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RESEARCH ARTICLE

Molecular characterization and Probiotic Potential of indigenous Streptomyces Isolates

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A B S T R A C T

Probiotics have emerged as a natural and effective alternative to antibiotics due to their relevancy to gut health and immune booster functions both in animals and humans. In this study, five indigenous *Streptomyces* strains—*Streptomyces rochie*, *Streptomyces fimbriatus*, *Streptomyces WSN2*, *Streptomyces globiosporus*, and *Streptomyces toxytricin*—were selected for molecular characterization and *in vitro* evaluation of their probiotic potential. These strains were isolated from native soil samples and identified using 16S rRNA gene sequencing. Each strain was screened for key probiotic traits, including resistance to pH, antimicrobial activity against common pathogens, and compatibility with standard antibiotics. The results indicated that all five strains exhibited notable tolerance to gastrointestinal-like conditions, with *S. WSN2* and *S. fimbriatus* showing the most robust antimicrobial effects. In conclusion, the selected indigenous *Streptomyces* species possess promising traits to be considered as probiotic candidates, with possible applications for the improvement of human and animal health.

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INTRODUCTION

Microorganisms are ubiquitous and can cause many diseases, which are characterized by the presence of bacteria, viruses, fungi, and certain parasites. The genus *Streptomyces* belongs to the phylum Actinobacteria, a diverse group of filamentous, Gram-positive bacteria known for producing a wide variety of secondary metabolites. This production plays a significant role in microbiology and biotechnology. *Streptomyces* play a significant role in the production of two-third of all antibiotics, most common are chloramphenicol, streptomycin, tetracycline, and neomycin, those are currently used in clinical settings (Maqbool *et al.,* 2023; Verma *et al.,* 2023: Tariq *et al.,* 2025). In microbiology and agricultural biotechnology, *Streptomyces* plays a significant role since its discovery. Most of the studies show that *Streptomyces* isolation from natural habitat produce variety of probiotics that can suppress harmful microbes, adapt to immunological response ultimately can help the gut environment improvement (Butt *et al*., 2023; Maqbool *et al.,* 2023). Like other species, *Lactobacillus*, *Bifidobacterium*, *Actinobacteria* species, and *Streptomyces* are considered as potential next-generation probiotics (Akram *et al*., 2017). Genus *Streptomyces* can produce a variety of secondary metabolite compounds those have strong antibacterial, antioxidant, and anti-inflammatory characteristics, especially those isolated from natural habitats like soil or other sources like the human gut, fermented food (El-Tarabily *et al*., 2021; Yaseen *et al*., 2025). One of the most important *Streptomyces levis* strains, HFM-2, was isolated from a human fecal sample. This strain has high antioxidant potential, antibiofilm activity that helps in eliminating strong, harmful biofilm-forming bacteria, and has strong antioxidant and high antibiofilm activities (Verma *et al.,* 2023).

For the declaration of potential probiotic strains of *Streptomyces*, molecular characterization is considered most important for their confirmation. For this purpose, identification, evolutionary connection with identified

probiotics or pathogenic strains and species validation is done through different methods including 16S rRNA gene sequencing, phylogenetic analysis, and whole genome analysis (Rijia *et al.,* 2024). Following the process of isolation and identification to select the probiotics strains, *in vitro* screening is considered most important. These *in vitro* tests proved the ability of *Streptomyces* strains to withstand acid and bile salt levels, their ability to adhere to intestinal epithelial cells, and their potential as antibacterial against the pathogenic bacteria in gut microbiota and antibiofilm. Inhibitory action against fungal phytopathogens like *Sclerotinia sclerotiorum* also has been demonstrated by certain strains, including *Streptomyces sampsonii* and *Streptomyces rochei*, suggesting potential cross-applications in plant and human health (El-Tarabily *et al.,* 2021). Furthermore, the role of *Streptomyces* in gut ecology is being acknowledged widely. Although *Streptomyces* are often not considered resident gut microbes, studies have discovered their DNA sequences in fecal samples both from humans and animals. This implies that *Streptomyces* might temporarily colonize or behave symbiotically in the gastrointestinal tract. Their natural abilities to resist environmental stress, produce antimicrobial peptides, and low-level inflammatory responses make them particularly desirable for the production of probiotics. Additionally, their ability to produce siderophores and other advantageous compounds also assist them to compete with pathogenic bacteria for nutrients or attachment sites, so preserving the microbiota's equilibrium (Butt *et al.,* 2023; Maqbool *et al.,* 2023).

Some negative traits of this bacterial genus have also been reported in the literature, like some strains of *Streptomyces* species have genes linked to virulence or can create toxic compounds. Before any declaration of the strains 'probiotics use in medicine or food, strict safety measures must be adopted, including preclinical safety evaluations and molecular screening for toxic genes. To make the strains more effective with strong probiotics properties, different techniques have been developed in synthetic biology and genetic engineering, which made it possible to modify strains to improve their functional efficacy and safety by deleting or silencing unwanted genes (Rijia *et al*., 2024). Poultry, being the more economical and richer source of protein has demand globally, and all the efforts are put to meet this demand. Due to this increase, growth promoters including antibiotics, phytochemicals, secondary metabolites from microbes are being used to improve the feed consumption ratio and gut health. As non-judicious antibiotics use may contribute to antimicrobial resistance, phytochemicals and probiotics are emerging as safer alternatives globally (Gul and Alsayeqh, 2022; Gul and Alsayeqh, 2023; Maqbool *et al.,* 2023; Tariq *et al.,* 2024; Yue *et al.,* 2025). Keeping in view these global trends, there is an urgent need to explore the alternatives to antibiotics that may assure the safety and economic viability in poultry production, not only in developing as well as in developed countries. So, the current project was designed to find the potential of local strains of *Streptomyces* to be used as probiotics in poultry. The data related to impact of these strains on gut health has already been published, this manuscript is emphasizing more on the microbiological aspects of the *Streptomyces*.

MATERIALS AND METHODS

Isolation and identification of *Streptomyces:* Soil samples were taken at depths ranging from just below the surface to 20cm, from variety of settings, such as agricultural fields and plant rhizospheres. Samples were collected in sterile plastic tubes and appropriately tagged for identification. Later, samples were allowed to cool to ambient temperature, and then stored at 4℃ after being oven-dried for two hours at 40-50°C. One gram of each soil sample was suspended in sterile water, shaken in an orbital shaker at 30°C for 1-2hours, and used as stock cultures for the isolation of *Streptomyces* in order to perform microbiological isolation. Following serial dilutions, the diluted samples were plated onto starch-casein nitrate agar and incubated for seven days at 30°C. Separate colonies were kept for additional screening after being purified.

Screening of *Streptomyces* for antibacterial activity: Using the agar streak method, the *Streptomyces* isolates were first screened for antibacterial activity against test bacteria such as *Salmonella* Typhi, *Escherichia coli*, and *Bacillus subtilis*. For morphological, cultural, and biochemical evaluation, colonies exhibiting potent antibacterial activity were chosen. In order to assess different characteristics including colony morphology, pigment generation, and hyphal structure under a microscope, they were cultivated on a variety of International *Streptomyces* Project (ISP) media. Biochemical profiling was carried out by additional assays such as nitrate reduction, xylanase activity, proteolytic and amylolytic activities, and the formation of acid and H_2S. A carbohydrate assimilation test using ISP-9 medium was used to assess the isolates' capacity to use various carbon sources.

For secondary screening and antibiotic production, selected isolates were subjected to fermentation in a production medium incubated for 10 days. After fermentation, cultures were centrifuged, and the supernatant was extracted with chloroform. The crude extract was tested for antibacterial activity using the cup well diffusion method against standard bacterial strains.

Molecular characterization and phylogenetic analysis of *Streptomyces* isolates: DNA was isolated from potent strains using a modified protocol, and genomic DNA was verified using agarose gel electrophoresis. DNA purity and concentration were measured using a Nanodrop spectrophotometer. Amplification of the 16S rRNA gene was carried out via PCR using specific primers, followed by sequencing and BLAST analysis to confirm species identity by following the method described by Ashraf *et al.,* 2022.

RESULTS

Isolation and screening of *Streptomyces* colonies: A total of 10 different soil samples were spread on casein nitrate agar plates and incubated for seven days. After incubation, 50 colonies were randomly selected. To purify these colonies, they were further streaked on agar plates to

study their morphological characteristics, and the results has been shown in Fig. 1.

Colonial morphology characteristics: To study the morphological and cultural characteristics of the colonies, International *Streptomyces* Project (ISP) media was used. For this purpose, different ISP media such as ISP-1, ISP-4, ISP-5, ISP-6, ISP-7, ISP-9 were used to study the morphological characteristics. Different growth feature like aerial mycelium, pigment production, on these different media was observed and results have been shown in Table 1 and 2 for all the selected isolates. On all tested media of ISP filamentous growth was observed in all the selected colonies, but the pigment production was only observed in the casein starch nitrate medium of 5 isolates as shown in Fig. 2A.

Microscopic characterization: Powdery spore formation of different shades in the late growth phase shows that all isolates belong to *Streptomyces* Species. Although a microscopic study revealed the characteristic mycelium-like growth identified by filamentous appearance at 1000X magnification under a phase contrast microscope and shown in Fig. 2B.

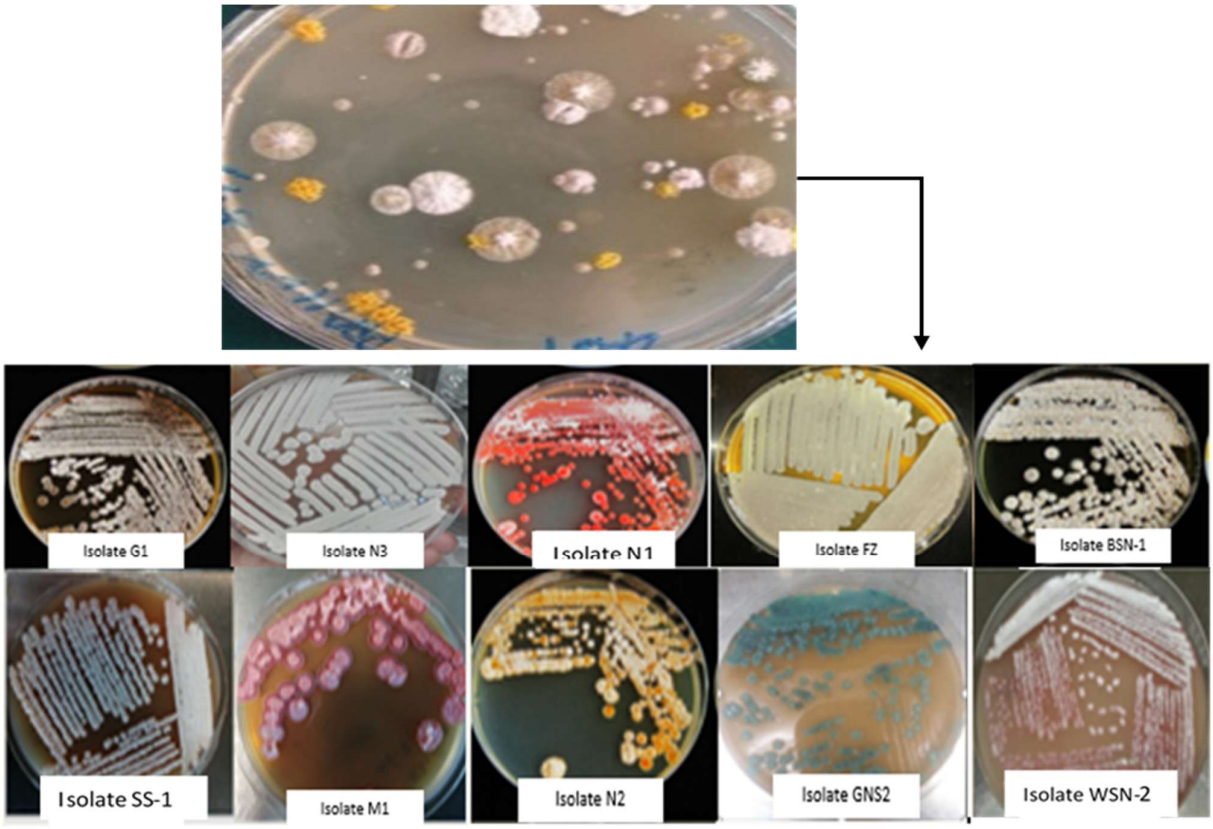

Fig. 1: *Streptomyces* colonies on starch-casein media plates obtained by spreading serially diluted soil samples.

Table 1: Morphological and cultural characteristics of *Streptomyces* isolates on different media

Sr. No	Medium used	Growth	Colour of aerial mycelium	Colour of substrate	Pigment production
Morphological and cultural characteristics of *Streptomyces* isolates SS1					
1	(ISP-1) Tryptone- yeast extract broth	Good	Whitish	Light Yellow	No
2	(ISP-2) Yeast extract malt extract agar	Very good	Creamy	Light brown	No
3	(ISP-3) Oat meal agar	Poor	White	Yellow	No
4	(ISP-4) Inorganic salt starch agar	Good	Whitish	Light brown	No
5	(ISP-5) Glycerol asparagine agar	Very good	White	White	No
6	Starch casein agar	Excellent	Whitish Grey	Light yellow	Light yellow
Morphological and cultural characteristics of *Streptomyces* isolates G1					
1.	(ISP-1) Tryptone- yeast extract broth	Good	Whitish Grey	white	No
2.	(ISP-2) Yeast extract malt extract agar	Good	Whitish Grey	Brown	No
3.	(ISP-3) Oat meal agar	Poor	Light Grey	Yellow	No
4.	(ISP-4) Inorganic salt starch agar	Very good	Grey	Light brown	Light brown
5.	(ISP-5) Glycerol asparagine agar	Very good	Whitish grey	White	Light yellow
6.	Starch casein agar	Excellent	Grey	Yellow	Light yellow
Morphological and cultural characteristics of *Streptomyces* isolates WSN2					
1.	(ISP-1) Tryptone- yeast extract broth	Good	Whitish pink	White	No
2.	(ISP-2) Yeast extract malt extract agar	Good	Pink	Pinkish	No
3.	(ISP-3) Oat meal agar	Poor	Light pink	Brown	No
4.	(ISP-4) Inorganic salt starch agar	Very good	Pinkish	Light brown	Light pink
5.	(ISP-5) Glycerol asparagine agar	Very good	Light pink	white	Pinkish
6.	Starch casein agar	Excellent	Pinkish	Pinkish	Pinkish

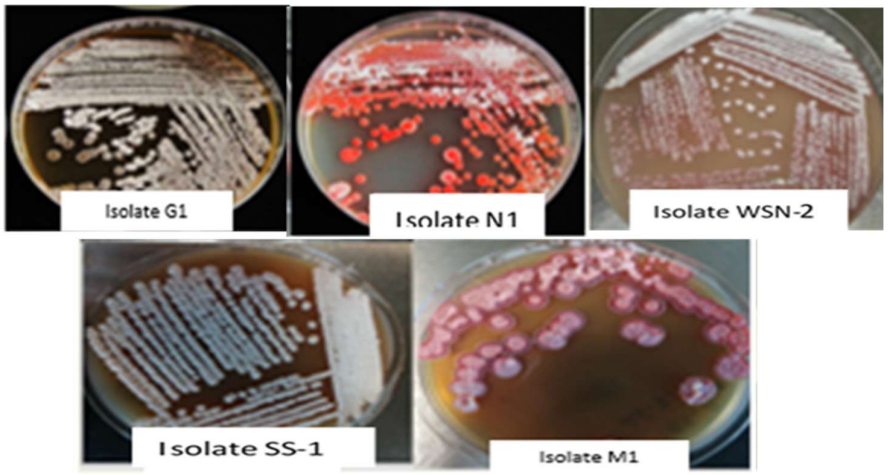

Fig. 2A: Colony characteristics of axenic growth of different isolates of *Streptomyces* showing characteristic pigment production.

Fig. 2B: Mycelial growth of *Streptomyces* spp. as examined by a phase contrast microscope (1000X magnification)

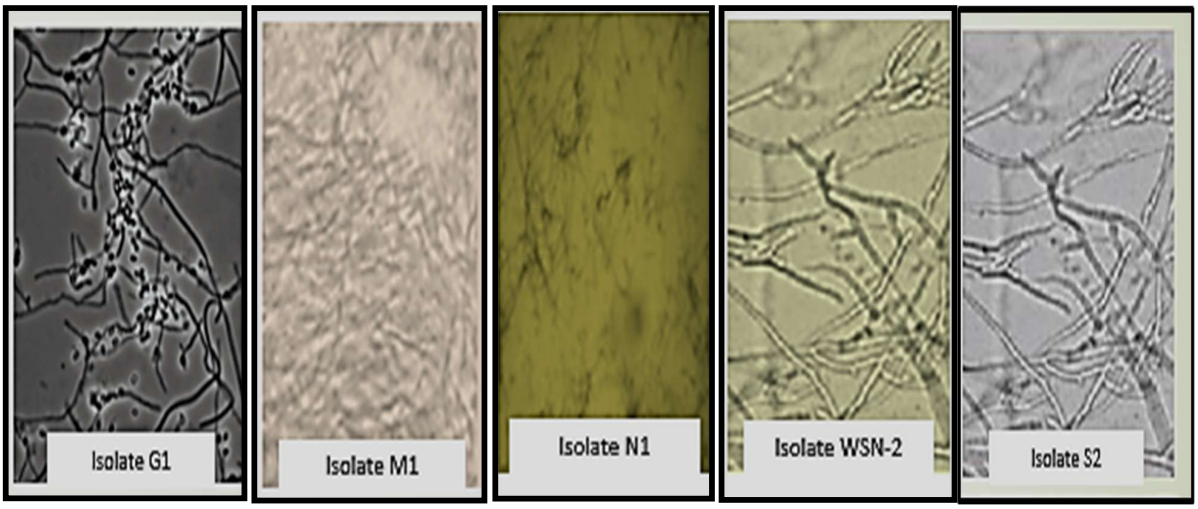

Table 2: Morphological and cultural characteristics of *Streptomyces* isolates on different media

Sr. No	Medium used	Growth	Colour of aerial mycelium	Colour of substrate	Pigment production
Morphological and cultural characteristics of *Streptomyces* isolates M1					
1	(ISP-1) Tryptone- yeast extract broth	Good	Light yellowish	Yellowish	No
2	(ISP-2) Yeast extract malt extract agar	Good	whitish	Light yellow	Yellowish pigment
3	(ISP-3) Oat meal agar	Good	Whitish	Brown	Yellowish pigment
4	(ISP-4) Inorganic salt starch agar	Very Good	whitish	Yellow	Yellowish pigment
5	(ISP-5) Glycerol asparagine agar	Very Good	Creamy	Yellow	Yellowish pigment
6	Starch casein agar	Excellent	Light yellowish	Yellow	Light yellowish
Morphological and cultural characteristics of *Streptomyces* isolates N1					
7.	(ISP-1) Tryptone- yeast extract broth	Good	Whitish orange	White	No
8.	(ISP-2) Yeast extract malt extract agar	Good	Whitish orange	White	No
9.	(ISP-3) Oat meal agar	Poor	Light orange	orange	No
10.	(ISP-4) Inorganic salt starch agar	Very Good	Orange	Orange	Light orange
11.	(ISP-5) Glycerol asparagine agar	Very Good	Orange	Orange	Light orange
12.	Starch casein agar	Excellent	Orange	Orange	Orange

Preliminary screening for antimicrobial agents production by cross-streak method: To check the antimicrobial activity of all the selected isolates, initial screening of antimicrobial agents was established against Gram-negative bacteria (*E. coli* and *Salmonella* Typhi) as well as Gram-positive bacteria (*Bacillus subtilis*) by the cross-streak method. The results details have been shown in Table 3. Out of all the isolates, only 05, encoded as SS1, G1, WSN-2, M1, and N1, show strong inhibitory activity against all the tested microorganisms as shown in Fig. 3A. Therefore, these 5 isolates (SS1, G1, WSN-2, M1, and N1) were selected for further studies.

Biochemical characterization of antimicrobial agents producing *Streptomyces* isolates: Those five (05) isolates with strong antimicrobial activity were further studied for chemical characterization. All the isolates have the potential to produce the melanin pigment. Isolates coded with SS1, G1, produce light yellow to brown pigment in casein starch nitrate medium. While isolates WSN2, M1, and N1 produce a light pinkish, orange colour, light yellow colour pigment in most of the ISP media. All of these isolates produced substrate-based extracellular enzymes such as amylase, protease, xylanase etc. All of the isolates showed starch hydrolysis, proteolytic, and gelatine liquefaction activities. Nitrate reduction test was also

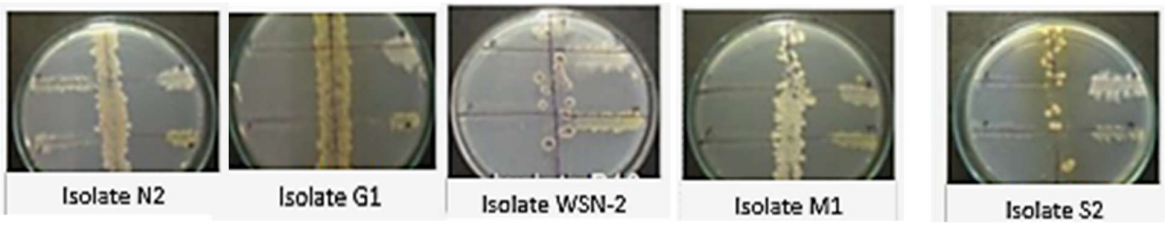

Fig. 3A: Results of growth inhibition of *E. coli, Salmonella* Typhi, *Bacillus subtilis* by *Streptomyces* species (SS1, G1, WSN-2, M1 and N1).

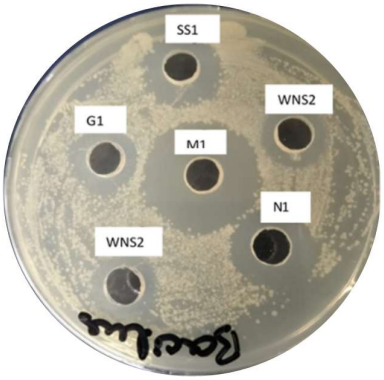

Fig. 3B: Zone of inhibition against *Bacillus subtilis* produced by (a) isolate SS-1, (b) isolate G-1(c) isolate M1 (d) isolate N1 (e) WSN2.

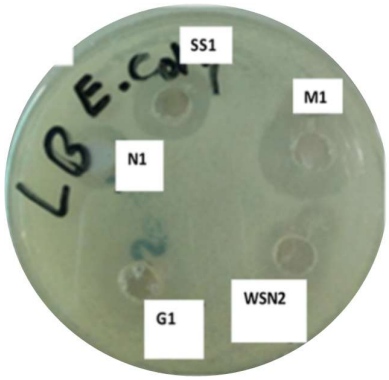

Fig. 3C: Zone of inhibition against *E. coli* produced by (a) isolate SS-1, (b) isolate G-1(c) isolate M1 (d) isolate N1 (e) WSN2.

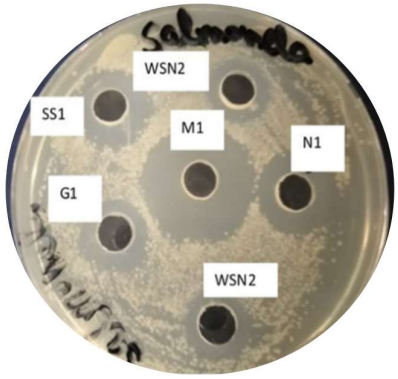

Fig. 3D: Zone of inhibition against *Salmonella typhimurium* produced by (a) isolate SS-1, (b) isolate G-1(c) isolate M1 (d) isolate N1 (e) WSN2.

Table 3: Results of antagonistic activity of isolated *Streptomyces* species against Gram-positive and Gram-negative bacteria

Sample no.	Isolate	Antimicrobial activity		
		Bacillus subtilis (ATCC6633)	*E. coli* (ATCC25922)	*Salmonella* Typhi (ATCC13311)
1	SS1	+++	++	++
2	G1	+++	++	+
3	WSN-2	++	+	+
4	M1	++++	++++	+++
5	N1	+++	+++	++

++++: Maximum activity; +++: Moderate activity; ++: Minimum activity, +: Least active.

positive for all the isolates, but no acid was produced in any ISP media used for the growth of these five isolates (Table 4). All of these *Streptomyces* isolates can grow from 25-45°C, but optimum growth was obtained between 28-34°C. Likewise, these isolates showed the grow between the pH range of 6.0-9.0, but optimum growth was observed at pH 8.0-9.0. It has also been indicated that all of these isolates could utilize a wide range of carbon sources such as glucose, lactose, maltose, fructose, sucrose, starch, and glycerol with slight variation in growth.

Molecular characterization and phylogenetic analysis: Genomic DNA of all the isolates S2, G1, M1, WSN2 and N1 was extracted (Fig. 4A). For molecular identification 16S rRNA gene of these bioactive *Streptomyces* isolates was amplified (1500bp) through PCR as shown in Fig. 4B and sequenced. These sequences were submitted to GenBank, and accession numbers were obtained and has been mentioned against each isolate. Species level characterization of these isolates was done through phylogenetic analysis. Phylogenetic tree construction and their potential relation with some already described *Streptomyces* species was determined by neighbour-joining method, using online available MEGA 11 software as shown in Fig. 5A. Sequence comparison study of isolate SS1(GenBank Accession # MF407458.1) , G1(GenBank Accession # MH341628.1), M1(GenBank Accession # OL744614.1), WSN2 (GenBank Accession # MN128377.1) and N1 (GenBank Accession # MH341623.1) showed above 99% identities with 16S rRNA gene sequences of *Streptomyces rochei, Streptmyces Fambritius Streptomyces globiosporious and Streptomyces toxytricin,* WSN2, respectively.

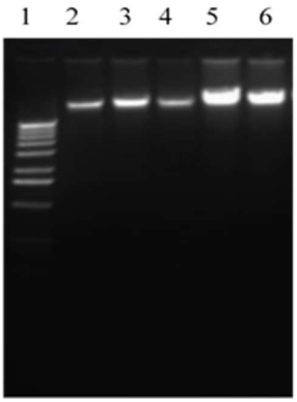

Fig. 4A: Genomic DNA isolation of indigenous *Streptomyces* isolates (SS1, G1, M1, N1, WSN-2). Lane 1= 1Kb DNA Ladder, Lane 2 = Genomic DNA of isolate SS1, Lane 3= Genomic DNA of isolate G1, Lane 4 = Genomic DNA of isolate M1, Lane 5= Genomic DNA of isolate N1, Lane 6= Genomic DNA of isolates WSN-2

Fig. 4B: PCR amplification of 16S rRNA gene of isolated *Streptomyces*. Lane 1= 1Kb DNA Ladder; Lane 2 = negative control; Lane 3= 1500 bp products of 16S rRNA gene of isolate SS1; Lane 4 = 1500 bp products of 16S rRNA gene of isolate G1; Lane 5= 1500 bp products of 16S rRNA gene of isolate M1; Lane 6= 1500 bp products of 16S rRNA gene of isolates WSN2; Lane 7= 1500 bp products of 16 rRNA gene of isolates N1.

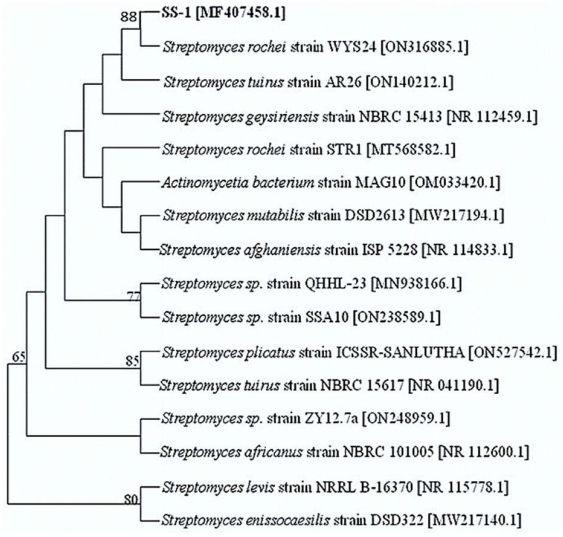

Fig. 5A: Phylogenetic tree obtained by neighbor joining method using bootstrap analysis of 16S rRNA gene sequences and SS1 isolate,

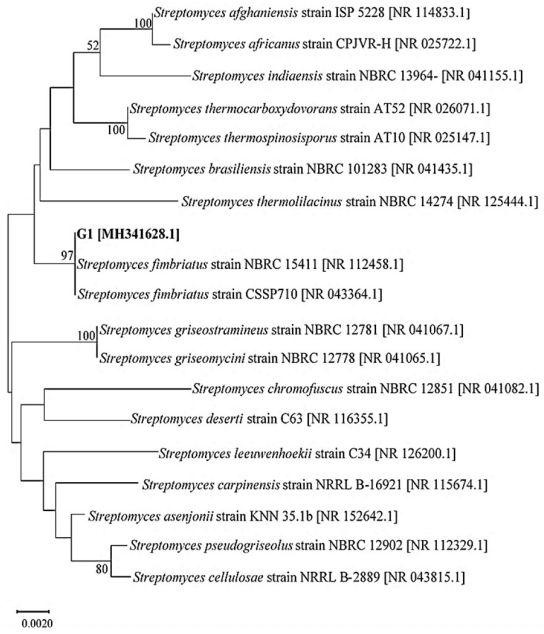

Fig. 5B: Phylogenetic tree obtained by distance matrix analysis of 16S rRNA gene sequences and isolate G1. Note: Numbers at branch nodes are bootstrap values, expressed as percentages of 100 replicates (only values >50% are shown). Bar, 0.1 substitutions per nucleotide position. The phylogenetic tree was constructed by using MEGA 11tree explorer.

The phylogenetic tree of Streptomyces isolate SS1 showed that this isolate occupies a position clustering with Streptomyces rochei strain WYS24 (ON316885.1) although the bootstrap value is less than 90%. These observations indicated that this isolate belongs to the S. rochei species but may be slightly different from already available strains of Streptomyces rochei. The phylogenetic tree has been shown in Fig. 5B. While Streptomyces isolate G1 was found to cluster with Streptomyces fimbriatus strain NBRC 15411 (NR112458.1) as shown in Fig. 6A, which indicated its similarity with Streptomyces fimbriatus showing the boot strap value of 97%.

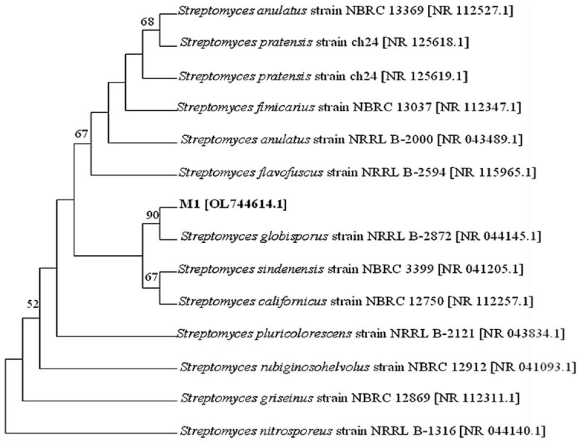

Fig. 6A: Phylogenetic tree obtained by neighbour joining method using bootstrap analysis of 16S rRNA gene sequences of *Streptomyces* isolate WSN2.

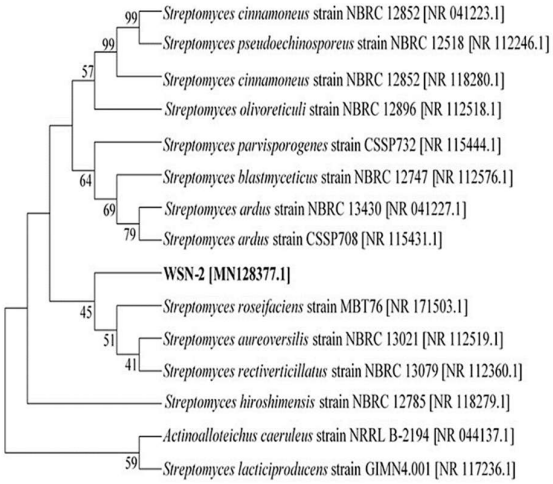

Fig. 6B: Phylogenetic tree obtained by neighbour joining method using bootstrap analysis of 16S rRNA gene sequences of Streptomyces isolate N1. Note: Numbers at branch nodes are bootstrap values, expressed as percentages of 100 replicates (only values >50% are shown). Bar, 0.1 substitutions per nucleotide position. The phylogenetic tree was constructed by using MEGA 11tree explorer.

Table 4: Biochemical Characterization of Streptomyces Isolates Showing Antimicrobial Activity

Biochemical Characteristics	*Streptomyces* Isolates				
	SS1	G1	WSN2	M1	N1
Optimum temperature	30°C	28°C	34°C	30°C	32°C
pH range	8.0	8.0	9.0	8.0	9.0
Starch hydrolysis	+++	++	+	+++	++
Gelatin hydrolysis	++	+	+	++	+
Proteolytic activity	++	++	++	+++	++
Casein hydrolysis	++	++	++	+++	+
Citrate utilization	+	+	+	++	+
H_2S production	-	-	-	-	-
Nitrate reduction	++	+	+	+	-
Urease	+	-	-	+	-
Catalase	+	+	+	++	+
Oxidase	++	+	+	+	+
Phosphate solubilization	+	-	+	+	-
Acid production	-	-	-	-	-
Glucose utilization	++	+	+	++	++
Lactose utilization	+++	++	+	+++	++
Maltose utilization	++	++	+	+	+
Fructose utilization	++	++	+	++	++
Dextrose utilization	++	+	+	+	+
Xylanase	+	+	+	++	+
Amylase	+	+	+	+	+

DISCUSSION

Multidrug resistant organisms are becoming a serious concern around the globe and despite the wide range of activity, antibiotics have historically been the most researched bioactive metabolites. Multiple antibiotic resistance is now becoming more alarming and compromises the clinical management of infectious diseases both in animals and humans (Ahmad *et al*., 2021; Pinheiro *et al*., 2020). So, there is an urgent need for potentially beneficial microbes/bacteria to fight against antimicrobial resistance and to control and opportunistic pathogens. *Streptomyces* strains have been proved to be the richest source of secondary metabolites and a potential source of bioactive chemicals (Suthindhiran and Kannabira, 2009; Butt *et al.,* 2023; Maqbool *et al.,* 2023).

*Streptomyc*es isolation and characterization from diverse habitat has a lot of interest because it is believed that screening of these organisms enhance the probability of finding new natural compounds by utilizing the biotechnological resources (Bredholt *et al*., 2008; Eccleston *et al*., 2008; Butt *et al.,* 2023; Maqbool *et al.,* 2023). This is the sound reason that new *Streptom*yces isolates from diverse habitats are proven to be a valuable source of fresh bioactive compounds. (Fiedler *et al*., 2005; Bull and Stach, 2007). Therefore, expanding this strategy to additional unexplored regions appears appropriate.

However, the study was aimed to lay the basis for future research by isolating, characterization, and screening native *Streptomyces* species. It was the goal of the current investigation to compile an exhaustive list of the different types of *Streptomyces* found in the local soil or to extract and characterise novel bioactive substances. Strenuous culture conditions and different chemical and physical conditions of soil samples were employed to isolate the *Streptomyces* strains on selective media by Hayakawa *et al*. 2004.

Identification of *Streptomyces* from all other bacterial group can be done because of their distinctive growth pattern, dense and embedded colonies, diffuse pigments and coloration of both substrate and aerial mycelia (Anderson and Wellington, 2001). So, in this study, fifteen soil samples were tested for the isolation of *Streptomyces,* their bioactivity against some Gram positive (*Bacillus subtilis*) and Gram negative (*E. coli*, *S.* Typhi) bacteria. Only five isolates were capable to prove their antimicrobial potential, and they were selected for further analysis.

The remarkable taxonomic and metabolic diversity found within *Streptomyces* species becoming more evident as new and potentially novel *Streptomyces* species were isolated during this study and demonstrated their potential for bioactive materials. It has been established in the literature that the taxonomic and ecological positions of antibiotic producing microorganisms are an integral part in antimicrobial agents' development programme. Comprehensive understanding of the microorganisms and their secondary metabolites being produced can be very helpful (Adegboye and Olubukola, 2012; Maqbool *et al.,* 2023).

Streptomyces is being identified on the basis of morphology, physiology, ecology and molecular characterization etc, however, to ensure the novelty of the metabolites being helpful as antibiotic is deemed necessary to be identified at species level. Although the habitat and suborders might be helpful for identification of secondary metabolites production and their potential effects, still further exploration can be more promising (Adegboye and Olubukola, 2012). Keeping in view these shortcoming, local isolates were tested for tested for carbon assimilation, gelatin liquefaction, H_2S production, melanoid formation, milk coagulation, nitrate reduction for their taxonomical characterization in this study. As per directive mentioned in already published literature, this analysis helped us to identify the morphology and culture characteristics of local Streptomyces isolates and their authentic categorization in the genus *Streptomyces* (Lorková *et al.,* 2025).

The further characterization of 16S rRNA, sequencing and phylogenetic analysis are important and evolutionary component of this study. Because for evolutionary analysis of multiple organisms, the analysis of family needs to be studied. Hence the most closely related sequences can be identified those lie just closer to them as the neighbouring branches of the tree. This objective is well accomplished through phylogenetic analysis and those microorganisms who share the same origin can be easily identified through this method (Krieger *et al.,* 2024). As a result, identification of new microbial species can lead to discovery of novel secondary metabolites (Thumar *et al*., 2010; Krieger *et al.,* 2024). In this study, 16S rRNA gene analysis of the locally isolated Streptomyces was done and their sequence were aligned with the available complete sequences of family Streptomyccetaceae. The potential isolates including SS1, G1, M1, N1 and WNS2 shared 99% similarity with this genes sequences of various strains including *Streptomyces rochie,*

The potent isolate SS1, G1, M1, N1, WNS2 showed 99% identity with 16S rRNA gene sequences of various strains of *Streptomyces rochie, Streptomyces fambritius, Streptomyces globiosporius, Streptomyces toxitricin and Streptomyces WNS2,* respectively. The phylogenetic analysis indicated that these strains were quite different from the other strains of *Streptomyces* including *Streptomyces hygroscopicus*, and *Streptomyces rimosus*, which have earlier been documented for their antimicrobial activities. For the production of secondary metabolites, it is important to design an appropriate fermentation medium and hence a previous knowledge and experience can be important factor for its optimization. Various environmental factors can also play a key role during the fermentation process for the production of secondary metabolites including nutrients (nitrogen, phosphorous & carbon source), growth rate, feedback control, enzyme inactivation and variable conditions (oxygen supply, temperature, light & pH) (Sanchez *et al*., 2010). Additionally, based on the strains used in fermentation process can influence the quantity and quality of the secondary metabolites during the fermentation process. The important role of the precursors and energies are also crucial for the synthesis of building blocks and secondary metabolites (Wang *et al*., 2010; Krieger *et al.,* 2024; Lorková *et al.,* 2025).

Conclusions: Hence this study concludes that medium components and environmental conditions play primary role for the production of secondary metabolites from genus *Streptomyces*. Indigenous *Streptomyces* isolates

exhibit key probiotic traits such pH tolerance, and antimicrobial activity against livestock pathogens, supporting their candidate status for veterinary probiotic development. *S. WSN2* and *S. fimbriatus* showed particularly strong antimicrobial potential. Future research should prioritize *in vivo* validation and thorough safety evaluations to enable their use as antibiotic alternatives in poultry and livestock production.

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