Original Research Article

Taxonomic Profile And Antimicrobial Potential Of *BrachybacteriumConglomeratum*VLCH–15 Isolated From Mangroves Of Machilipatnam, Andhra Pradesh, India

ABSTRACT: Actinobacteria of mangrove origin become a vital source of novel antibiotics with high therapeutic potential. Hence, the current study has been focused on antimicrobial potential of secondary metabolites produced by rare actinobacterium isolated from mangrove ecosystemof Machilipatnam, Andhra Pradesh, India. Sediment samples collected from mangrove habitats were shade dried at room temperature and employed for isolation of actinobacteria by dilution plate technique. Yeast extract malt extract dextrose (YMD) agar, starch casein agar (SCA), actinomycetes isolation agar (AIA) and humic acid vitamin agar (HV) media amended with NaCl @3% were employed for isolation of actinobacteria. A total of 15 actinobacterial strains were isolated and coded as VLCH-1 to VLCH-15. All the isolates were screened for their antimicrobial metabolite production against test bacteria and fungi. Out of 15 strains, VLCH-15 exhibited broad spectrum of antagonistic activity. Based on polyphasic taxonomic studies, the strain was identified as *Brachybacterium conglomeratum* VLCH-15.The growth curve and antimicrobial profile of *B. conglomeratum* VLCH-15were studied at regular intervals up to 10 days in batch culture. The secondary metabolites obtained from 7-day-old culture showed high antimicrobial activity against *X. campestris, E. coli* and *A. flavus.* Among the fungi tested,*Aspergillus flavus* showed high sensitivity. Further studies on optimization, purification and characterization of bioactive metabolites of *B. conglomeratum* VLCH-15are in progress.

Key words: Mangrove actinobacteria, Polyphasic taxonomy, Brachybacteriumconglomeratum VLCH-15, Antagonistic activity.

1. INTRODUCTION

The discovery of novel microbes that produce new secondary metabolites can be expected to remain significant in the race against emerging diseases and antibiotic resistant pathogens. The excess use of large amounts of antibiotics resulted in microbial pathogens become resistant to multiple drugs and developed into Multi-Drug Resistant (MDR) microbes. The development of MDR microbes leads to facing difficulties in their treatment. Because of this reason, we are searching for new bioactive compounds from under explored mangrove niches that can have greater growth controlling capabilities.

Mangroves are woody and saline resistant forest ecosystems with extreme environment. Mangrove habitats show extreme variations in available nutrients, light, oxygen concentration, pressure, salinity and temperature. These soils provide a unique ecological niche for the growth of diversified microorganisms especially actinobacteria which have unique biochemical metabolic and physiological capabilities and also provide potential for the production of novel metabolites (Bull&Stach, 2007; Skropeta& Wei,2014).
Mangrove actinobacteria are widely distributed group, they are the most economically important and biotechnologically valuable prokaryotes as potential provider of novel antimicrobial agents and have the capacity to synthesis many biologically active natural compounds. The secondary metabolites like

antibiotics, herbicides, pesticides, anti-parasitic and enzyme inhibitors are obtained from actinobacteria, they possess diverse biological activities like antimicrobial, antioxidant, antitumor and antiviral **(**Berdy, 2012; Barka*et al*., 2015). Two-third of the commercially obtained antibiotics were isolated from actinobacteria, more than 23,000 bioactive secondary metabolites are produced by the microbes, of these 10,000 compounds are produced by actinobacteria.

Actinobacteria are aerobic, branched, unicellular, Gram positive bacteria with high percentage of GC (70%) in their DNA. Members of the genus *Streptomyces* are major inhabitants of soil and 'rare actinobacteria' are isolated less frequently than that of the routinely isolated *Streptomyces*. Some of the rare actinobacteria genera are *Actinoplanes, Nocardia, Amycolatopsis, Pseudonocardia*and*Saccharomonospora* (Tiwari & Gupta, 2012; Jose &Jebakumar, 2014).Currently, the discoveries of new natural metabolites are focusing on rare actinobacteria.The bioactivity studies of natural metabolites from the mangrove rare actinobacteria have become popular. Compounds discovered from the mangrove rare actinobacteria are uniquely structured and lead directly to the development of novel antibiotics that are effective against antibiotic-resistant pathogens (Ushakiranmayi*et al.,* 2016).

It is predicted that there would be a greater demand for new bioactive compounds synthesized by rare actinobacteria. In the present study, an attempt has been made to isolate and screen the rare actinobacterium*Brachybacteriumconglomeratum*VLCH-15 from the unexplored mangroves of Andhra Pradesh (Machilipatnam), which can be wealthy valuable resource of secondary metabolites.

2. MATERIALS AND METHODS

2.1 COLLECTION OF SOIL SAMPLES AND SAMPLE PROCESSING

The mangrove soil samples were collected at a depth of 20 cm by using an aseptic metal trowel from different locations of mangrove region in Machilipatnam, Andhra Pradesh, India. The samples were randomly collected, labeled then brought to the laboratory in sterile zip log bags and stored them in refrigerator at 4ºC. Air dried samples were ground with mortar and pestle. All the samples were subjected to dry heating (50°C/30min) to control unwanted microbial contamination, pre treated with calcium carbonate (10:1 w/v) and phenol (1.5%) to enrichment of actinobacteria (Manimaran *et al.*, 2017).

2.2 ISOLATION OF MANGROVE ACTINOBACTERIA

Actinobacteria were isolated by employing dilution plate technique using selective media ISP-2 (YMD), starch casein agar (SCA), Actinomycetes isolation agar (AIA) and humic acid vitamin (HV) agar media supplemented with 3% NaCl and at pH 7.0. The four selective media were amended with rifampicin (25µg/mL) and nystatin (25µg/mL) to minimize the bacterial and fungal contamination**.** The pretreated samples were dissolved in 1ml of sterile water and diluted up to 10^{-5} dilutions. Aliquots of 0.1 ml of each dilution were spread evenly on plates of above media in triplicates. The plates were incubated at 30±2°C up to 3 weeks. After incubation, the plates were examined for actinobacteria colonies based on color, dryness, rough, convex colony**.** The fifteen prominent colonies were selected, purified and maintained on YMD slants at 4ºC for further study(Mangamuri*et al.,* 2019).

2.3 PRELIMINARY SCREENING OF ACTINOBACTERIA FOR ANTIMICROBIAL ACTIVITY

All the isolated actinobacteria were screened for their antagonistic activity against bacterial and fungal pathogens by using primary screening (Cross streak technique). Activities were assessed using nutrient agar for test bacteria and Czapek-Dox for test fungi. The actinobacteria were streaked at the centre of plates and test organisms were streaked perpendicularly. The plates were incubated at room temperature for 24h for bacteria and 48h for fungi. After incubation, the potent isolates were selected based on the antagonism against each test organism.

2.4 SECONDARY SCREENING OF POTENT ACTINOBACTERIAL STRAINS FOR BIOACTIVE METABOLITES

Based on primary screening, a total of eight predominant actinobacterial isolates were selected to screen their antimicrobial potential for bioactive compounds. The pure cultures of the strains were transferred

aseptically into the seed medium (YMD broth). After 24h of incubation, the seed culture was inoculated into the production medium of the same composition. Fermentation was carried out for one week at room temperature under agitation at 120rpm. The flasks were harvested and the biomass was separated from the broth. Antimicrobial crude compounds were recovered from the filtrate by solvent extraction with ethyl acetate in the ratio of 1:1 (v/v). Solvent extract was evaporated to dryness in water bath and the precipitate obtained was used to determine antimicrobial activity by agar well-diffusion method (Janaki, 2016). In this method, 8 mm diameter wells were made on plates by using sterile steel cork borer. Further, each well were loaded with 80µL of crude extracts and incubated at 35°C for 24 h. and streptomycin was used as positive control(Swapna & Vijayalakshmi, 2019). After incubation, the zone of inhibition was expressed as mm in diameter.

2.5 POLYPHASIC TAXONOMY OF THE POTENT ACTINOBACTERIAL STRAIN

2.5.1 PHENOTYPIC CHARACTERIZATION

The potent actinobacterial strain was characterized by cultural, morphological, physiological, biochemical and molecular methods. The nature of mycelium and spore arrangement of the strain was observed by slide culture method under a compound microscope (model Motic- BA410)(Kavitha, 2010; Pridham & Lyons, 1980).

In SEM microscopy, the morphological characteristics of 7-day old culture grown on YMD agar were assessed at various magnifications of scanning electron microscope. For SEM, samples were fixed in 2.5% glutaraldehyde in phosphate buffer (0.1M; pH 7.2) at 4ºC for 24 h and post fixed in osmium tetroxide (2%) for 4h. Samples were carefully affixed onto the copper stud, dehydrated with serial grades of 30, 50, 70 and 90% alcohol. The studs were then kept in desiccators for final drying. The surface containing organisms was coated with a gold film about 150-200Å thickness and observed under SEM for spore surface ornamentation (Williams & Davies, 1967).

The cultural characteristics such as color of both aerial and substrate mycelia, colony texture were examined by culturing the potent strain on seven ISP media such as tryptone yeast extract agar (ISP-1), yeast extract malt extract dextrose agar (ISP-2), oat meal agar (ISP-3), inorganic salts starch agar (ISP-4) , glycerol asparagine agar (ISP-5), peptone yeast extract agar (ISP-6), tyrosine agar (ISP-7) and three non-ISP media such as actinomycetes agar, Czapek-Dox agar (CDA) and nutrient agar (NA).

2.5.2 PHYSIOLOGICAL AND BIOCHEMICAL ANALYSIS

Physiological characteristics such as the effect of pH ranges (pH 5.0-9.0), temperature (20-45°C) and different salinity concentrations (0–9%) on the growth of the strain were tested. Biochemical characteristics of the strain were studied to identify and confirm the strain at genus and species level (Naraganiet *al.,* 2020). Different biochemical testes were assessed to the potent strain VLCH-15 such as the IMVIC tests, catalase production, urease production, gelatin liquefaction, hydrolysis of starch, hydrolysis of casein, nitrate reduction and H_2S production were tested[9]. Utilization of various sources of carbohydrates were tested by culturing the strain in ISP-2 broth supplemented with 0.4% respective carbon sources and incubated for one week at 30±2°C (Mangamuriet *al.,* 2020).

2.5.3 *IN-VITRO* SCREENING FOR ANTIBIOTIC SUSCEPTIBILITY

The antibiotic sensitivity test was conducted to assess the susceptibility of the strain to different antibiotics by following the Kirby-Bauer disc diffusion method. Nine antibiotic discs *viz*., azithromycin (15µg), chloramphenicol (10µg), Gentamicin (10µg), kanamycin (30µg) were used and procured from Hi-Media Pvt. Ltd., India. The plates were inoculated with the potent actinobacteria, specified antibiotic disc was placed and incubated them at 30±2°C for 48 h. After incubation, the inhibition zones around the antibiotic discs were observed. Based on observation, the strain was considered as either sensitive (S) or resistant (R) to an antibiotic (Ragalatha, 2020).

2.5.4 GENOTYPIC CHARACTERIZATION OF THE POTENT STRAIN VLCH-15

The genomic DNA used for the polymerase chain reaction (PCR) was prepared from the colonies grown on YMD agar for 3 days. The total genomic DNA extracted from the isolate was isolated by employing the DNA purification Kit (Pure Fast® Bacterial Genomic DNA purification kit, Helini Bio molecules, India) according to the manufacturer protocol. Conditions of the PCR were standardized with initial denaturation at 94°C for 3 minutes followed by 30 cycles of amplification (Denaturation at 94°C for 60 seconds, annealing temperature of 55°C for 60 seconds, and extension at 72°C for 60 seconds and an addition of 5 minutes at 72°C as final extension).The amplification reactions were carried out with a total volume of 50µL in a gradient PCR (Eppendorf, Germany). Each reaction mixture contained 1 µL of DNA, 1 µL of 10 *P* mol forward 16S actino specific primer (5'AAATGGAGGAAGGTGGGGAT-'3), 1 µL of 10 *P* mol reverse 16S actino specific primer (5'- AGGAGGTGATCCAACCGCA-'3), 25 µL of master mix, and 22 µL of molecular grade nuclease free water. The separation was carried out at 90 Volts for 40 minutes in TAE buffer with 5 µL of ethidium bromide. PCR product was analyzed using agarose gel (1%) and the fragment was purified (Helini Pure Fast PCR clean up kit, Helini Bio molecules, India) as per the manufacturer's instructions. The bands were analyzed under UV light and documented using Gel Doc. The direct sequencing of PCR products was performed by dideoxy chain termination method using 3100-Avant genetic analyzer (Applied Biosystems, USA).

The gene sequence of the strainVLCH-15 was aligned using BLAST against the gene library available for *Brachybacterium*species in the NCBI and the GenBank. Pairwise evolutionary distances were computed by MEGA-6 software. The phylogenetic analysis was conducted using the maximum parsimony method of the isolate using BLAST and CLUSTAL W. The closely related homologous isolates were identified, retrieved and compared to the sequence of the isolated strains using CLUSTAL W available with the MEGA 6 Version (Tamura *et al*., 2013). The 16S rRNA gene sequence of the strain VLCH-15 was registered in the GenBank database.

2.6 DETERMINATION OF GROWTH PATTERN

To determine the growth pattern, the strain was inoculated in 100 ml YMD broth and incubated at 30±2°C on a rotary shaker at 120 rpm for ten days. The flasks were harvested at 24 hrs interval and the growth of the strain was estimated by taking the dry weightof biomass. The culture filtrates obtained after separating the biomasswere extracted with ethyl acetate and antimicrobial activity of crudeextract was determined by agar well diffusion method(Managamuri& Vijayalakshmi, 2016).

2.7 *IN-VITRO* ANTIMICROBIAL ACTIVITY OF VLCH-15

The antimicrobial activity of the strain was determined by agar well diffusion assay. YMD broth was used as a production medium for the extraction of crude secondary metabolites. The potent actinobacterium VLCH-15 was inoculated and the fermentation was carried out at 30°C for 120hrs under agitation at 120 rpm. Bioactive compounds were recovered from the filtrate by solvent extraction method. Ethyl acetate was added to the filtrate (1:1 v/v) and shaken vigorously. The ethyl acetate phase that contains bioactive secondary metabolites was separated from the aqueous phase using separating funnel. The ethyl acetate extract was evaporated to dryness in water bath (40-45°C) and the residue thus obtained was used to determine antimicrobial activity (Naragani*et al.,* 2021). A volume of 80µL of crude extract was carefully dispensed into separate well and allowed to diffuse followed by incubation at 37°C for 24h. Ethyl acetate itself was used as negative control and standard antibiotic (streptomycin 80µL) was used as a positive control. After incubation, the zone of inhibition (mm) was measured and recorded.
Microbial pathogens: Thetested bacterial pathogens were*Staphylococcus aureus* (MTCC 3160), *Klebsiella pneumoniae* (MTCC 109),*Bacillus subtilis*(NCIM 2187), *Xanthomonas campestris* (MTCC2286), *Pseudomonas aeruginosa* and *Escherichia coli* (ATCC 9027). The fungal pathogens used for this bioactivity were*Aspergillus flavus, Penicillium citrinum*and *Candida albicans* (MTCC 183).

2.8 *IN-VITRO* SCREENING FOR INDUSTRIALLY IMPORTANT ENZYMES

The potent actinobacteria VLCH-15 was screened for the production of different extracellular and industrially important enzymes such as amylase, cellulase, asparaginase, glutaminase and tyrosinase. The strain which showed positive enzyme production was selected for further enzymatic assay.

2.8.1 SCREENING OF AMYLASE ENZYME

ISP-4 (starch casein agar) medium was incorporated with 1% soluble starch and sterilized at 121ºC for 15min was employed to screen the amylase production of the strain VLCH-15. The strain was inoculated and incubated at 28±2ºC for 3-4 days. Thereafter, plates were flooded with Gram's iodine solution (1%) and amylase production was confirmed by decolonization or clear zone around the culture.

2.8.2 DETERMINATION OF CELLULOLYTIC ACTIVITY

Carboxyl methyl cellulose (CMC) agar medium was used for preliminary screening for cellulolytic potential of the strain VLCH-15. The culture VLCH-15 was screened qualitatively for cellulolytic potential by observation of a clear zone around the growth on CMC agar. The culture was inoculated into the medium plates and incubated at 28±2ºC for seven days. After incubation, the cellulolytic activity was visualized by plates were flooded with Gram's iodine (1%) for 3-5min (Yeoh *et al.,* 1985).

2.8.3 SCREENING OF L-ASPARGINASE PRODUCTION

The strain VLCH-15 was screened for potential, anti-carcinogenic L-asparginase enzyme production through rapid plate assay (Gulati *et al.,* 1997). The preliminary screening of L-asparginase activity was carried out by using aspargine dextrose (ADS) agar, pH was adjusted to 7.0 and phenol red (0.009%) was supplemented as pH indicator. The ADS agar medium was sterilized in an autoclave for 20 min at 15 lbs pressure. The strain was inoculated and plates were incubated for 5 days at 28±2ºC.

2.8.4 SCREENING FOR L-GLUTAMINASE ACTIVITY

The strain VLCH-15 was screened for L-glutaminase production by preparing minimal glutamine agar medium (MGA) containing 1% L-Glutamine as substrate and supplemented with 0.03% phenol red as pH indicator then final pH was adjusted to 6.8. The strain was inoculated into the prepared MGA medium and incubated at 28±2ºC for five days. L-glutaminase production was observed by change in color from yellow to pink indicating the strain ability to hydrolyse L-glutamine to L-glutamic acid and ammonia.

2.8.5 SCREENING OF TYROSINASE PRODUCTION

Skim milk agar (pH 6.5) and tyrosine agar (pH 7.2) media were employed for tyrosinase screening. The strain VLCH-15 was inoculated and plates were incubated at 28±2ºC for 3-4 days. The appearance of clear zone around the colony and observation of brown pigmentation which gradually changed to black (melanin formation) was indicated as tyrosinase positive(Chang *et al.,* 2006).

3. RESULTS AND DISCUSSION

The combined pretreatment of mangrove soils with dry heat (50°C/30min), calcium carbonate (10:1 w/v) and phenol (1.5%) reduced the growth of contaminant microbial species and also enrich the rare actinobacteria. In isolation, the more number of actinobacteria were recovered from actinomycetes agar and humic acid vitamin agar media, for purification and preservation ISP-2 medium was employed. A total of fifteen actinobacterial strains were isolated from the mangrove ecosystem of Machilipatnam and they were designated as VLCH-1 to VLCH-15. The isolates were evaluated for their antimicrobial potential. In preliminary screening, eight predominant actinobacterial isolates exhibited antagonistic activity and they were selected for secondary screening of antimicrobial activity.Among them, one strain such as VLCH-15 exhibited high bioactivity hence it was selected for further studies (Table 1).

3.1 POLYPHASIC TAXONOMY OF THE POTENT STRAIN VLCH-15 (MORPHOLOGICAL, CULTURAL, BIOCHEMICAL AND PHYSIOLOGICAL CHARACTERISTICS)

For micro-morphological study of the strain, 4 day-old culture of VLCH-15 was examined under Scanning electron microscopy.The strain VLCH-15 displayed the massive sporulation with branched mycelium and abundant aerial hyphae. Colonies are aerobic, slightly slimy and the color of aerial mycelium was creamy gray and the substrate mycelium was mild black. Spores of VLCH-15 strain were non-motile, spherical to elongated form smooth surface, spore edges were low convex (Fig.1A&B)(Takeuchi *et al.,* 1995).
Cultural characteristics and growth properties of the strain was studied on both ISP and non-ISP agar media (Table2). Strain VLCH-15 exhibited good growth on tryptone yeast extract agar (ISP-1), yeast extract malt extract dextrose agar (ISP-2) and inorganic salts starch agar (ISP-4). The strain also displayed moderate growth on Oat meal agar (ISP-3), glycerol asparagine agar (ISP-5), actinomycetes isolation agar and tyrosine agar (ISP-7). Whereas poor growth was observed on peptone yeast extract agar (ISP-6), nutrient agar and no growth was found on Czapek-Dox agar. Melanin pigmentation was produced on tyrosine agar (ISP-7). The color of aerial mycelium was creamy gray and substrate mycelium was mild black on different media tested.
The carbon utilization of potent strain VLCH-15 was tested on 12 different carbon sources and they represented in table 4.The strain displayed excellent utilization of D-glucose, maltose, mannose while moderately utilized in sucrose, lactose, fructose, galactose and utilization of starch, mannitol are very poor. However, the carbon sources such as raffinose and xylose were not utilized. In screening of preliminary enzymatic activity, the strain VLCH-15 displayed positive response for amylase, asparginase, glutaminase, tyrosinase, cellulose and negative response for pectinase and chitinase (Table 5).

The details of the physiological and biological characteristics of the strain has represented in table 3. The potent strain VLCH-15 could grow well in the medium addition with 4% salinity and showed tolerance up to 9%.The strain exhibited good growth at pH range between 5 to 9 with the optimum being 7 and the range of temperature for growth was 25-40°C with the optimum being 30°C. The strain exhibited positive response to Gram's reaction, catalase production, indole test, urease test, nitrate reduction, citrate utilization, H_2S production and showed negative response to oxidase, gelatin liquefaction, starch hydrolysis, casein hydrolysis, methyl red and Vogues-Proskauer tests.

3.2 *IN-VITRO* SCREENING FOR ANTIBIOTIC SUSCEPTIBILITY

In the screening of *in-vitro* antibiotic susceptibility, the strain exhibited resistance to amoxyclav, chloramphenicol, cephalexin, Gentamicin, azithromycin and streptomycin where as it displayed sensitivity to vancomycin, neomycin and kanamycin (Table 6).

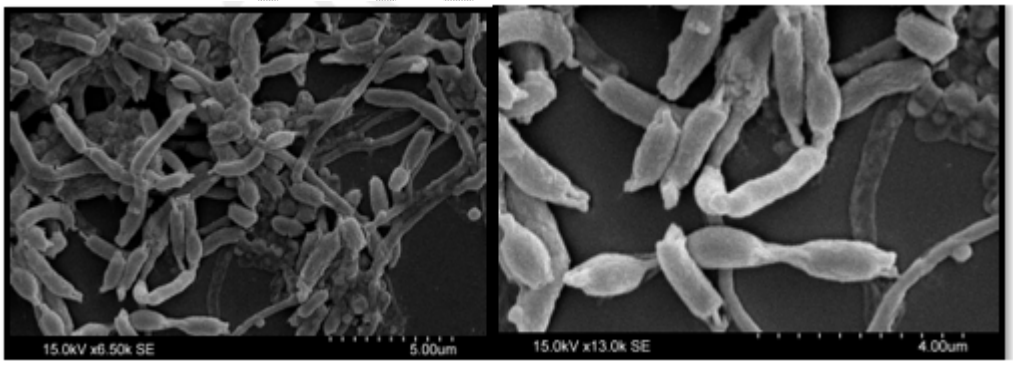

Fig. 1A&B. SEM photographs of *Brachybacteriumconglomeratum*VLCH–15

3.3 GENOTYPIC CHARACTERIZATION OF THE POTENT STRAIN VLCH-15

The isolated potent strain was identified as *Brachybacterium conglomeratum* VLCH–15 on the basis of 16S rRNA sequencing and a phylogenetic tree was constructed by the Maximum parsimony method (Fig. 2). The phylogenetic tree of the potent strain clearly revealed its evolutionary relationship with a group of *Brachybacterium* species were generated by a Maximum parsimony method with the aid of MEGA

software version 6. DNA sequences of *Brachybacterium conglomeratum* were deposited in GenBank with the accession number MK205372.

Table 2: Cultural characteristics of the strain VLCH-15

Medium	Growth	AM	SM	Pigmentation
Tryptone yeast extract agar (ISP-1)	Good	Creamy grey	Mild black	Nil
Yeast extract malt extract dextrose agar (ISP-2)	Good	Creamy grey	Mild black	Nil
Oat meal agar (ISP-3)	Moderate	Creamy grey	Mild black	Nil
Inorganic salts starch agar (ISP-4)	Good	Creamy grey	Mild black	Nil
Glycerol asparagine agar (ISP-5)	Moderate	Creamy grey	Mild black	Nil
Peptone yeast extract agar (ISP-6)	Poor	-	-	Nil
Tyrosine agar (ISP-7)	Moderate	Creamy grey	Mild black	Black
Actinomycetes agar	Moderate	Creamy grey	Mild black	Nil
Czapek-Dox agar (CDA)	Poor	-	-	Nil
Nutrient agar (NA)	-	-	-	Nil

AM- Aerial mycelium, SM-Substrate mycelium, -: No growth, ISP-International *Streptomyces* Project

Table 3: Morphological, physiological and biochemical characteristics of the strain VLCH-15

Character	Response
Morphological characters	
Sporophore morphology	Fragmentation
Color of aerial mycelium	Creamy grey
Color of substrate mycelium	Mild black
Physiological characters	
Grams reaction	Gram Positive
Acid – fast reaction	-
Production of melanin pigment	+
Range of temperature for growth	25–40^{o}C
Optimum temperature for growth	30^{o}C
Range of pH for growth	5–9
Optimum pH for growth	7
NaCl tolerance	9%
Optimum NaCl%	4%
Biochemical characters	
Catalase production	+
Urease production	+

H_2S production test	+
Nitrate reduction	+
Starch hydrolysis	-
Gelatin liquefaction	-
Methyl red test	-
Voges-proskauer test	-
Indole production	+
Citrate utilization	+
Casein hydrolysis	-

+:Positive; -:Negative

Table 4: Utilization of carbon sources of the strain VLCH-15

Carbon Sources	Response
D-Glucose	+++
Maltose	+++
Sucrose	++
Lactose	++
Arabinose	-
Fructose	++
Galactose	++
Mannose	+++
Starch	+
Mannitol	+
Xylose	-
Raffinose	-

Excellent (+++), Moderate (++), Poor (+), Not utilized (-)

Table 5: Enzyme activity of the strain VLCH-15

Enzymatic activity	Response
Amylase	+
Tyrosinase	+
Cellulase	+
Asparaginase	+
Glutaminase	+
Chitinase	-
Pectinase	-

+:Positive; -:Negative

Table 6: Antibiotic susceptibility/resistance of the strainVLCH-15

Antibiotics (µg)	Susceptibility (S)/Resistance (R)
Azithromycin (15)	R
Cephalexin (15)	R

Penicillin (10)	R
Gentamicin (10)	S
Chloramphenicol(10)	R
Vancomycin (30)	S
Streptomycin (25)	R
Amoxyclav (30)	R
Kanamycin (30)	S

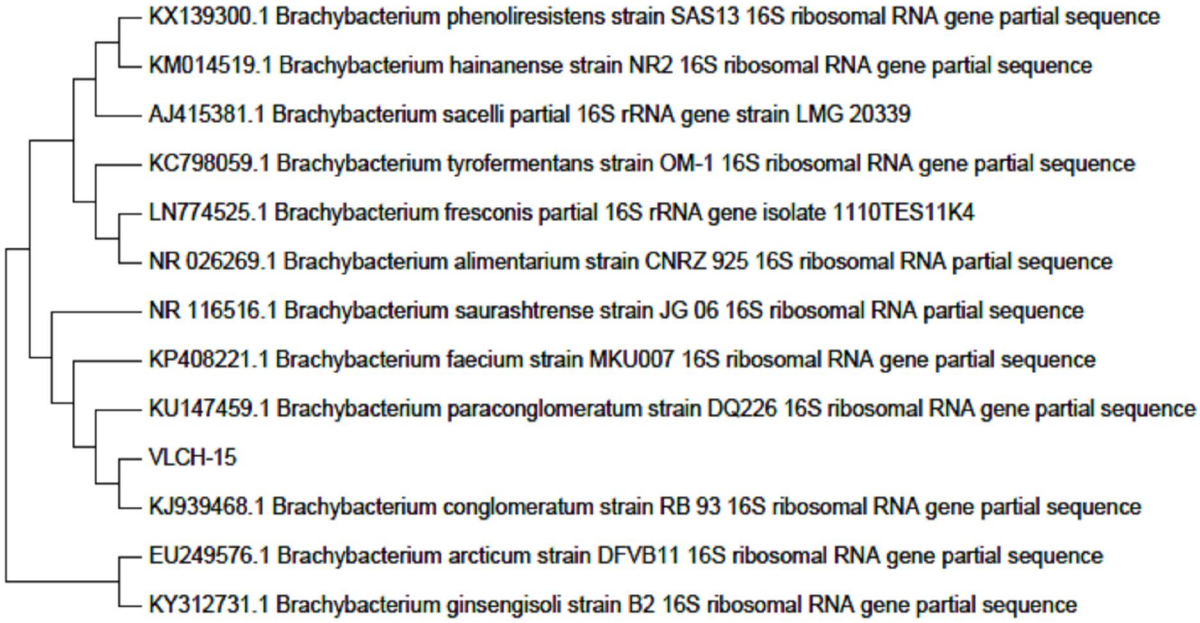

Fig. 2. Maximum Parsimony method based on 16S rRNA gene sequences showing relationship between strain VLCH-15 and related members of the genus *Brachybacterium*

3.4 GROWTH PROFILE AND ANTIMICROBIAL POTENTIAL OF *BRACHYBACTERIUM CONGLOMERATUM* VLCH-15

The growth curve and antimicrobial profile of *B. conglomeratum* VLCH-15were studied at regular intervals up to 10 days in batch culture. The culture broth was harvested at 24 h intervals and dry weight of biomass was expressed in mg/100 ml. The stationary phase of the strain extended from 168 h to 192 h of incubation (figure3). The culture filtrate was extracted with ethyl acetate and evaporated to dryness in water bath. The secondary metabolites obtained from 7-day-old culture showed high antimicrobial activity against *X. campestris, E. coli* and *A. flavus*(figure 4A&B). Metabolites extracted from eight day old culture of *Saccharomonosporaoceani*VJDS-3 exhibited strong antimicrobial activity(Indupalli*et al.,* 2016). The bioactive compounds derived from eight day old cultures of *Nocardiopsisdassonvillei*VJRM-7 showed high antimicrobial potentiality (Ragalatha*et al.*, 2020).The antimicrobial profile of the strain was tabulated (Table 7).

Table 7: Antimicrobial assay of *Brachybacterium conglomeratum* VLCH–15

Test organism	Zone of Inhibition (mm)
Bacteria	**VLCH-15**
Staphylococcus aureus	19.52±0.38
Escherichia coli	20.21±0.21
Xanthomonas campestris	24.21±0.11

Pseudomonas aeruginosa	17.26±0.12
Bacillus subtilis	19.45±0.27
Streptococcus mutans	16.28±0.18
Klebsiella pneumoniae	17.49±0.16
Fungi	
Candida albicans	12.48±0.15
Aspergillus flavus	18.39±0.26
Penicillium citrinum	09.26±0.13

Statistical analysis: Values are the means of three replicates ± SD

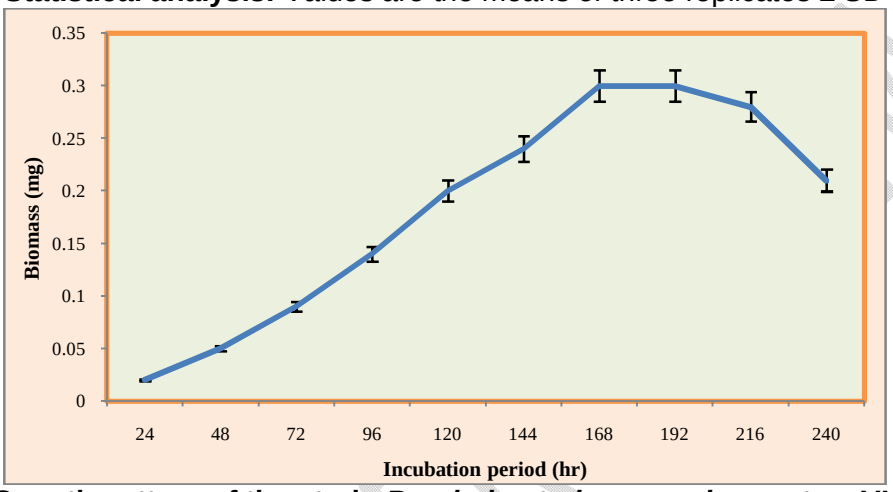

Fig. 3. Growth pattern of the strain *Brachybacterium conglomeratum* VLCH–15

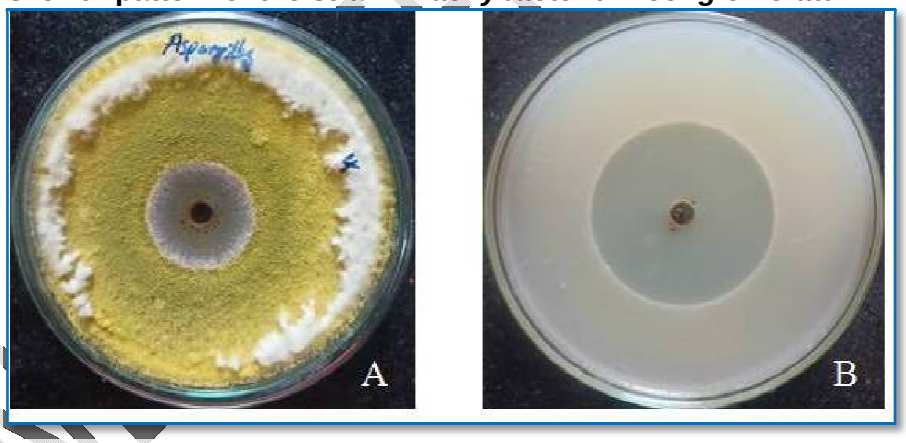

Fig. 4. Antimicrobial activity of VLCH–15 extract against A) *Aspergillus flavus* andB) *Xanthomonas campestris*

4. CONCLUSION

*Brachybacteriumconglomeratum*VLCH-15 isolated from mangrove origin can have an immense potential to produce high quality of bioactive compounds and thestudy opens scope for extensive exploration against the multiple drug resistant pathogens (MDR). This is the first report on taxonomic profile of mangrove *Brachybacterium conglomeratum* VLCH–15with enzymatic and antagonistic potential.

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