacterium sp. LS-736 16S ribosomal RNA gene, partial sequence	2484	2484	99%	0.0	98.18%	KP162070.1
Methylobacterium sp. strain Isolate_32S1 16S ribosomal RNA gene, partial sequence	2483	2483	99%	0.0	98.04%	MN982845.1
Methylobacterium terrae strain 17Sr1-28 chromosome, complete genome	2483	22088	99%	0.0	97.92%	CP029553.1
Uncultured bacterium clone BJ201110-32 16S ribosomal RNA gene, partial sequence	2479	2479	99%	0.0	98.11%	KX507412.1
Uncultured bacterium clone B112-70 16S ribosomal RNA gene, partial sequence	2479	2479	99%	0.0	98.11%	KF010770.1
Methylobacterium sp. strain DB0501 16S ribosomal RNA gene, partial sequence	2477	2477	99%	0.0	98.17%	MN396448.1
Methylobacterium currus strain PR1016A chromosome 1	2477	22201	99%	0.0	97.97%	CP028843.1
Methylobacterium sp. CP-2018 strain MP1016A_00022 16S ribosomal RNA gene, partial sequence	2477	2477	99%	0.0	97.97%	MH158285.1
Methylobacterium platani gene for 16S ribosomal RNA, partial sequence, strain: KB7	2477	2477	99%	0.0	97.97%	LC025994.1
Methylobacterium sp. AMS19 gene for 16S rRNA, partial sequence	2477	2477	99%	0.0	97.97%	AB600008.1
Methylobacterium sp. F91 16S ribosomal RNA gene, partial sequence	2477	2477	99%	0.0	98.11%	HQ680432.1

Fig. 7: Sequence similarity search result of C4.

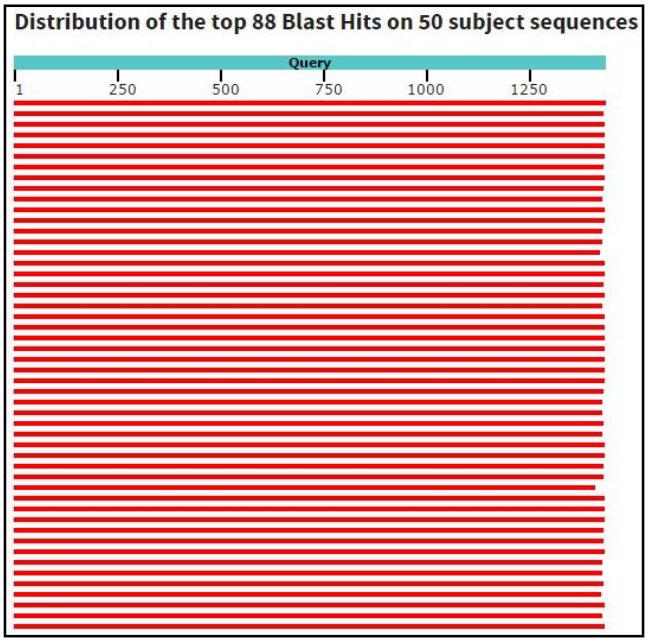

Fig. 8. BLAST hits of C4.

both solvents table 3.

Isolation of Endophytes

Four colonies were observed in the nutrient agar plate (C2, C3, C3 and C4) and a single colony in the Mac Conky agar plate (C1). Colonies were absent in EMB agar medium and on urea agar medium.

Optimization of Growth

Optimum temperature for the growth of endophytes is 30^0 C and optimum pH is 7. table 4 and 5.

Identification of Endophytes

Gram staining and motility test

All the endophytic bacteria were gram positive except C4. Except C1 all other bacteria were rod shaped. C1 bacteria were ovoid in shape. All the endophytic bacteria were motile except C1 table 6.

Biochemical analysis

The C1 shows positive results in for the citrate utilization test, MR test and the starch hydrolysis test. C2 shows positive results in sugar fermentation test, nitrate reduction test, citrate utilization test, MR test and starch hydrolysis test. C3 shows positive results in sugar fermentation test, nitrate reduction test, citrate utilization test, MR test and starch hydrolysis test. C4 shows positive results in sugar fermentation test, citrate utilization test, MR test and starch hydrolysis test. The C5 shown positive results in sugar fermentation test, nitrate reduction test, citrate utilization test, MR test and starch hydrolysis test table 7.

Sequence analysis

The DNA isolates were amplified by PCR and sequenced to identify the species of organism. The C1 is *Nocardia* sp, which shows greatest similarity to *Nocardia*

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Paenibacillus cathormii 16S ribosomal RNA, partial sequence	2638	2638	100%	0.0	100.00%	NR_148814.1
Paenibacillus timonensis strain N67 16S ribosomal RNA gene, partial sequence	2274	2274	96%	0.0	96.32%	KP410787.1
Paenibacillus timonensis strain N41 16S ribosomal RNA gene, partial sequence	2274	2274	96%	0.0	96.32%	KP410785.1
Paenibacillus timonensis strain N5 16S ribosomal RNA gene, partial sequence	2274	2274	96%	0.0	96.32%	KP410782.1
Paenibacillus timonensis strain 3584BRRJ 16S ribosomal RNA gene, partial sequence	2274	2274	96%	0.0	96.32%	JF309265.1
Paenibacillus timonensis strain N44 16S ribosomal RNA gene, partial sequence	2272	2272	96%	0.0	96.32%	KP410789.1
Paenibacillus sp. C39 16S ribosomal RNA gene, partial sequence	2270	2270	96%	0.0	96.33%	KF479621.1
Paenibacillus sp. strain RT10 16S ribosomal RNA gene, partial sequence	2268	2268	96%	0.0	96.32%	MK014240.1
Paenibacillus timonensis strain N34 16S ribosomal RNA gene, partial sequence	2268	2268	96%	0.0	96.25%	KP410790.1
Paenibacillus timonensis strain N23 16S ribosomal RNA gene, partial sequence	2268	2268	96%	0.0	96.25%	KP410788.1
Paenibacillus timonensis strain N42 16S ribosomal RNA gene, partial sequence	2268	2268	96%	0.0	96.31%	KP410783.1
Paenibacillus sp. MS5-14 16S ribosomal RNA gene, partial sequence	2268	2268	96%	0.0	96.31%	KC117520.1
Paenibacillus timonensis strain SCTB120 16S ribosomal RNA gene, partial sequence	2268	2268	96%	0.0	96.32%	JN650285.1
Paenibacillus sp. Marseille-P2973 partial 16S rRNA gene, strain Marseille-P2973	2266	2266	96%	0.0	96.31%	LT598593.1
Paenibacillus timonensis strain MER_TA_27 16S ribosomal RNA gene, partial sequence	2266	2266	96%	0.0	96.25%	KT719432.1
Paenibacillus sp. strain XAAS.x162 16S ribosomal RNA gene, partial sequence	2263	2263	96%	0.0	96.25%	MN187276.1
Paenibacillus timonensis strain PF4H_2.1 16S ribosomal RNA gene, partial sequence	2263	2263	96%	0.0	96.18%	KT720082.1
Paenibacillus timonensis strain N63 16S ribosomal RNA gene, partial sequence	2263	2263	96%	0.0	96.30%	KP410786.1
Paenibacillus timonensis strain N1 16S ribosomal RNA gene, partial sequence	2263	2263	96%	0.0	96.24%	KP410784.1
Paenibacillus sp. strain KCOM 3021 16S ribosomal RNA gene, partial sequence	2259	2259	96%	0.0	96.19%	MK748165.1

Fig. 9: Sequence similarity search result of C5.

Fig. 10. BLAST hits of C5.

callitridis. C2 is *Sporolactobacillus spathodeae,* C3 *Bacillus subtilis,* C4 *Methylobacterium platani,* C5 *Paenibacillus cathormi* respectively tables 8 - 12 Fig. 1-10.

Transformation

E. coli cells were transformed by the insertion of plasmid DNA of endophytes. The transformation is confirmed by antibiotic sensitivity test. Transformed *E coli* were grown in the medium containing chloramphenicol. The recombinants are named as RC1, RC2, RC4 and RC5. The transformed *E coli* show variation in biochemical test. In sugar fermentation test the donor strain and recipient strain of recombinant *E coli,* RC2, RC4 were shown positive result but the recombinant shows negative results. In nitrate reduction test the donor and recipients of the recombinant RC2 were shows positive result, but the recombinant shows negative results. The donor and recipient strains of RC2 and RC4 were starch hydrolyzing organisms, recombinants shows variation from their parents. RC2 and RC4 shows negative result in starch hydrolyisis test table 7.

Discussion

Phytochemical compounds like saponins, proteins, carbohydrate, alkaloid and flavanoids were present in the bark of *A. venaneta*. Endophytic bacterias including *Nocardia callitridis, Sporolactobacillus spathodeae, Bacillus subtilis, Mycobacterium platani and Paenibacillus cathormi* were isolated from the bark of *A. venanata*. The optimum temperature and pH for the growth of these microbes were 30^0 C and 7 respectively.

The transformation of the plasmids of these endophytes to *E. coli* reveals that the recombinants *(*RC2 and RC4) show variations from both the donor and recipient.

Conclusion

The result of the study reveals the presence of endophytes and phytochemicals in the bark of *A. venaneta*. The endophytes present in the plants have the ability to alter the nature of the host cells. In future the activity of endophytes on the production of bioactive compounds in *A. venaneta* can be studied and the bioactive components can be produced industrially without exploiting the host plant.

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References

Chen, S.L., H. Yu, H.M. Luo, Q. Wu, C.F. Li and A. Steinmetz (2016). Conservation and sustainable use of medicinal plants: problems progress and prospects. *Chinese Medicine*, **11:** 37.

Halberstein, R.A. (2005). Medicinal plants: historical and cross cultural usage patterns. *Ann. Epidemiol.,* **15(9):** 686-99.

Jack, D.B. (1997). One hundred years of aspirin. *Lancet*, **350(9075):** 437-439.

Jamshidi-Kia, F., Z. Lorigooini and H. Amini-Khoei (2018). Medicinal plants: Past history and future perspective. *J. Herbmed. Pharmacol*., **7(1):** 1-7.

Khan, M.A. (2016). Introduction and importance of medicinal plants and herbs. National health portal.

Kusari, S., C. Hertweck and M. Spiteller (2012). Chemical ecology and endophytic fungi: origins of secondary metabolites. *Chemistry and Biology*, **19:** 792-798.

Pant, S.C. and G. Pandey (1995). Ethnobotanical studies on medicinal flora in Tharu tribal pockets in Kumaon region in Uttar Pradesh. B.M.E.B.R, XVI pp. 1-10.

Prakasha, H.M., M. Krishnappa, Y.L. Krishnamurthy and S.V. Poornima (2010). Folk medicine of NR Purataluk in Chikmagalur district of Karnataka. *Ind. J. Trad. Knowl*., **9(1):** 55-60.

Prasad, P.N., A.W. Jabadhas and E.K. Janakiammal (1987). Medicinal plants used by the Kanikkars of South India. *J. Econ. Tax. Bot*., **11:** 149 -155.

Smith-Hall, C., H.O. Larsen and M. Pouliot (2012). "People, plants and health: a conceptual framework for assessing changes in medicinal plant consumption". *J. Ethnobiol. Ethnomed*., **8:** 43.

Venieraki, A., M. Dimou and P. Kartinakis (2017). Endophytic fungi residing in medicinal plants have the ability to produce the same or similar pharmacologically active secondary metabolites as their hosts. *Hellenic Plant Protection Journal*, **10:** 51-66.