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Streptomyces sp SM01 isolated from Indian soil produces a novel antibiotic picolinamycin effective against multi drug resistant bacterial strains 2020

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Streptomyces sp SM01 isolated from Indian soil produces a novel antibiotic picolinamycin effective against multi drug resistant bacterial strains

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Abstract

- A Kashmir Himalayan **(India) soil** isolate, *Streptomyces* sp. SM01 was subjected to small scale fermentation for the production of **novel antimicrobials**, picolinamycin (SM1).
- The production has been optimized which found to be maximum while incubated in **AIA medium** (pH 7) for 7 days at 30 °C.
- showed a **larger zone of inhibition against *Staphylococcus aureus*** compared to **streptomycin (5µg) and ampicillin (5µg).**
- has been proved to be a **new class of antibiotic** with 1013 dalton molecular weight.
- We have named this new antibiotic as **picolinamycin.**
- antimicrobial **potency** of this newly characterized antibiotic found to be **higher against Gram-positive** organisms than the tested **Gram-negative organisms**.
- it showed strong growth impairments of several multidrug resistance **(MDR).**
- It also showed **anti-mycobacterial** potential.
- **Picolinamycin** however did not show toxicity against tested A549 **human cell line**

Background

- About **one out of ten patients** are acquiring nosocomial infection as hospitals are the principal source of multidrug-resistant (MDR) pathogens .
- From January **2000 to October 2019**, only **44 compounds under 7 novel** classes have been introduced in clinical pipeline.
- Despite the discovery of new antimicrobials, the rise of antibiotic resistance is very fast .
- Among the microbes**, actinobacteria** are one of the principal factories for **novel antibiotic** production because nearly two-third of all known antibiotic found to be derived from them.
- Although **soil** contains diverse species of Streptomyces, for isolation of novel antibiotic-producers, **the target soil** sample **needs to be very specific to avoid the re-isolation** of the same species and same antibiotics.
- This study delineates about the identification of an isolate, ***Streptomyces* sp. SM01.**
- **Purification** of the antimicrobial compound has been done through **chromatography** followed by structural elucidation **through various spectrometry** and **CHN-analysis.**
- The **MIC and MBC** of the purified compound against several test organisms ranging from **non-pathogenic, opportunistic, eight MDR strains.**
- **picolinamycin** does not have any cytotoxicity on A549 **human cell line** indicating its spectrum of activity limited within bacteria.

Hypothesis

- Isolation of actinomycetes from soil samples at Rangreth of Kashmir Himalaya, India could indicate their novelty as an isolate and their ability to produce non-redundant antimicrobials

Methods

1- Isolation

- The soil samples were collected from Rangreth of Kashmir Himalaya, India
- Soil samples were pre-treated with $CaCO_3$ for 7 days followed by heat treatment for 2 hr in a hot air oven at 65 °C to enrich and selectively isolate actinobacteria
- After that 1 g of soil was dissolved in 1 ml of 0.9% NaCl and a serial dilution up to 10−5 has been made.
- From these dilutions, each 0.1 ml of sample was spread on Actinomycetes isolation agar (AIA) medium .
- supplemented with 50 µg/ml of cycloheximide and nystatin to inhibit the unwanted fungal growth.
- The inoculated plates were incubated for 3–4 days at 28 °C. Incubated plates showed various actinobacterial colonies.
- 135 strains isolated, based on morphometry (substrate and aerial mycelium, soluble pigments, diffusible pigments).
- SM01 colony has been selected for its unique features, picked by the sterile toothpick, and streaked on fresh (International Streptomyces Project) ISP-2 plate to get pure cultures.

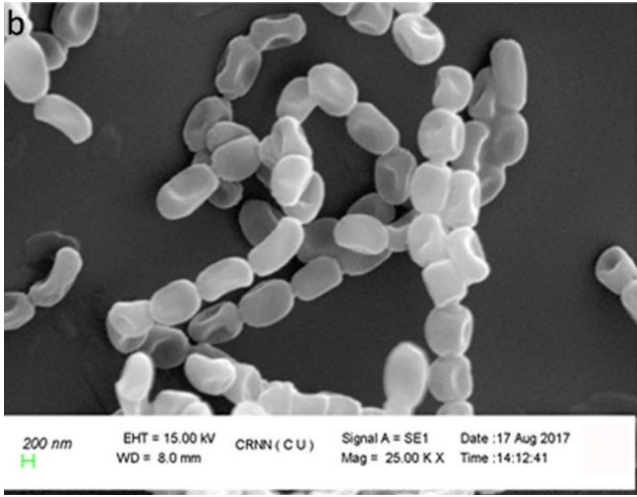
Methods

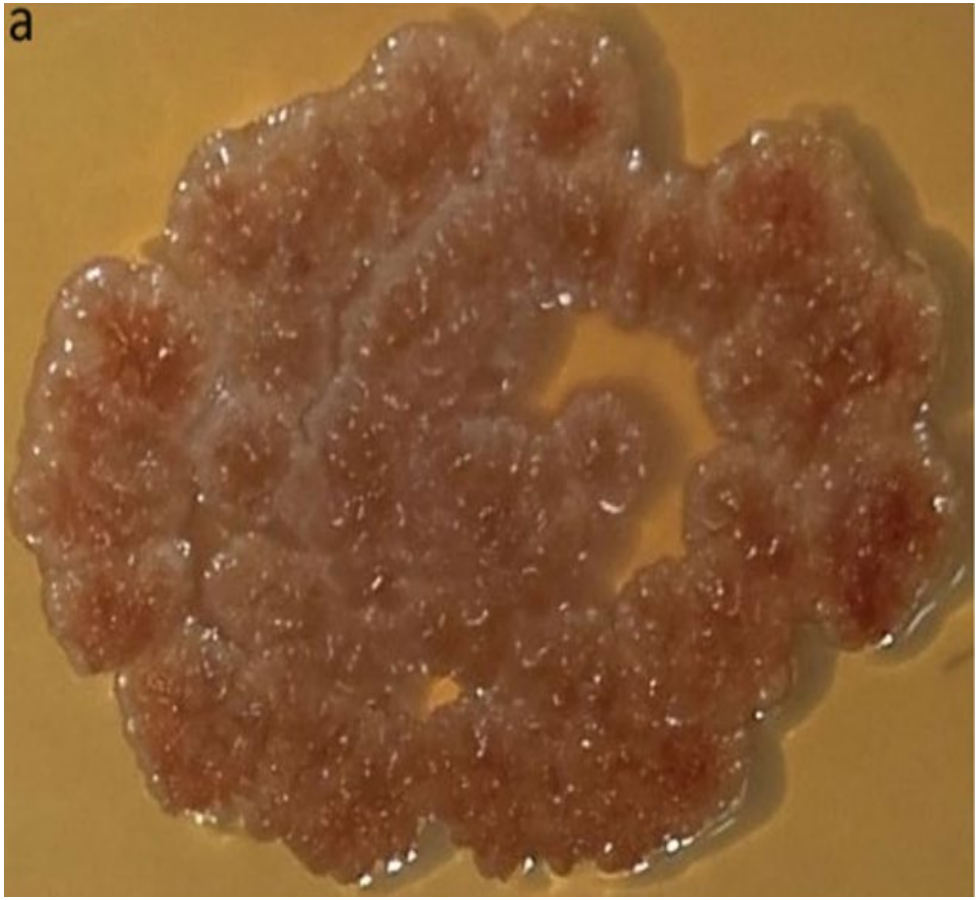
2- Identification of SM01.

- After 14 days of incubation on ISP-2 medium. Colony morphology which includes size, shape, margin, texture,form, optical property of the colony
- Scanning electron microscopy was performed
- The 16 S rDNA gene was amplified by using universal primer 8 F and 1492 R followed by sequencing and sequence analysis using both the BLAST program

Results

- SM01 was isolated from an unexplored region of Rangreth,
  Himalayan Kashmir, India, in AIA medium. SM01 identified as a Streptomyces .
- Scanning electron micrograph shows that the aerial mycelium produces spore chain and the spore surfaces are smooth .
- The 16 S rDNA gene sequence of SM01 shows that SM01 belong to separate cluster which might indicate that SM01 is a new species





SM01 colony

appearance of SM01 colony in ISP-2 agar medium

Methods

3-screening for antimicrobial property

- Antimicrobial activity was assessed by the agar-diffusion method on Mueller-Hinton (MH) agar media.
- For preliminary assay two Gram-positive (S. aureus MTCC 96, B. cereus MTCC 1272), two Gram-negative (E. coli MTCC 1687, P. aeruginosa) bacteria and one yeast (Saccharomyces cerevisiae) were used as test organisms.

Results

- From preliminary screening it has been found that SM1 inhibits the growth of both Gram-positive and Gram-negative organisms however, the growth impairment of yeast is very less.

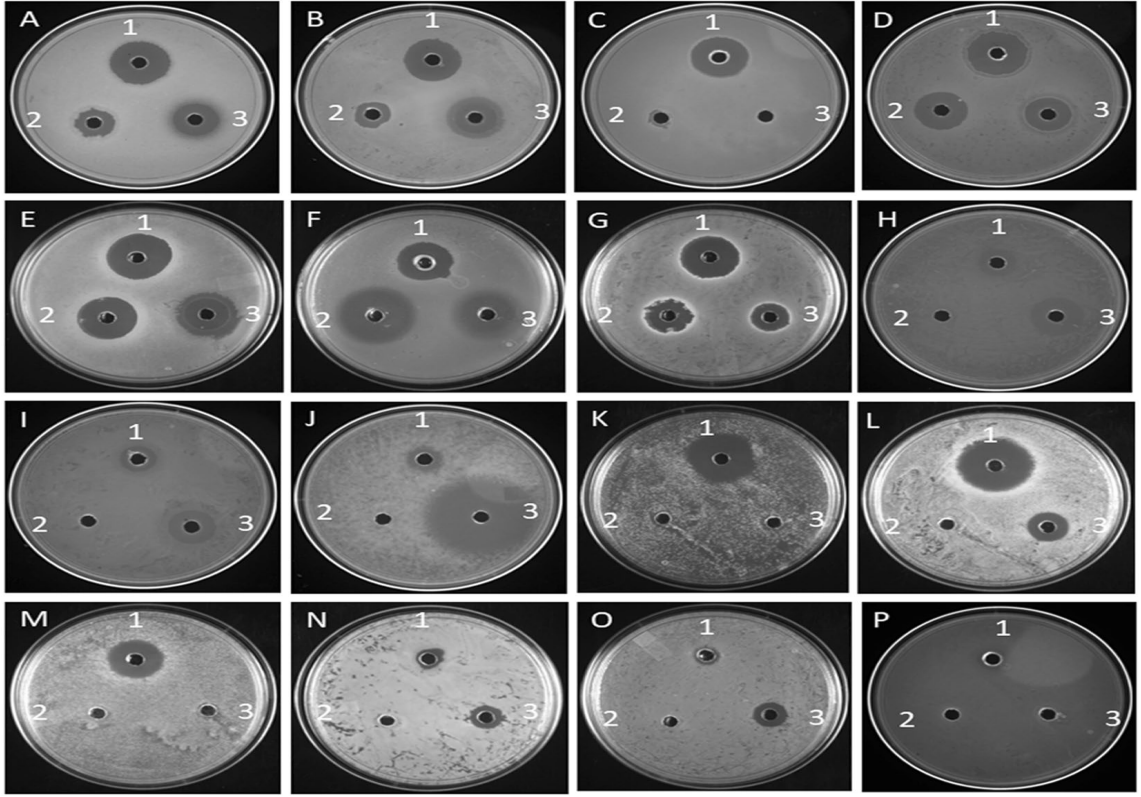
Methods

4-Determination of antimicrobial potency of SM01 culture supernatant.

- the culture extracts (50µL) were examined in detail to check the efficacy of the antimicrobial against different test organisms and compared with standard **ampicillin** and **streptomycin** antibiotics. The amount of ampicillin for Gram-positive and Gram-negative organisms was 0.1 µg and 5 µg, respectively whereas for streptomycin, it was 5 µg for both organisms.

- **Result**

In a qualitative assay, it has been found that 50 µL of culture supernatant showed a larger zone of inhibition against Gram-positive organisms compared to standard ampicillin and streptomycin



Antimicrobial efficacy of crude SM1. Efficacy detected by zone of inhibition against different organisms using SM1, ampicillin, streptomycin [A =S. aureus MTCC 96, B =S. epidermidis MTCC 3086, C=B. cereus MTCC 1272, D =B. stratosphericus MCC 2251, E =E. faecalis, F=S. Typhi, G = K. pneumoniae, H=E. coli MTCC 1687, I = P. aeruginosa, J =M. smegmatis mc2 155, K = S. haemolyticus (MDR)., L =S. aureus (MDR), M =Enterococcus sp, N = Enterococcus sp. 291, O =P. aeruginosa MV36846 (MDR), P =S. flexneri IDH 07210 (MDR)]; 1 = 50µl medium supernatant of SM01, 2 = Ampicillin (5 µg for Gram-negative bacteria, 0.1 µg/ml for Gram-positive bacteria), 3 = Streptomycin (5 µg).

Methods

5-Antibiotic sensitivity test by disc diffusion method..

- To understand the probable antimicrobial categories of the compound produced by SM01, **thirty standard antibiotics** were tested against SM01. 0.1 ml of the fresh culture of SM01 was spread and antibiotic discs were placed on MH agar plate.

Result

Interestingly it has been found that strain SM01 is sensitive against most of the tested antibiotics, weakly sensitive against seven antibiotics and resistance against none (see Supplementary Table S1). This indicates that SM01 should not be the producer strain of any of these tested antibiotics, as in general the strain become resistant against the self-produced antibiotics. Thus we predict that the SM1 produced by strain SM01 is different from these tested antibiotics and might have the possibility to be a new antimicrobial agent.

Methods

6-Production, extraction, and purification.

- 3-liter AIA medium with 1% inocula of SM01 was incubated at 28 °C for 7 days and medium supernatant was collected by centrifugation for 15 min at 13000 rpm. The active compounds have been extracted after adding an equal volume of ethyl acetate with the medium supernatant. The resulting active organic phase collected and dried by rotary evaporator

Methods

7-MIC and MBC of bacteria.

- Minimum inhibitory concentration (MIC) has been estimated in the microplate using MH agar medium.
- Minimum bactericidal concentrations (MBC) were analyzed in microplates through broth dilution

Test organisms	MIC (μg/ml)	MBC (μg/ml)
S. aureus MTCC 96	0.01	1.28
S. epidermidis MTCC 3086	0.01	>50
B. cereus MTCC 1272	0.01	5.12
B. stratosphericus MCC 2251	0.01	20.48
E. faecalis MCC 2041^{T}	0.01	50
S. Typhi	0.08	25
K. pneumoniae	0.02	>50
E. coli MTCC 1687	2.56	50
P. aeruginosa	5.12	20.48
M. smegmatis mc^{2} 155	10.24	>50
MDR strains		
S. haemolyticus	0.08	>50
S. aureus	0.08	5.12
Enterococcus sp.	0.04	1.28
Enterococcus sp.291	5.12	>50
P. aeruginosa MV36846	2.56	>50
S. flexneri IDH07210	2.56	50

MIC and MBC of picolinamycin

Antimicrobial activity of SM1.

- Interestingly among the five tested media for antibiotic production-optimization study, all media (except AIA) i,e starch casein, ISP-2 and TSB support larger quantity [wet weight (g)] of cell mass but showed smaller zone of inhibition against tested bacteria. We have observed that the growth and antibiotic production are not proportional as some medium support larger cell. mass without enhancement in antibiotic production.

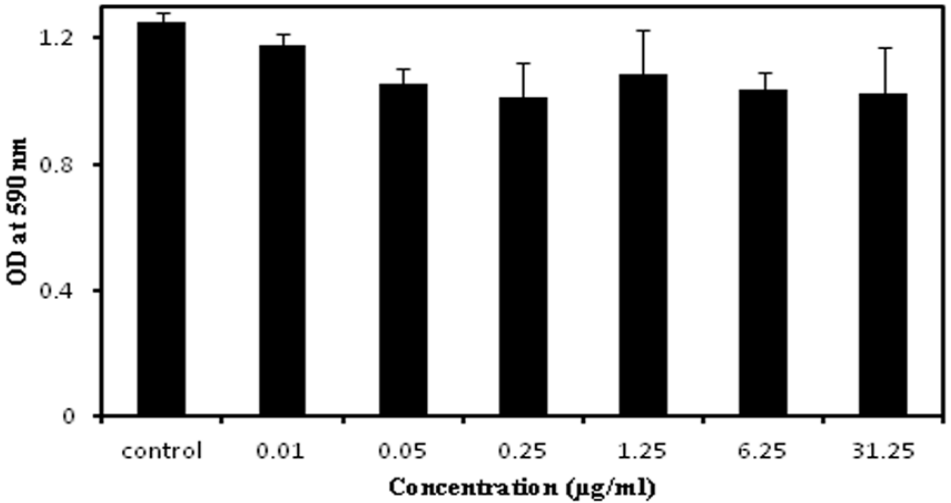
Medium	Wet weight (g)/100 ml	Zone of inhibition (mm)		
		S. aureus	*B. cereus*	*E. coli*
AIA	2.135	26	21	11
ISP-2	2.71	18	14	14
ISP-3	4.53	23	19	10
Starch casein	5.43	25	21	10
Tryptic soya broth	2.59	24	11	05

Methods

8-Cytotoxicity assay.

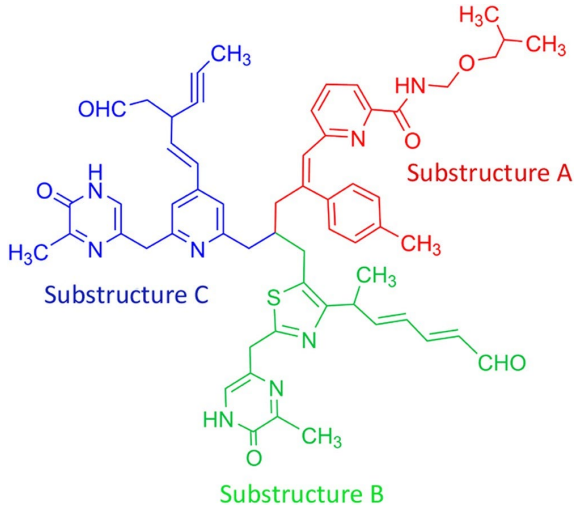
- Newly discovered antimicrobials should be assessed for its possible cytotoxicity on the mammalian cell lines.

- Result



Structural elucidation of compound SM1

- The total structure of SM1 is consists of substructure A, B, and C . SM1 contains a central picolinamide moiety. As this antibiotic compound produced by Streptomyces sp and having a picolinamide moiety we name this as picolinamycin.



Discussion

- The 16 S rDNA sequence-based phylogenetic analysis along with morphological and biochemical analysis has been indicated strain SM01 as a species of Streptomyces.
- the AIA medium has been considered as the most favorable medium for the highest amount of antibiotic production.
- Considering these nutrients factors, one can predict that the components of AIA like sodium propionate, L-asparagine and glycerol might have roles for higher yield of the antimicrobial compound.
- It has been found that the purified compound picolinamycin is highly effective against many hospital-borne deadly pathogens having vancomycin and oxacillin resistance phenotype.
- Picolinamycin up to a concentration of 31.25 µg/ml could not exert any cytotoxicity against the tested human A549 cell line.
- More precisely Streptomyces sp SM01 is a novel antibiotic producer that was isolated from Indian soil.
- It is active against both drug resistance and sensitive Gram-positive and Gram-negative bacteria. Hence picolinamycin has the potentiality to use industrially as **next-generation** drug of choice for MDR strains.

THANKS