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Research Article

OPTIMIZATION OF CULTURAL PARAMETERS FOR ANTIFUNGAL AND ANTIBACTERIAL METABOLITE FROM MICROBIAL ISOLATE; *STREPTOMYCES RIMOSUS* MTCC 10792 FROM SOIL OF CHHATTISGARH

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ABSTRACT

A microbial isolate, isolated from forest soil of Chhattisgarh, (India) was found to show significant antimicrobial activity against gram positive and gram negative bacteria as well as filamentous fungi including human pathogens. Based on physiological, biochemical characteristics the isolate was identified as *Streptomyces rimosus* subsp*. rimosus* and score id was calculated by the PIBWin software 1.9.2. This strain was further cultivated in different conditions fermentation in order to optimize biosynthetic process of antibiotic production including: different pH values, different temperatures, different carbon and nitrogen sources were fully investigates. Minimum inhibitory concentration of the crude extract was found ranged between 6.25-12.5µg/ml, 12.5- 25µg/ml and 25-50µg/ml against the filamentous, unicellular fungal and bacterial test strains. This strain is a found to be a potential antibiotic producer strain and may be commercially exploited for the development of novel drugs.

Keywords: *Streptomyces rimosus* Y8, Antifungal and antibacterial activity, Classical optimization.

INTRODUCTION

The substances that can inhibit pathogens and have little toxicity to host cells are considered candidates for developing new antimicrobial drugs[48]. *Streptomyces* are the microbes that occur in a multiplicity of natural and man-made environments[34] and a unique group having different morphological, cultural, biochemical and physiological characters[35]. *Streptomyces* sp. are known to be greatest sources of bioactive metabolites which has antibiotic antiparasitic, antitumor, insecticide, herbicide, alkaloid, enzyme inhibitor, immunoactive peptide, antithrombotic agent, and so forth[15,29,38]. Among natural sources, several antifungal and antibacterial compounds are reported from the genus *Streptomyces*[6,36, 4,12,8]. In recent years these metabolites are being used, either directly as precursors or as lead compounds in the pharmaceutical industry to make drugs against microorganisms[49]. In past 20 years due to increase in resistance of numerous pathogens including fungi to common antifungal drugs as the frequency of systemic fungal infections has increased drastically[7]. Therefore new sources and strategies are required to find new broad-spectrum antimicrobial molecules to combat these pathogens[13, 1]. The present piece of work represents taxonomy characterization of the new isolate as *Streptomyces rimosus* MTCC 10792. This strain is notably most characterized producer of oxytetracycline and other tetracycline antibiotics however *Streptomyces rimosus* is not reported to exhibited significant broad spectrum antifungal activity. Our isolate has shown broad spectrum antifungal activity along with strong antibacterial activity. Activity profile of the isolate requires detailed investigations on the nature of the active molecules produced and the optimum fermentation conditions for their production. We are reporting here classical optimization studies on the metabolites produced by the new isolate.

MATERIALS AND METHODS

Collection of Soil samples

Soil samples were collected from the different preserved forest ecosystems, these habitats included the rhizosphere of plants, mountains soil, hot sediments dung and forest soils Chhattisgarh, India. Soil samples were collected by scraping off an approximately 4-5 cm of surface material with a spatula in sterile plastic bags, transfer to the laboratory and stored at the 4°C until use for analysis. Physical treatment was given at 45°C for 16 h to separate spores from vegetative cells and chemical treatment was giving by cycloheximide[19] and nystatin, each at concentration of 50 µg/ml of medium to inhibit the fungal growth and the isolation of the microbes which are resistant to these antibiotics.

Isolation and screening of antimicrobial producing actinomycetes

Actinomycetes were isolated on ISP-2 medium supplemented with (yeast extract 4 g, malt extract 10 g, glucose 4 g, $CaCO_3$ 2 g) by the serial dilution method 1**.** All the isolates which show *Streptomyces* appearance were purified by streak plate technique[16]. Selected colonies of *Streptomyces* were transferred from the plates onto respective agar slants for long term preservation at 4°C. These isolates were subjected *in-vitro* antimicrobial activity by agar well diffusion method[40]. Flasks having culture broth were inoculated with the loop full spores of *Streptomyces* isolates and incubated at 28°C at rotary shaker (Tempo) at 180 rev/min. after fermentation culture broth were centrifuged at 8000 rev/min for 15 min, three cores were removed from the agar plates with the help of borer that have pre-seeded with test organisms by using the sterile swab stick. Holes were filled with the 100µl culture supernatant. The plates were incubated at 37°C for 24 h for antibacterial activity and at 28°C for 48 h for antifungal activity inhibition zones were visualized and recorded. Each experiment was repeated three times and means value of inhibition zones was calculated.

Test organisms used

Test organisms used for screening of antimicrobial activity of isolates, were obtained from Microbial Type Culture Collection and gene bank (MTCC), IMTECH, Chandigarh, they are as *Bacillus subtilis* (MTCC 1789), *Bacillus pumilus* (MTCC 1607), *Bacillus megaterium* (MTCC 1684), *Bacillus cereus* (MTCC 1305), *Bacillus cereus* (ATCC 10876), *Staphylococcus aureus* (MTCC 96), *Staphylococcus aureus* (MTCC 737), *Staphylococcus epidermis* (MTCC435), *Salmonella typhi* (MTCC 531), *Proteus vulgaris* (MTCC 1771), *Klebsiella pneumoniae* (MTCC 2405), *Escherichia coli* (MTCC 1667), *Escherichia coli* (MTCC 739), *Escherichia coli* (MTCC 1687), *Escherichia coli* (ATCC 35218),*Candida albicans* (MTCC 1637), *Candida albicans* (MTCC 184), *Candida albicans* (MTCC 183), *Candida tropicalis*(MTCC 3017), *Aspergillus niger* (MTCC 872), *Aspergillus fumigatus* (MTCC 2544), *Alterneria alternate (*MTCC 1779*)*, *Penicillium citrinum* (MTCC 1751), *Sachromyces cereviseae* (MTCC 170)*, Tricophyton rubrum* (MTCC 296). Bacterial cultures were grown at 37°C on nutrient agar medium and fungi were grown at 28°C on Sabouraud's dextrose agar. All the cultures were preserved at 4°C and sub-cultured regularly.

Physiological, biochemical and molecular characterization

Most active antimicrobial producing isolate designated as Y8, characterized on the basis of morphological, physiological, cultural and biochemical features. The morphology of the spore bearing hyphae with entire spore chain along with the substrate and aerial mycelium was examined under scanning electron microscope (SEM). For electron scanning microscopy specimens were fixed (4 h or overnight) by immersing them in 2 % paraformaldehyde and 2.5 % glutaraldehyde for 3 hr. The fixative was made up in 0.1 M phosphate buffer (pH 7.4). Osmication (2 hr) was done with OsO_4 and then it was washed thrice with 0.1M phosphate buffer. Specimens were then dehydrated through a graded ethanol series (30, 50: 15 min; 70, 90: 30 min; 100: 1hr), followed by critical point drying using liquid carbon dioxide (CO_2). The specimens were mounted on brass studs with aluminum conducting tape and coated with a 20 nm layer of gold in a JFC-1 100 ion sputterer (JEOL, Tokyo, Japan). The specimens were examined with a scanning electron microscope (JEOL) operated at 10 kV.

Cultural characteristics (growth pattern, color of aerial and substrate mycelia, formation of pigment) was studied on different media yeast extract-malt extract-dextrose agar (ISP-2), Oat meal agar (ISP-3), Starch inorganic salt agar (ISP-4), Asparagine glycerol agar (ISP-5), Tyrosine agar (ISP-7), Starch casein nitrate agar, Czapek-Dox agar, Sabouraud's agar, Asparagine glucose agar and Nutrient agar.

Ability of isolate producing different type of enzymes such as lecithinase, amylase, lipase, pectinase, gelatinase and catalase was examined by using standard methods[14]. Degradation of esculin, xanthine and hypoxanthine nitrate reduction[25], hydrogen sulphide reduction, citrate utilization, methyl red and oxidase test were studied, growth in presence of chemical inhibitors 0.001% potassium tellurite, 7% NaCl, 1% phenol, 0.01% sodium azide were determined. The chemo taxonomical property such as cell wall amino acid for 2, 6 diaminopimelic acid was studied[1]. Antibiotic sensitivity against the different antibiotics was performed on Muller Hinton agar media by paper disc method[3].

The carbohydrate utilization was determined by growth on carbon utilization medium (ISP-9)[42]. Supplemented with 1% carbon sources, the ability to utilize nitrogen was determined in a basal medium (glucose 1.0 g, $MgSO_4.7H_2O$ 0.05 g, $FeSO_4.7H_2O$ 0.001 g, K_2HPO_4 0.01 g, NaCl 0.05 g, agar 2.0 g and distilled water 100 ml) to which 0.1% nitrogen sources were added.

Fermentation studies and optimization of cultural conditions

Effect of incubation time on antimicrobial compound and biomass production of the isolate was studied in every 24h interval on glucose soybean meal broth for 14 days at 28°C. Seed inoculums were prepared by growing producer strain on ISP-2 broth at 28°C for 48-72 h. All the flasks were inoculated with 2% seed inoculums and incubated at 28°C shaking with 180rev/min.

Cultural characteristics and media composition were optimized by a systematic study and the suitability of various carbon and nitrogen sources were evaluated and correlated. Strain was subjected to grow on different media as per International Streptomyces Project (ISP). The dry mycelia weight was measured to optimize the media and cultural characteristics were noted down.

Selection of production media

Seven different broth media (Yeast Extract Malt Extract Dextrose broth (YEMEB), Glucose Soybean meal Broth (GSB), Nutrient Broth (NB), Starch-Casein-Nitrate-Broth (SCNB), Inorganic Salt Starch Broth (ISSB), Sabouraud's Dextrose Broth (SDB), Czapek-Dox Broth (CZB) were inoculated and incubated at 28°C for 96 h at180 rev/min. The antimicrobial activity was tested by agar well diffusion method against the *C. albicans* and *B. cereus* as target test organisms for antifungal and antibacterial activity.

Effect of carbon and nitrogen sources

Different carbon sources such as starch, glycerol, fructose, lactose, xylose, maltose, and sucrose were tested at 1% (v/v) to the production media for maximum antimicrobial compound production. Glucose was used as a control for each carbon source. Whereas the impact of nitrogen sources such as urea, beef extract, malt extract, peptone, yeast extract, sodium nitrate, and potassium nitrate was studied with 1% (v/v) nitrogen sources to production media with best carbon sources by using soybean meal as a control.

Effect of pH, temperature and NaCl concentration

Different ranges of pH (4.0, 5.0. 6.0, 7.0, 7.5, 8.0, 9.0 and 10), temperature (20, 25, 28, 28, 30, 35, 40 and 45°C) and NaCl concentration (2, 4, 6, 8, 10, 12, 14, 16, 18 and 20% w/v) were tested for antimicrobial compound production separately while keeping other parameter constant. The pH of medium was adjusted with 1M HCl and 1M NaOH by using pH meter (ELICO-L1 127).

Minimum inhibitory concentration (MIC) of extracellular crude extracts of strain Y8

Minimum inhibitory concentration (MIC) values of the crude extract of Y8 which inhibited growth of *C. albicans* and *B. cereus* pathogens was determined by the broth dilution technique recommended NCCLS, 2002 [41]. Tubes having two fold dilution of antimicrobial compound with inoculums incubated at 28°C for 48 h for antifungal activity and at 37°C for 24 h for antibacterial activity. After incubation, the antimicrobial compound concentration appeared in the first clear test tube was considered to be the MIC. MIC was determined as the lowest concentration resulting in no growth on sub culturing[24]. From the tube with no turbidity 100µl aliquot was taken and spread over the surface of Sabouraud's Dextrose agar and nutrient agar plates and incubated at 28°C for 48 h and 37°C for 24 h respectively. After incubation MIC values were recorded as the minimum concentration that inhibits the growth of test microorganisms (causing 99.9% death of test organism). Minimum bactericidal concentration (MBC) was defined as the lowest concentration yielding negative subcultures.

RESULTS

Isolation and screening of antimicrobial producing actinomycetes

The selective isolation procedure resulted in isolation of eighteen *actinomycetes* isolates based on their colony morphology, resembling that of *Streptomyces* and matched the genus description as reported by Shriling and Gottlieb and Nonomura[18, 26]. All the isolates were subjected to their *in-vitro* antimicrobial activity, one promising isolate Y8, exhibited strong antifungal and antibacterial activity against the all tested organisms in glucose soybean meal broth was selected for the further study [Table 1].

Physiological, biochemical and molecular characterization

Based on morphological, physiological and biochemical characteristics, strain Y8 seems to be closely related to the genus *Streptomyces* [Table 2]. This gram positive organism has off white aerial mycelia and yellowish substrate mycelium [Figure 1A and 1B]. When observed under the scanning electron microscope (SEM) appears rod like structures (chains of cells) and often branched to form a network of filaments (mycelium), smooth surface of the spore (5µm in length) developed on the terminal of the aerial mycelium [Figure 1C].

Fermentation studies

Antimicrobial compound and biomass production was monitored over a period of 14 days. Atypical time course of fermentation is shown in [Figure 2]. Rate of antimicrobial metabolite production correlated with biomass. Antibiotic production was detected in culture broth after 24 h of incubation and reached maximum at stationary phase after 96 h of incubation. Mycelium growth gradually increased up to 96 h of incubation and entered in stationary phase. In our case maximum antimicrobial metabolite production was took place at late log phase indicating that metabolite production was directly proportional to the growth rate. It is reported that antibiotic production usually occurs in stationary phase[33]. The condition of incubation influenced quantitatively the biosynthesis of antibiotics as well as biomass reported by the Al-

Zahrani, 2007 [45]. It has been observed that accumulation of antibiotics, cephalomycin C and clavulanic acid has been occurring in parallel with growth in a defined medium[23].

Optimization of cultural condition in antimicrobial compound production

Variation in fermentation environment like cultural characteristic and media composition often resulted in change in antibiotic production in terms of either the yield or composition of compounds[9]. It has been reported that nutritional requirement play an important role during metabolite synthesis. Nutritional requirement and cultural conditions affects production of secondary metabolites such as carbon sources, nitrogen sources, pH, temperature, salt concentration and incubation time.

Selection of broth medium

Results showed that isolate Y8 had highest antimicrobial activity and biomass production in glucose soybean meal broth having medium composition glucose 10 g, soybean meal 10 g, NaCl 10 g, $CaCO_3$ 1 g, pH-7.5 followed by Sabouraud's Dextrose Broth (SDB), Yeast Extract Malt Extract Dextrose broth (YEMEB), Inorganic Salt Starch Broth (ISSB), Nutrient Broth (NB), Starch Casein Nitrate Broth (SCNB), Czapek Dox Broth (CZB) when compared with the control. Incubation was carried out at 28°C for 96 h [Figure 3].

Carbon sources

Form the experiment it observed that no significant difference in antimicrobial activity and biomass production when isolates were cultivated in media having glucose or glycerol as a carbon source followed by the starch, maltose, xylose whereas lactose showing the good antibacterial activity but low antifungal activity. In contrast, low activity was observed with fructose, sucrose [Figure 4]. Glycerol is reported in the literature as an important medium component for the production of antifungal compounds from microorganisms [4, 12, 28]. Maximum growth and antibiotic production is found to occur when glycerol used as the sole source of carbon[30]. Similar results have been with *Streptomyces violatus* in batch cultures [27]. It was reported that glucose was the best carbon source for production of magnamycin antibiotic by *Streptomyces halstedii*[17]. It has been glucose was the best carbon source for the production of antifungal polyenes by *Streptomyces griseocarneus*[47]. Generally a quickly metabolized substance like glucose is responsible for catabolite repression but in some cases it is also reported to enhance antifungal metabolite production[32,44, 10].

Nitrogen sources

Soybean meal and peptone were found best nitrogen source for the highest antimicrobial activity and biomass production followed by the yeast extract, beef extract in contrast low activity was observed with malt extract, potassium nitrate, sodium nitrate, urea [Figure 5], same results were investigated that soybean meal and peptone are the best organic nitrogen sources for production of oxytetracycline production by a strain of *Streptomyces rimosus* 93060[27]. Soybean meal considered as suitable medium component for the production of antibiotic from the *Streptomyces capoamus* [39]. In literature it has been reported that medium supplemented with soybean meal was found to be suitable for maximum antimicrobial metabolites production[32].

Optimum pH, temperature and NaCl concentration for production of antimicrobial compounds

Optimum pH for production of antimicrobial compound was found 7.5 [Figure 6]. It has been observed that maximum antibiotic production was obtained at pH 7.5 by *Streptomyces* strains[46]. The change in pH of the culture media induces production of new substances that affect antibiotic production[3]. The importance of pH for antifungal production by *Streptomyces* was reported by several investigators who observed that the optimum pH for antibiotic production range between 7.0 and 7.5[21]. The optimum temperature for maximum antimicrobial activity and biomass production was found to be 28°C [Figure 7]. It is reported that *Streptomyces* usually produce antibiotics at a temperature near 27°C. Usually cultivation for antibiotic production is performed under constant temperature from the beginning to the end [20]. It was observed that optimal NaCl concentration for production of antimicrobial activity and biomass production was 10g/l [Figure 8]. However, antibiotic production was occurs with all NaCl concentrations. All NaCl concentration was found to be significantly different from each other for antimicrobial activity and biomass production.

Minimum inhibitory concentration (MIC) of extracellular crude extracts of *Streptomyces rimosus* Y8

Minimum inhibitory concentration of crude extract was determined by the serial dilution assay starting with the 50µg/ml. MIC values of the crude extract was ranged between 25-50µg/ml and 12.5-25µg/ml for filamentous and unicellular fungi and 6.25-12.5µg/ml for bacterial test organisms respectively [Table 3]. The minimum inhibitory concentration of the crude extract is relatively high, which needs further study using purified active component.

Table 1: Activity profile of the *Streptomyces rimosus* Y8 against tested organisms

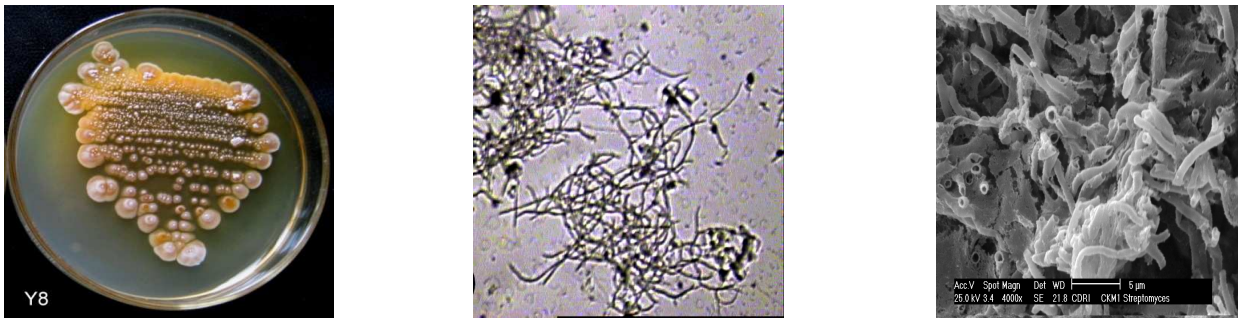
Test organisms	Zone of Inhibition (mm)
Bacillus subtilis (MTCC 1789)	35.0±0.0
Bacillus pumilus (MTCC 1607)	33.0±0.0
Bacillus megaterium (MTCC 1684)	30.6±0.57
Bacillus cereus (MTCC 1305)	40.0±0.0
Bacillus cereus (ATCC 10876)	35.0±0.0
Staphylococcus aureus (MTCC 96)	45.0±0.0
Staphylococcus aureus (MTCC 737)	40.0±0.0
Staphylococcusepidermis (MTCC435)	25.6±0.57
Salmonella typhi (MTCC 531)	39.3±0.57
Proteus vulgaris (MTCC 1771)	23.6±0.57
KlebsiellaPneumoniae (MTCC 2405)	26.3±1.15
Escherichia coli (MTCC 1667)	28.0±0.0
Escherichia coli (MTCC 739)	19.0±0.0
Escherichia coli (MTCC 1687)	15.0±0.0
Escherichia coli (ATCC 35218)	27.0±0.0
Candida albicans (MTCC 1637)	28.6±0.57
Candida albicans (MTCC 184)	26.0±0.0
Candida albicans (MTCC 183)	28.0±0.0
Candida tropicalis(MTCC 3017)	29.0±0.0
Aspergillus niger (MTCC 872)	32.0±0.0
Aspergillus fumigatus (MTCC 2544)	30.0±0.0
*Alterneria alternate (*MTCC 1779*)*	37.0±0.0
Penicillium citrinum (MTCC 1751)	27.3±0.57
Sachromyces cereviseae (MTCC 170)	28.0±0.0
Tricophyton rubrum (MTCC 296)	29.0±0.0

Table 2: Morphological and biochemical characteristics of *Streptomyces rimosus* Y8

Characteristics	Y8	characteristics	Y8
Morphological characteristics		Growth with (%w/v)	
Spore chain	Spiral	NaCl (7%)	+
Spore mass color	White	Sodium azide (0.01)	-
Substrate mycelium color	yellow	Phenol (0.1)	-
Diffusible pigment produced	+	Potassium tellurite (0.001)	+
Biochemical characteristics:		Potassium tellurite (0.01)	-
Lecithinase	+	Crystal violet (0.0001)	+
Amylase	+	Growth at 45°C	-
Lipolysis	+	pH (4.3)	+
Nitrate reduction	+	Utilization of carbon sources:	
H2S production	-	Sucrose	-
Gelatinase	+	Starch	+
Urease	+	Mannitol	+
Catalase	+	Rahamnose	-
Caseinase	+	Raffinose	+
Oxidase	+	Galactose	+
Citrate utilization	+	Arabinose	+
Methyl reduction	+	Meso-inositol	+
Degradation:		Adonitol	+
Xanthine	-	Fructose	+
Hypoxanthine	+	Sorbitol	+
Arbutin	+	Maltose	+
Adenine	+	Lactose	+
Pectin	-	Cellibiose	+
Esculin	+	Xylose	+
L-tyrosine	-	Dextran	-
Xylan	-	Utilization of nitrogen sources:	
Guanine	-	Cystein	-
Resistance:		Valine	+
Rifamycin (R^5)	R	Phenylalanine	+
Cephalosporin (CR^{30})	R	Histidine	+
Amphotericin B (AP^{30})	R	Hydroxyproline	-
Amoxicillin (AM^{10})	R	Asparagine	+
Methicillin (M^{10})	R	Arginine	+
Nalidixic acid (NA^{30})	R	α-aminobutyric acid	-
Penicillin G (P^{10})	R	Potassium nitrate	+
Fluconazole (F^{10})	R	Serine	+
Polymixim B (PB^{100})	R	Methionine	+

Table 3: Minimum Inhibitory Concentration (MIC) of crude extract of active strain Y8

MIC (µg/ml)	Test strains
6.25-12.5	*B. cereus, B. subtilis, S. aureus*
12.5-25	*C. albicans*
25-50	*T. rubrum, A. niger*



1A **1B** **1C**

Fig. 1A: Growth pattern of *Streptomyces rimosus* strain Y8 on starch casein nitrate agar media, 1B Micrpscopic observation of *Streptomyces rimosus* Y8 under the light microscope (1000X), 1C SEM (scanning electron microscope) examination of the producer strain of *Streptomyces rimosus* Y8 showing its morphology feature and dimension of the microbe.

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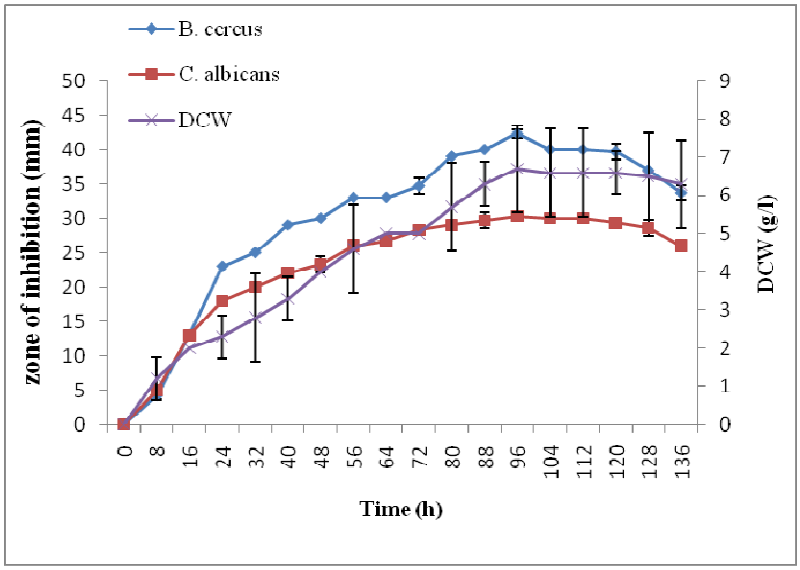

Fig. 2: Effect of incubation time on the antimicrobial activity and biomass production.

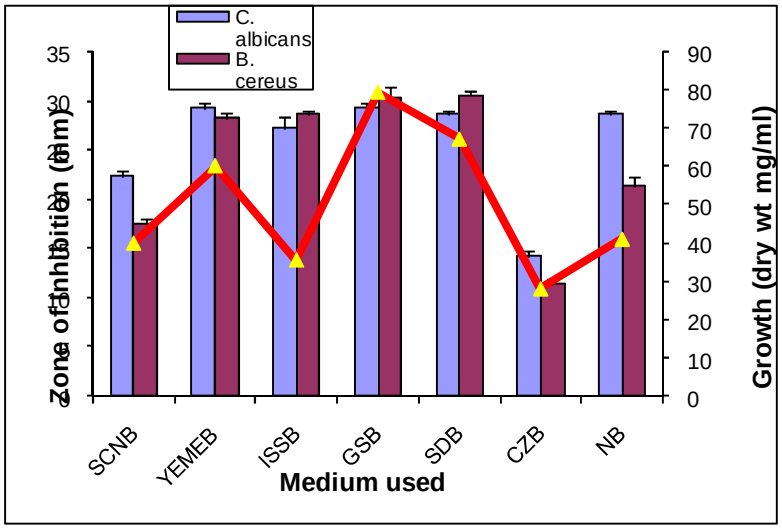

Fig. 3: Optimization of production media in order to attended the maximum antimicrobial activity and biomass production of the antimicrobial metabolites.

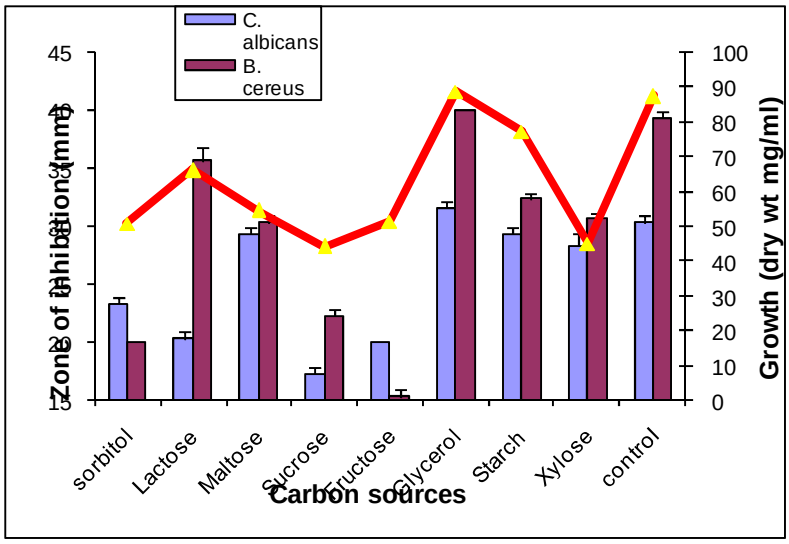

Fig. 4: Effect of carbon sources (1%) on antimicrobial activity and biomass production from the *Streptomyces rimosus* Y8 strain.

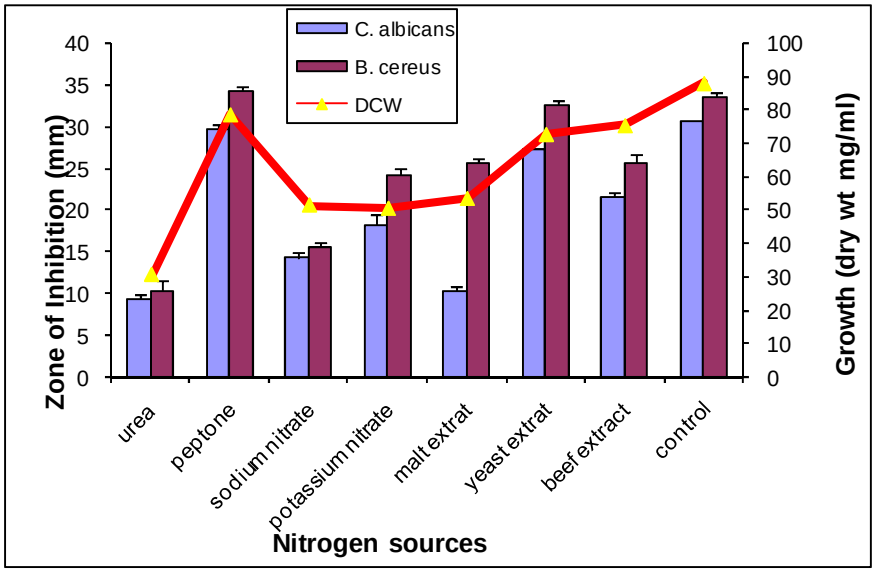

Fig. 5: Effect of different source of nitrogen sources on antimicrobial activity and biomass production from *Streptomyces rimosus* Y8.

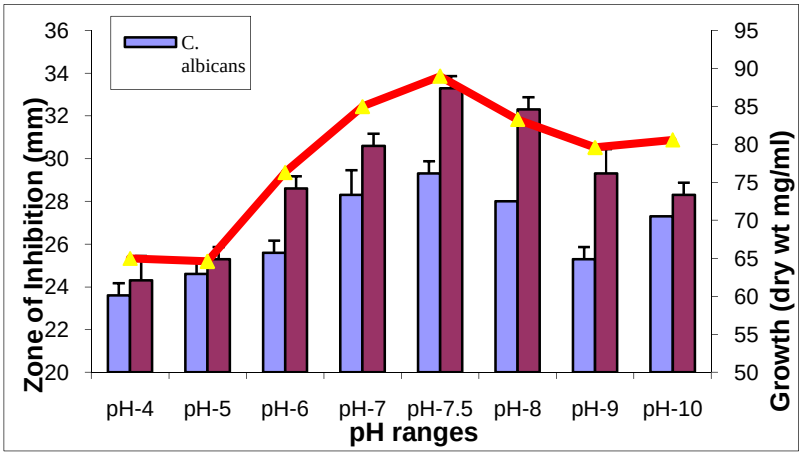

Fig. 6: Effect of pH on antimicrobial activity and biomass production from *Streptomyces rimosus* Y8.

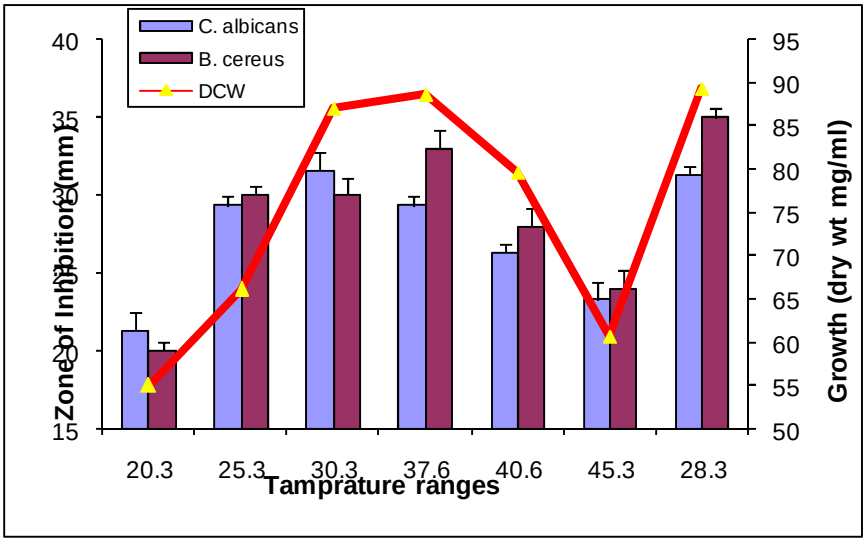

Fig. 7: Effect of temperatures on antimicrobial activity and biomass production from *Streptomyces rimosus* Y8.

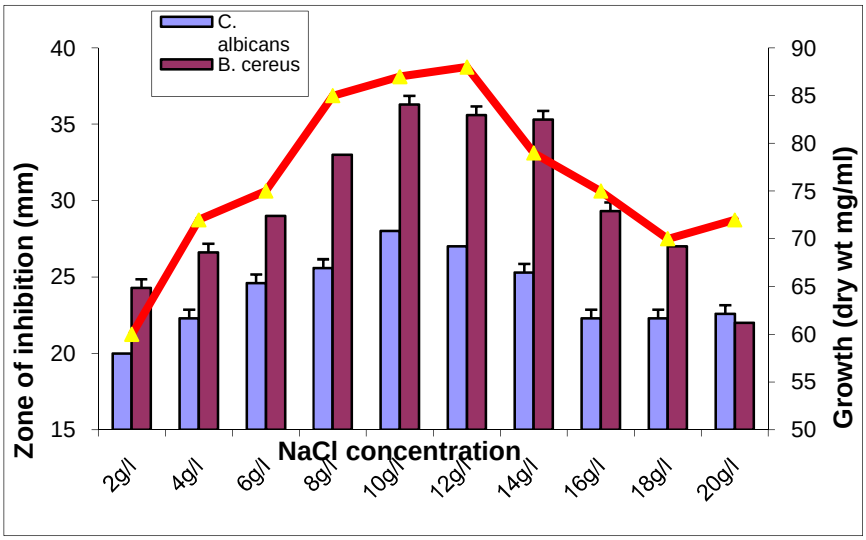

Fig. 8: Effect of NaCl concentration on antimicrobial activity and biomass production from *Streptomyces rimosus* Y8.

DISCUSSION

Screening and isolation of microorganisms isolated from the soils of Chhattisgarh region was identified as *Streptomyces rimosus* Y8. *Streptomyces rimosus* have been reported as notably producer of oxytetracycline and other tetracycline class of antibiotics. In our case strain Y8 showed the strong antifungal activity against the various fungal pathogens with broad spectrum antibacterial activity which shows the novelty of active metabolite produced by our isolate. Therefore *Streptomyces rimosus* Y8 can be employed as a target to search for a new active metabolite or drug to satisfy public demands. In this study, we focused on the optimization of culture conditions for production of antibiotics by a new isolate *Streptomyces rimosus* Y8. The optimization of fermentation medium is as important as selection of an organism to obtain antibiotic production. The source of carbon and nitrogen in the fermentation media plays an important role, since microbial and fermented products are largely composed of these elements. It is usual that the production of antibiotic is promoted after readily utilizable sugars as a carbon source. It has been reported in literature that the highest antibacterial activity of *Streptomyces sannanensis* strain RJT-1 was obtained when glucose at 1% (w/v) was used as a carbon source followed by xylose and arabinose [22]. It is well known that changes in the kind and concentration of nitrogen source influence greatly antibiotic production. Soybean meal is a complex nitrogen source and contains a number of amino acids (lysine, methionine, threonine, tryptophan, aspartic acid, glutamine, proline, alanine, valine, isoleucine, etc). The presence of tryptophan in soybean meal increases antibiotic production up to a certain level [5].Other environmental factor such as temperature, pH and NaCl concentration of the solution was also tested in order to establish the suitable cultural conditions for the optimal production of antibiotics. However, more studies should be conducted with regard to statistical optimization, purification and characterization of bioactive metabolite produced by the active strain *Streptomyces rimosus* Y8.

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REFERENCES

1. Abou-Zeid A, EL-Diwany A. Utilization of molassess as a natural medium for production of magnamycin by *Streptomyces halatedii*. Agr Wastes 1980; 2: 23-30.
2. Abou-Zeid A, Baeshin NY. Utilization of date-seed lipid and hydrolysate in fermentative formation of oxytetracyline by *Streptomyces rimosus*. Bioresour Technol 1992; 41: 41
3. Al-Zahrani SHM. Studies on the antimicrobial activity of *Streptomyces* sp. isolated from Jazan. JKAU 2007; 19: 127-138.
4. Banna NE, Winkelmann G. Pyrrolnitrin from Burkholderia cepacia: antibiotic activity against fungi and novel activities against streptomycetes. J Appl Microbiol 1998; 85: 69-78.
5. Basilio A, Gonzalez I, Vincente MF, Gorrochatgui J, Cabello A. Patterns of antimicrobial activities from soil actinomycetes isolated under different conditions of pH and salinity. Appl Microbiol 2003; 95: 814-823.
6. Castillo UF, Strobel GA, Ford EJ, Hess WM, Porter H, Jensen JB, Albert H, Robinson R, Condron MAM, Teplow DB, Stevens D, Yaver D. Munumbicins, wide-spectrum antibiotics produced by *Streptomyces* NRRL 30562, endophytic on Kennedia nigriscans. Microbiol 2002;148: 2675–2685.
7. Cruze R, Arias ME, Soliveri J. Nutritional requirements for the production of pyrazoloisoquinolinone antibiotics by *Streptomyces griseocarneus*. Appl Microbiol Biotechnol 1999; 53: 115-119.
8. Desphande BS, Ambedkar SS, Shewale JG. Biologically active secondary metabolites from *Streptomyces*. Enzy Microbio Technol 1988; 10 : 455-473.
9. Didomenico B. Novel antifungal drugs. Microbiol 1999; 2: 509-515.
10. Fukuda T, Matsumoto A, Takahashi Y, Tomoda H, Omura S. Phenatic acids A and B, new Potentiators of antifungal miconazole activity produced by *Streptomyces* sp. K03–0132. J Antibiot 2005; 58: 252-259.
11. Fushimi S, Nishikawa S, Shimazu A, Seto H. Studies on new phosphate ester antifungal antibiotics phoslactomycins. J Antibiot 1989; 42: 1019-1025.
12. Georgopapadakou NH. Antifungal mechanisms of action and resistance established and novel drugs. Microbiol 1998; 1: 547-557.
13. Goolfellow M, Williams ST. Ecology of actinomycetes. Ann Rev Microbiol 1983; 37: 189-92.
14. Gordon RE, Barnett DA, Handehan JE, Pang CH. *Nocardia coeliaca, Nocardia autotrophica* and *Nocardia* strain. Int J Sys Bacteriol 1974; 24: 54-63.
15. Gramer A. Antibiotic sensitivity and assay test. *In:* Microbiological Methods, Collins CH, Lyne PM (eds) 1976; Butterworth and Co. London, pp 235.
16. Hayakawa Y, Iwakiri T, Imamura K, Seto H, Otake N. Studies on the isotetraacenone antibiotics I. Capoamycin, a new antitumor antibiotic. J Antibiot 1985; 38: 957-959.
17. Holding AJ, Collee JG. Routine biochemical tests. Meth Microbial 1971; 6: 1-31.
18. Kumar S, Kannabiran K. Antifungal activity of *Streptomyces* VITSVK5 spp. against drug resistant *Aspergillus* clinical isolates

from pulmonary tuberculosis patients. J Mycol Medicale 2010; 20: 101-107.

19. Lavermicocca P, Valerio F, Visconti A. A antifungal activity of Phenyl lactic acid against molds isolated from bakery products. Appl Env Microbiol 2003; 69: 634-40.
20. Lechevalier HA, Lechevalier MP. Biology of actinomycetes. Ann Rev Microbiol 1967; 21: 71-100.
21. Locci R. Streptomycetes and related genera. In: Bergey's manual of systematic bacteriology 1989
22. Marwick JD, Wright PC, Burgess JG. Bioprocess intensification for production of novel marine bacterial antibiotics through bioreactor operation and design. Mar Biotechnol 1999; 1: 495-507.
23. Mustafa O. Antifungal and antibacterial compounds from Streptomyces strains. Afri J Biotechnol 2009; 8 (13): 3007-3017
24. Narayana KJP, Vijayalakshm M. Optimization of antimicrobial metabolites production by *Streptomyces albidoflavus*. Res J Pharmacol 2008; 2(1): 4-7
25. National Committee for Clinical Laboratory Standards Methods for Dilution Antimicrobial Susceptibility test for Bacteria That Grow Aerobically- Fifth Edition, Approved Standard M7-A5, NCCLS, and Wayne, PA, USA; 2002.
26. Nonomura H. Key for classification and identification of 485 species of the *Streptomyces* included in the ISP. J Ferment Technol 1974; 52: 78-92.
27. Omura S, Nakagawa A, Yamada H, Hata T, Furusaki A, Watanabe TC. Structure and biochemical properties of Kanamycin A, B, C and D. Chem Pharm Bull 1973; 21: 931-34.
28. Oskay M. Antifungal and antibacterial compound from *Streptomyces* strains. Afr J Biotecnol 2009; 8 (13): 3007-3017.
29. Ouhdouch Y, Barakata M, Finance C. Actinomycetes of Moroccan habitats: Isolation and screening for antifungal activities. Soil Biol 2003; 37: 59-74
30. Pridham TG, Gottlieb D. The utilization of carbon compounds by some actinomycetales as an aid for species determination. J Bacteriol 1948; 56: 107-114.
31. Qureshi A, Mauger JB, Cano RJ, Galazzo JL, Lee MD. MF-EA-705a & MF-EA-705b, new metabolites from microbial fermentation of a *Streptomyces* sp. J Antibiot 2001; 54: 1100–1103.
32. Reichenbach H, Gerth K, Irschikunze HB, Hofle G. Myxobacteria: A source of new antibiotics. Trends Biotechnol 1988; 6: 115-21.
33. Sahin N, Ugar A. Investigation of antimicrobial activity of some *Stretomyces* isolations. Turk J Biol 2003; 27: 73-78.
34. Saitou N**,** Nei M**.** The neighbor-joining method: a new method for reconstructing phylogenetic trees Mol Biol Evol 1987; 4(4): 406-25.
35. Shiling EB, Gottlieb D. Methods for characterization of *Streptomyces* species. Int J Syst Bacteriol 1966 ;16: 312-340.
36. Singh V, Khan M, Khan S, Tripathi C K M. Optimization of actinomycin V production by *Streptomyces triostinicus* using artificial neural network and genetic algorithm. Appl Microbiol Biotechnol 2009; 82 (2): 379-385.
37. Singh V, Tripathi CKM, Bihari V. Production, optimization and purification of an antifungal compound from *Streptomyces capoamus* MTCC 8123. Med Chem Res 2008; 17: 94-102.
38. Sirvastava S, Singhal V. Advances in general microbiology. Fundamental Microbiol 1994; 3: 176-177.
39. Ueki M, Abe K, Hanafi M, Shibata K, Tanaka T, Taniguchi M. UK-2A, B, C, D. Novel antifungal antibiotics from *Streptomyces* sp. 517–02 I. Fermentation Isolation and Biogical Properties. J Antibiot 1996; 49: 639–643.
40. Upadhyay MK, Kumar R, Kumar A, Gupta S, Kumari M, Singh A, Jain D, Verma HN. Optimization and characterization of an extracellular proteases from *Aspergillus flavus* "MTCC 277" Afr J Agr Res 2010; 5[14]: 1845-1850.
41. Vahidi H, Kobarfard F, Namjoyan F. Effect of cultivation conditions on growth and antifungal activity of Mycena leptocephala. Afr J Biotechnol 2004; 3: 606–609
42. Vasavada S H, Thumar J T, Singh SP. Secretion of a potent antibiotic by salt-tolerant and alkaliphilic actinomycete *Streptomyces sannanensis* strain RJT-1. Curr Sci 2006; 91[10]: 1393-1397.
43. Watve MG, Tickoo R, Jog MM, Bhole BD. How many antibiotics are produced by the genus *Streptomyces*? Arch Microbiol 2001; 176: 386–390.
44. Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 16S rDNA amplification for phylogenetic study. J Bacteriol 1991; 173: 697-703.
45. Williams ST, Holt JG. editors. Baltimore: Williams and Wilkins pp 1983; 451-91.
46. Wilson K. Preparation of genomic DNA from bacteria. In: Ausubel Kingstan RE, Moore DD, Smith JA, Seidman JG, Struhl K (eds) Current protocol in molecular biology, 1990; pp 2-4 Greene publishing and Wiley Inter Science, New York.
47. Zheng Z, Zeng W, Huang Y, Yang Z, Li J, Cai H, Su W. Detection of antitumor and antimicrobial activities in marine organism associated actinomycetes isolated from the Taiwan Strait, China. *FEMS* Microbiol Lett 2000; 188: 87-91.
48. Kachhawa JBS. Sharma N. Tyagi S. Gupta RS. Sharma KK. *In vitro* evaluation of antibacterial activity of *Pterocarpus marsupium* roxb. Int J Pharm Pharm Sci 2012; 4[1]: 67-68
49. Leelaprakash G. Mohan SD. Antimicrobial activity and phytochemical screening of methanol extract of *Enicostemma axillare* Int J Pharm Pharm Sci 2012; 4[1]: 342-348.