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Antimicrobial and Enzymatic potential of *Streptomyces* sp. KAS-1 isolated from the microbiologically unexplored estuary of Kali river ecosystem

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ABSTRACT

In the present study, a *Streptomyces* sp. KAS-1 was isolated from sediment soil samples of the Kali river estuary, Karwar, Karnataka, India. The isolated Actinomycetes strains were screened against pathogenic microorganisms and for the production of hydrolytic enzymes. Later, the strongest strain *Streptomyces* sp. KAS-1 was taken for further analysis based on primary characterizations. The morphological, physiological and biochemical characterizations of *Streptomyces* sp. KAS-1 were carried out, followed by molecular characterization through 16S rRNA gene sequencing. The 16S ribosomal RNA gene sequencing and analysis of the phylogenetic tree showed a 98.97% sequence similarity with *Streptomyces thermocarboxydus* strain SDT64 (KJ018992). Finally, the intracellular methanol extract was characterized through UV-Visible and FTIR spectrophotometer. The *Streptomyces* sp. is a gram-positive, aerobic, non-motile, spore-forming bacterium and it was designated as *Streptomyces* sp. KAS-1 and was found to possess a strong antimicrobial activity against pathogenic microorganisms. The characterizations revealed the *Streptomyces* sp. KAS-1 was moderately thermophilic, neutrophile organism having the capacity to degrade a broad range of carbon sources. The UV-Visible spectrum of methanol extract of *Streptomyces* sp. KAS-1 revealed the presence of Muconomycin-B and the presence of different functional groups such as aldehydes, amines, and alkyl halides were indicated by the FTIR spectrum.

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INTRODUCTION

Actinomycetes are a group of gram-positive, filamentous bacteria and having high G+C content. They are physiologically versatile and found in most of the environments, including terrestrial and aquatic habitats (Pathalam *et al.*, 2017). The word Actinomycetes was coined from the Greek language where "*atkis*" means "*a ray*" and "*mykes*" means "*fungus*." This is why Actinomycetes are also called as Ray fungi due to close similarities with fungal counterparts. However, they even have many dis-similarities with fungi; due to unique properties and thus are restricted into the domain bacteria (Chaudhary *et al.*, 2013). There is an increasing realization of the potential for sediment soil

as sources of Actinomycetes that produce useful bioactive compounds. (Okami, 1986), reported that Actinomycetes of freshwater origin produce novel bioactive substances. However, the focus is increasing towards novel regions, estuarine ecosystems, and extreme environments for isolating novel bioactive strains, especially Actinomycetes, which produce nearly 80% of all known antibiotics.

Additionally, the microbial profiles also serve as an indicator of freshwater, estuarine ecological health. Microbes are the factors for a multitude of human diseases, but also equally owe a great extent to human medicine, disease treatment, and control. Marine actinobacteria are the rich source for the potent novel secondary metabolites and the majority of these compounds are derived from a single genus *Streptomyces* (Sharma *et al.*, 2014).

The bioactive secondary metabolites from the Actinomycetes are endowed with novel chemical structures with strong biological activities. Marine habitats provide more chances for isolating new species of *Streptomyces* with unique chemical structures repelling many microbial diseases and cancers (Janardhan *et al.*, 2014). *Streptomyces* are more useful in the field of pharmaceutical industries because of their outstanding capacity to produce antibiotics, enzymes, antimicrobial metabolites, mainly such as aminoglycosides, macrolides, polyenes, glycopeptides, polyketides, betalactides and actinomycins (Ding *et al.*, 2019).

Actinomycetes of marine and estuarine habitats are less explored because of the difficulties in the collection and isolation processes involved when compared to terrestrial Actinomycetes (Kamjam *et al.*, 2017). An estuary is a semi-enclosed coastal body of water estuaries act as a transition zone between two aquatic ecosystems, namely marine and freshwater. They are affected by both marine influences, such as waves, tides, a mixture of saline water, and sediments. As a result, they may contain many biological niches within a small area, and so are linked with large biological diversity (Parthasarathi *et al.*, 2012; Rosmine and Varghese, 2016). Estuaries provide some of the most productive habitats on earth because of the accumulation and availability of nutrients along with adequate light conditions that enhance the production of phytoplankton in the water (Cloern *et al.*, 2014).

The result of an intensive screening program carried out over the past many decades is that there is a growing problem of a rediscovery of already known bioactive compounds (Genilloud, 2018). An approach to address this problem is to expand the source of Actinomycetes by carrying out an ecological assessment of environments other than terrestrial soils. There is a growing interest in the *Streptomyces* from estuarine ecosystems and it was found that the majority of the isolates were *Streptomycetes*, indicating that the estuary soil is a suitable source of Actinomycetes to screen for production of novel bioactive compounds (Dharumadurai *et al.*, 2010).

Our review of the literature showed that there was no isolation study of Actinomycetes from the Kali river estuarine sediment samples of Karwar, Uttara Kannada (U.K) until today. Therefore a systematic screening program was undertaken and a *Streptomyces* sp. KAS-1 was isolated from sediment samples of the Kali river estuarine area on the south coast of India. Taxonomic characterization was carried out based on 16S rRNA sequence analysis in combination with morphological, physiological, hydrolytic enzymes and biochemical characterizations. The antibacterial and antifungal activity was also investigated.

MATERIALS AND METHODS

Collection of samples

12 sediment samples were collected from different locations of the Kali river ecosystem of Karwar, Karnataka, India. The sediment samples were collected at approximately 2 meter depth using a sediment sampler and stored in sterile containers at 4°C.

Isolation of Actinomycetes

To isolate Actinomycetes, the sediment samples were pre-treated by heating at 60°C for 15 minutes to kill unwanted bacteria and fungi. The standard serial dilution technique was employed to isolate Actinomycetes (Hayakawa *et al.*, 1988). Each sample was serially diluted and 100μL aliquot was spread evenly on starch casein agar (SCA) medium. Antifungal and antibacterial antibiotics supplements (Amphotericin B and Tetracyclin) at 25μg/mL respectively were used for Actinomycetes growth enrichment and also to prevent fungal and bacterial contaminations (Cuesta *et al.*, 2012; Zhang *et al.*, 2005). Later the plates were incubated at 30°C for 8 days and observed periodically for the growth of Actinomycetes. Finally, the selected Actinomycetes colonies were purified and maintained at 4°C for subsequent studies.

Primary screening of antimicrobial activities

To check the potentiality of isolated Actinomycetes, antimicrobial activity was studied by the perpendicular streak method (Hayakawa *et al.*, 1988; Williams *et al.*, 1983)against pathogenic bacteria and fungi. The test organisms such as *Aspergillus fumigatus* (MTCC8877), *Enterobacter aerogenes* (ATCC2822),

Table 1: Activity of isolated strains against different test pathogens. (++Strong antimicrobial activity; + Moderate antimicrobial activity; –No antimicrobial activity)

Strain No.	*A.fumigatus* (MTCC8877)	*E.aerogenes* (ATTC2822)	*S.epidermidis* (MTCC435)	*S.flexneri* (MTCC1457)
KAS-1	++	–	++	–
KAS-2	–	–	+	–
KAS-3	–	+	–	–
KAS-5	–	–	–	–
KAS-6	+	–	–	–
KAS-7	–	–	+	–
KAS-8	–	+	–	–
KAS-9	–	–	–	–
KAS-10	++	–	–	+
KAS-11	–	+	+	–
KAS-13	–	–	+	+
KAS-14	+	–	–	–
KAS-15	–	–	+	–
KAS-16	–	++	–	–
KAS-17	+	–	–	+
KAS-18	–	–	–	–
KAS-19	+	–	+	–
KAS-20	–	++	–	+
KAS-22	–	+	–	–
KAS-23	+	–	–	–

Table 2: Production of hydrolytic enzymes by Actinomycetes (++ Strong activity; + Moderate activity; –No activity)

Name of Isolates	Amylase	Esterase	Pectinase	DNase
KAS-1	++	+	++	++
KAS-2	-	-	+	-
KAS-3	+	++	-	+
KAS-5	+	-	-	+
KAS-6	++	++	-	-
KAS-7	-	-	-	+
KAS-8	+	+	+	+
KAS-9	+	-	-	-
KAS-10	-	+	-	+
KAS-11	+	++	+	-
KAS-13	+	-	-	-
KAS-14	+	-	+	+
KAS-15	-	++	-	-
KAS-16	+	-	+	+
KAS-17	+	+	-	++
KAS-18	-	++	+	-
KAS-19	+	+	-	-
KAS-20	++	-	-	-
KAS-22	-	+	+	-
KAS-23	+	+	++	-

Table 3: Biochemical characterizations of *Streptomyces* sp. KAS-1

Morphological Characterization	
Color of aerial mycelium	Shiny greyish
Color of substrate mycelium	Light brown
Pigmentation	Faint brown
Colony of *Streptomyces* sp. KAS-1	Irregular, rough surface and slightly raised
Physiological Characterization	
Growth at different temperatures	
20°C	-
25°C	-
30°C	+
35°C	++
40°C	++
45°C	++
50°C	+
55°C	-
60°C	-
65°C	-
70°C	-
Growth at different pH and NaCl concentration	
pH 5.0	-
pH 6.0	-
pH 7.0	+
pH 8.0	-
NaCl Concentration	3 to 4 %
Biochemical Characterizations	
Gram staining	Gram-positive
Acid fast staining	Positive
Starch	+
Urea	-
Casein	+
Gelatin	+
Esculin	+
Arbutin	+
D-glucose	+
Sucrose	-
D-lactose	+
D-mannitol	+
Trehalose	+
Raffinose	-
Galactose	-
Xylose	-
H_2S Production	+
Nitrate reduction	+

Table 4: FTIR absorption peaks and their associated functional groups

Sl. No.	Absorption peak (cm-1)	Functional groups
1	618	C-Br stretch alkyl halides
2	868	C-Cl stretch alkyl halides
3	1048	CO-O-CO stretch anhydride
4	1079	C-O stretch primary alcohol
5	1112	C-O stretch secondary alcohol
6	1241	C-N stretch amine
7	1386	O-H bending phenol
8	1404	O-H bending carboxylic acid
9	1465	CH_2 bending alkane
10	1580	N-H bend primary amines
11	1638	C=C stretch alkenes
12	2852	C-H stretch alkene
13	2923	C-H stretch alkanes
14	2957	C-H stretch alkanes
15	3420	N-H stretching primary amine

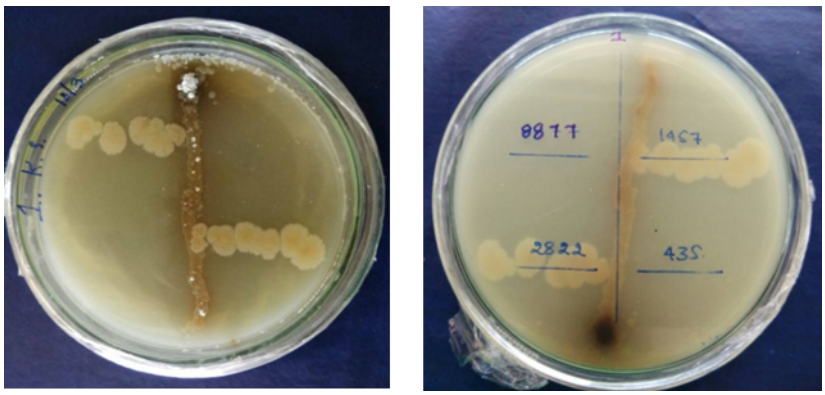

Figure 1: Primary screening of *Streptomyces* sp. KAS-1 using cross streak method against *A. fumigatus* (MTCC8877), *E.aerogenes* (ATCC2822), *S. epidermidis* (MTCC435) and *S. flexneri* (MTCC1457)

Staphylococcus epidermidis (MTCC435) and *Shigella flexneri* (MTCC1457) were streaked at an angle of 90° to Actinomycetes isolates on nutrient agar (NA) medium. Thereafter, the most active strain, which inhibited the growth of microbial pathogens during preliminary screening, was selected for further studies.

Studies on Enzyme activity

To study amylase activity, the isolates were grown on starch casein agar medium (pH 7.0 ± 0.5) for 6 days at 30°C. After incubation, each plate was flooded with 1% iodine solution and the formation of the halo zone around colonies was observed (Oumer and Abate, 2018). Esterase activity was studied by streaking Actinomycetes isolates on Tween-80 agar (7.2 ± 0.1) plates and incubating at 30°C for 6 days. The zone of hydrolysis around colonies was observed and recorded (Rai *et al.*, 2014). Thereafter, pectinase activity was carried out by inoculating isolated Actinomycetes on pecti-

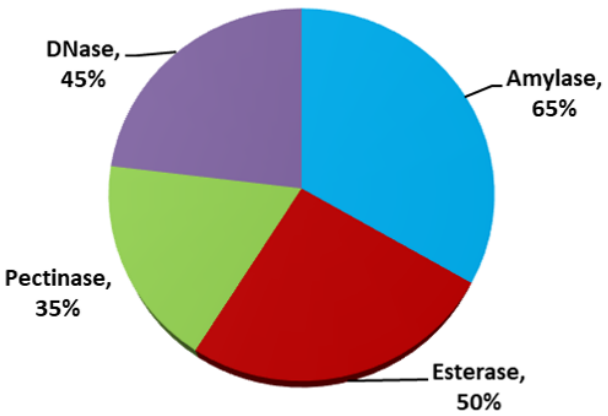

Figure 2: Graphical representation of hydrolytic enzyme activity

nase screening agar (MP-5 medium, pH 5.5 ± 0.1) plates and incubated at 30°C for 6 days. Later, after incubation, each plate was flooded with potassium iodide solution, the halo zones produced around each colony were observed and noted (Hamedi *et al.*, 2019).

Figure 3: Colony morphology of isolated *Streptomyces* sp. KAS-1

The isolated microorganisms ability to hydrolyze DNA was studied according to (Pradhan *et al.*, 2015; Rai *et al.*, 2014) by inoculating them on the DNase medium (pH 7.5 ± 0.5). After an incubation period of 6 days at 30°C, the DNase enzyme producing organisms were selected based on a clear zone around them. The Actinomycetes strain, which showed the best production of all hydrolytic enzymes, was selected for further studies.

Morphological, physiological and biochemical characterization

The most potent strain *Streptomyces* sp. KAS-1 was studied through morphological, physiological and biochemical analysis. For morphological characterization, the *Streptomyces* sp. KAS-1 was observed for shape, size, and pigmentation through the coverslip culture method (Kawato and Shinobu, 1959; Williams and Davis, 1965). Gram staining and acid-fast staining were done according to the method of (Fawole and Oso, 2004). Likewise, physiological characterizations were done by observing growth and tolerance in the range of temperature from 20°C to 70°C and pH in the range of 5.0 to 8.0. Salt tolerance was recorded by growing the isolate in the SCA medium supplemented up to 7% sodium chloride (NaCl) concentration. Further, biochemical characterization was done according to (Gottieb, 1976). *Streptomyces* sp. KAS-1 was characterized for hydrolysis of starch, urea, casein, gelatin, esculin, arbutin and carbon source utilization test for D-glucose, sucrose, D-lactose, D-mannitol, trehalose, raffinose, galactose and xylose. Hydrogen sulfide production and nitrate reduction tests were also done according to the method (Singh *et al.*, 2013).

Molecular characterization of *Streptomyces* sp. KAS-1

Streptomyces sp. KAS-1 was grown in medium and the total genomic DNA was extracted. The 16S rRNA gene was amplified using two eubacterial primers; forward primer: 5'-AGAGTTTGATCMTGGCTCAG-3'and reverse primer 5'-TACGGYTACCTTGTTACGACTT-3'. On the other hand, for PCR amplification; thermal cycling conditions were programmed as follows: initial denaturation at 96°C for 5 minutes; followed by denaturation at 96°C for 30 seconds, hybridization at 50°C for 30 seconds and elongation at 60°C for 90 seconds. The electrophoresis of the PCR products was accomplished on 1% agarose gel with 500 base pair DNA ladder as a size reference (Arasu *et al.*, 2008). The DNA data was sequenced, compiled, analyzed and matched with the Gene Bank database using the basic local alignment search tool (BLAST). Furthermore, the aligned DNA sequence was employed and a phylogenetic tree was constructed through the neighbor-joining method using the software MEGA 7.0 (Altschul *et al.*, 1990; Tamura *et al.*, 2007).

Production and extraction of bioactive compounds

Streptomyces sp. KAS-1 was grown for the production of bioactive compounds in a rotary shaker (150 rpm) at 33°C for 14 days. After fermentation, the biomass was separated by centrifugation at 8000 rpm at 4°C for 20 minutes and washed three times with sterile distilled water. The crude intracellular extract was prepared according to the method of (Saravana *et al.*, 2014). In short, the harvested biomass was taken in an autoclaved mortar and ground thoroughly. Methanol was added in 1:1 ratio (w/v) and the mixture was shaken vigorously overnight. Later, the mixture was centrifuged at 5000 rpm at 4°C for 30 minutes and the obtained supernatant was concentrated through evaporation using a rota-evaporator at 40°C.

Ultraviolet-visible (UV-Vis) spectroscopy

The crude intracellular methanol extract of *Streptomyces* sp. KAS-1 was analyzed qualitatively using Jasco V-670 UV-Visible spectrophotometer for the possible recognition of compounds.

Fourier transform infrared (FTIR) spectral analysis

The biological functional groups present in the methanol extract were interpreted by FTIR spec-

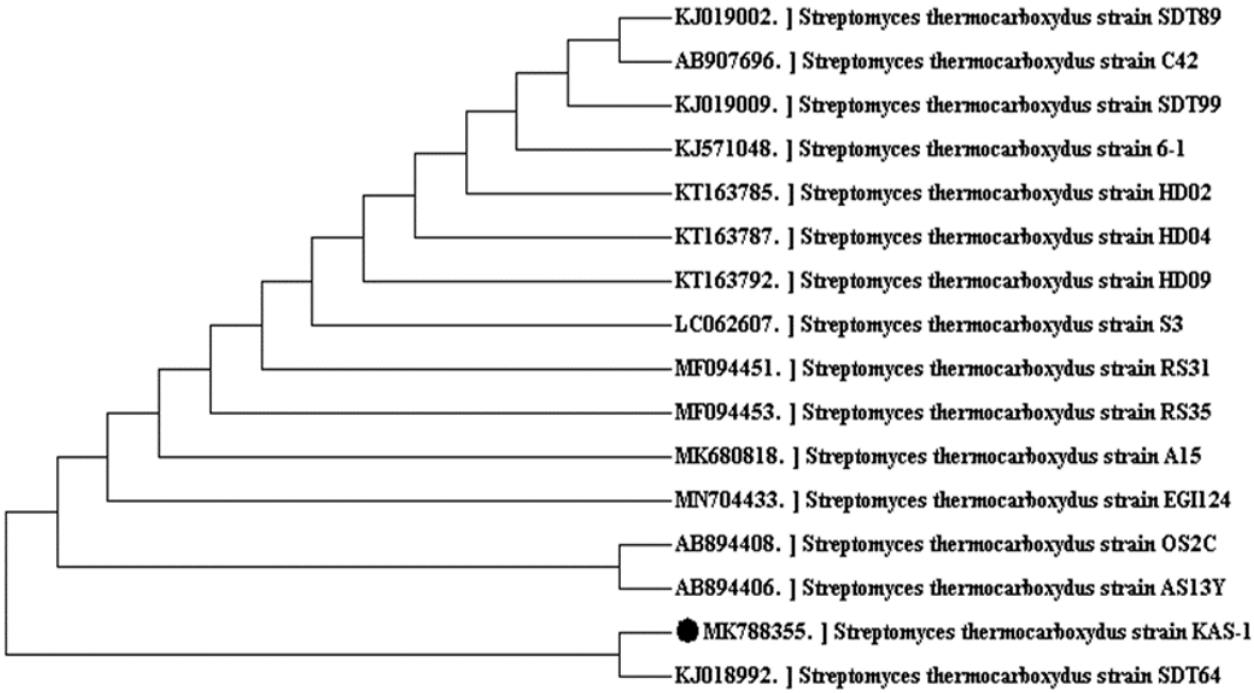

Figure 4: Dendrogram indicating the phylogenetic relationship of *Streptomyces sp*. KAS-1

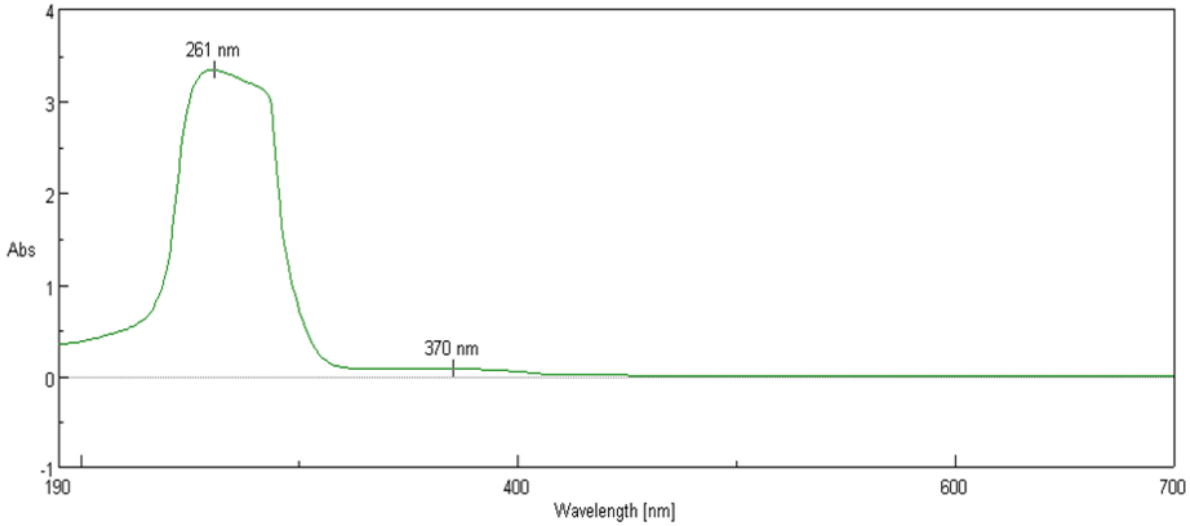

Figure 5: UV-Spectrum of methanol extract of *Streptomyces* sp. KAS-1

troscopy. Here, the sample was prepared by grinding a small pinch of KBr with methanol extract and thin discs were made through hydraulic pressing. Further, the FTIR spectrum of the active extract was detected in the range of 400 cm^{-1} to 4000 cm^{-1} using a Nicolet 6700 FTIR spectrophotometer.

RESULTS AND DISCUSSION

Isolation of Actinomycetes and primary screening

In this work, bioactive Actinomycetes were assessed from collected sediment samples of the Kali river ecosystem. A total of 23 Actinomycetes strains were selectively isolated from 12 sediment samples. All the isolates showed morphologically distinct colonies on starch casein agar supplemented with Amphotericin B and Tetracyclin. However, out of 23 Actinomycetes, 20 isolates were found to be gram-positive. The *Streptomyces* sp. KAS-1 showed total inhibition during primary screening against two pathogenic organisms, such as *Aspergillus fumigatus* (MTCC8877) and *Staphylococcus epidermidis*, as shown in Figure 1. Rest all organisms showed moderate or no inhibition against the pathogenic microorganisms, reported in Table 1. A similar result was observed by (Subhan *et al.*, 2015), where 33 Actinomycetes were isolated from soil samples,

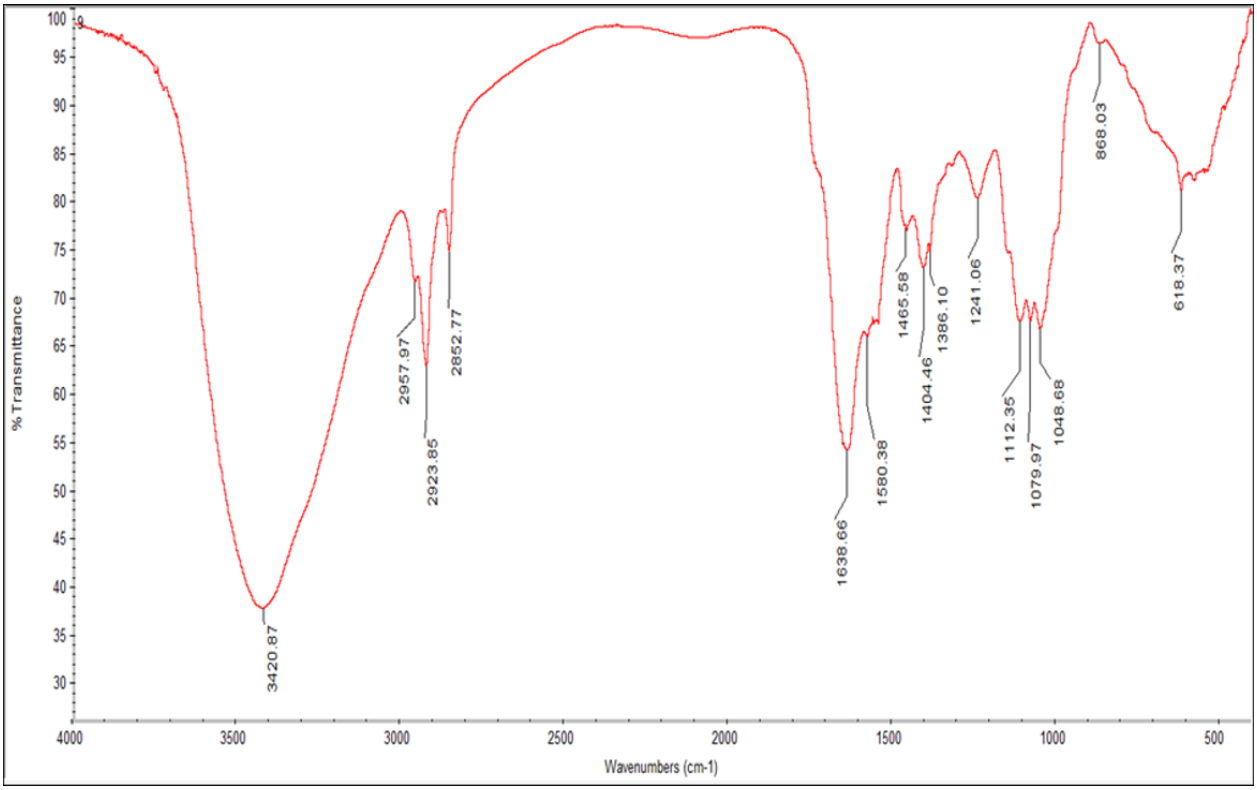

Figure 6: FTIR spectrum of methanol extract of *Streptomyces* sp. KAS-1

but only 17 isolates showed antimicrobial activity against pathogenic organisms.

Studies on Enzyme activity

The Actinomycetes can produce numerous enzymes, which are essential for their living and resistance against other pathogens. It is assumed that there may be an interrelationship between the production of enzymes and habitat, where the organism is living. These enzymes have various industrial applications. Here, the 20 strains of gram positive Actinomycetes were screened for enzymatic activity against amylase, esterase, pectinase and DNase, which was shown in Figure 2, Table 2. The *Streptomyces* sp. KAS-1 was found to produce all the four enzymes. Hence, the *Streptomyces* sp. KAS-1 was selected for further analysis based on its ability to produce enzymes and antimicrobial activities. Among the 20 isolates, 14 isolates produced amylase enzyme whereas, the esterase enzyme was produced by 12 isolates. However, the enzyme pectinase was produced by only 9 isolates While, DNase was produced by 9 isolates. Microorganisms utilize starch as a source of carbon, but since the starch molecules are too large and cannot be absorbed through the cell membrane, the organisms produce the extracellular enzyme amylase. The enzyme amylase reacts with starch molecules and breaks down into smaller units, which can be absorbed easily by the cell membrane of microorganisms. When organisms are grown on starch agar medium, they hydrolyze starch with the help of enzyme amylase; thereby produce a transparent halo surrounding the colony. The production of enzymes is dependent on methods of cultivation, the composition of medium, temperature, pH, incubation time, cell growth, nutrient requirements, pH, temperature, etc. Among these parameters, the production of the enzyme is mostly dependent on the pH and temperature of the medium (Kafilzadeh and Dehdari, 2015). Microorganisms produce extracellular esterase enzymes, which hydrolyze and splits esters in alcohols and acid. Generally, the esterase producing Actinomycetes release fatty acids due to the hydrolysis of tween-80. The fatty acids molecules released from Tween-80 bind to calcium ions present in the medium and produce a halo zone around the colonies (Kumar *et al.*, 2012). Here, 9 isolates out of 20 were able to hydrolyze pectin and used that as a source of carbon. A similar result was published by (Mohandas *et al.*, 2018), where 50 microorganisms isolated from spoiled fruits and vegetables indicated the presence of pectinase enzyme. The enzyme DNase was produced only by 9 Actinomycetes. DNA or Deoxyribonucleic acid is made of four nucleotides and it is a large polymer of nucleotides. Actinomycetes can utilize DNA as a source of nitrogen, carbon, and phosphate. The exo-enzyme DNase secreted by Acti-

nomycetes hydrolyzes and cleaves DNA molecules into free nucleotides and a phosphate group. These nucleotides than enter the cell membrane and can be used as a source of nutrition. The negatively charged DNA in the form of a polymer bind to positively charged methyl green indicator in the medium. After breaking down into free nucleotides, it is no longer bound to methyl green and forms a clear zone around the DNase producing organism.

Morphological, physiological and biochemical characterization

The *Streptomyces* sp. KAS-1 isolated from sediment samples was aerobic and gram-positive. The colony of the *Streptomyces* sp. KAS-1 was irregularly shaped with a rough surface, whereas the elevation of the colony was slightly raised. The branched vegetative mycelium was light brown, while the spore-bearing mycelium was shiny greyish. The strain also produced a faint brown pigment after incubation in the SCA medium for 8 to 9 days as shown in Figure 3. The strain was found positive for gram stain and acid-fast staining. This is because all the *Streptomyces* strains showed a positive result for acid-fast staining and gram staining. Our observations were supported with results by (Islam *et al.*, 2014). The strain was found to grow optimally in the range of temperature from 35°C to 45°C, but a moderate to no growth was found in the temperature below and above that. This proved that the *Streptomyces* sp. KAS-1 was a moderately thermophilic organism. These thermophilic Actinomycetes play a significant role in the mineralization of nutrients and degradation of organic matter. The pH of the surrounding environment has a great influence on the growth of Actinomycetes.

The pH, which favored the optimum growth of the *Streptomyces* sp. KAS-1 was found at pH 7.5, in presence of up to 7% NaCl tolerance with an optimum at 3 to 4%. But moderate growth was observed at pH 7.0 and 8.0; however, there was found no growth at pH below 7.0 and above 8.0. Therefore, *Streptomyces* sp. KAS-1 can be identified as a neutrophile, which is capable of growing in such an environment, where H^+ ion concentration is almost at equilibrium. Most of the Actinomycetes, especially *Streptomyces* grow better in pH ranging from 6.0 to 8.0 (Kim *et al.*, 1992). The organism showed optimum growth at 3 to 4% NaCl concentration. Therefore, it is obvious that the *Streptomyces* sp. KAS-1 was, not a halo-tolerant sp. because it can grow best at low salt concentration. It is noted that a smaller amount of metallic ion or NaCl concentrations in medium maintain an osmotic condition, which is similar to the cytoplasm of the microorganism. However, a high salt concentration leads to osmosis to the microorganisms and tends to denature proteins which, lead to the death of the cell. Bergey's Manual of Systematic Bacteriology: The Actinobacteria (Whitman *et al.*, 2012).

The *Streptomyces* sp. KAS-1 showed a positive result for starch, casein, gelatin, esculin and arbutin. *Streptomyces* sp. KAS-1 was able to hydrolyze starch, because of the production of enzyme α-amylase, which cleaves starch into simpler molecules and absorb through the cell membrane as a source of carbon. The organism showed a positive result for gelatin liquefaction. It implies that the organism can produce gelatinase, which is a protease enzyme. The organism extracellularly secretes the gelatinase enzyme and use gelatin as a source of protein followed by hydrolysis. Casein is made up of amino acid subunits and the major milk protein. Microorganisms cannot absorb casein through their cell membrane because of its large size. Therefore, the organisms secrete protease enzyme which, degrade casein into free amino acid molecules and make it easily absorbable through cell membranes.

The *Streptomyces* sp. KAS-1 was negative for the urea hydrolysis test, but it showed a positive result for esculin degradation. The organisms degrade esculin to esculetin, which shows a positive result. The *Streptomyces* sp. KAS-1 even showed a positive result for arbutin degradation. In addition to this, the organism was positive for hydrogen sulfide production and nitrate reduction tests. Hydrogen sulfide is produced by some microbes while degradation of amino acids containing a sulfur derivative, such as methionine, where as the positive result for nitrate reduction implies the ability to produce nitrate reductase enzyme. The microorganisms require nitrate as an electron acceptor during anaerobic respiration. The carbon sources utilized by the strain *Streptomyces* sp. KAS-1 were D-galactose, D-mannitol, trehalose, and D-lactose but no growth observed in sucrose, raffinose, galactose, and xylose. The morphological, physiological and biochemical characterizations of *Streptomyces* sp. KAS-1 was reported in Table 3. The results obtained from morphological, physiological and biochemical characterizations were compared with *Streptomyces* species reported in Bergey's manual of systematic bacteriology (Locci, 1989).

Molecular characterization

The genomic DNA of the *Streptomyces* sp. KAS-1 was extracted and analyzed by 16S rRNA gene sequencing. For the identification of microorganisms up to species level, the 16S rRNA gene sequencing is widely being used in the construction of phylogenies

because of the slow rate of evolution at that region of gene (Woese and Fox, 1977). The length of the complete gene sequence obtained was 1358 base pair and was submitted to NCBI gene bank with the Accession number MK788355. This sequence was later subjected in search of close relatives through nucleotide blast (BLASTN) in NCBI. The analysis of the 16S rRNA gene sequence of the *Streptomyces* sp. KAS-1 revealed 98.97% sequence similarity with *Streptomyces thermocarboxydus*. Further, the reconstruction of a phylogenetic tree was done through the neighbor-joining method with obtained gene sequences. The analysis of the phylogenetic tree revealed the strain *Streptomyces* sp. KAS-1 fell within the cluster of the genus *Streptomyces* and formed a branch with *Streptomyces thermocarboxydus* strain-SDT64 (KJ018992) as represented in Figure 4.

UV absorption analysis of *Streptomyces* sp. KAS-1 methanol extract

The qualitative UV-visible analysis of methanol extract exhibited maximum UV absorption at 261 nm as shown in Figure 5. The UV absorption at 261 nm reported the tentative identification of the compound muconomycin B (Jacqueline and Vittimberga, 1965). Muconomycin B is an aromatic sesquiterpene and a powerful antibiotic compound naturally present in microorganisms. There was a similar result obtained by (Maleki and Omid, 2011), where the ethyl acetate extract of bioactive compounds exhibited maximum UV absorption at the range 217-221nm and showed the presence of broad-spectrum antimicrobial compounds.

FTIR analysis of *Streptomyces* sp. KAS-1methanol extract

The biological functional groups present in methanol extract of *Streptomyces* sp.KAS-1 was determined by FTIR analysis, based on the peak values of the infrared spectrum Figure 6,Table 4 . It revealed that the presence of different functional groups based on the absorption peaks at 618 cm^{-1} and 868 cm^{-1} due to C-Br stretching alkyl groups and C-Cl stretch alkyl halides respectively, 1044 cm^{-1} C-N stretch aliphatic amines, 1119 cm^{-1} C-N stretch aliphatic amines, 1274 cm^{-1} C-H wag alkyl halides, 1315 cm^{-1} symmetric stretching aromatics, 1384 cm^{-1} CH_2 wag modes / C-H rock alkanes, 1415 cm^{-1} ring-stretching of phenazine constitutional unit , 1471 cm^{-1} CH_2 bending polyethylene, 1630 cm^{-1} N-H bend 1^0 amines, 2853 cm^{-1} C-H stretch alkanes, 2924 cm^{-1} H-C=O: C-H stretch aldehydes, 2960 C-H stretch alkanes and 3441 O-H stretch alcohols. This result was compared with the similar results of (Fatima *et al.*, 2017).

CONCLUSIONS

The current study shows that the isolated strain extract of *Streptomyces* sp. KAS-1 from sediment soil samples of Kali river of the Uttar Kannada region has the potential to act against pathogenic microorganisms. Here, we found that sediment soil samples of the Kali River Estuary, Karwar is a good region of biodiversity and has been adequately acceptable due to its vast flora and microbial diversity. Results showed that the strain *Streptomyces* sp. KAS-1 is dominant against two active test pathogens and showed the production of hydrolytic enzymes for all four enzyme activity tested. Thus, the study on this isolate can further be explored for the development of new antibiotic drugs to treat infectious diseases caused by pathogenic and drug-resistant strains as well as for the production of hydrolytic enzymes in industrial and pharmaceutical purposes.

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