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Original Research Article

Identification of *Streptomyces* spp and assessment of their inhibiting metabolic potency against some pathogenic micro-organisms

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Abstract

This study involved isolation and diagnosis of a few species of the filamentous bacteria of genus *Streptomyces* from 50 soil samples collected from different regions of Kirkuk province, Iraq. Thirteen *Streptomyces* spp, *S. bambergiensis*, *S. rimosus, S. longisporoflavus, S. canus, S. pactum, S. glaucescens S. chromogenus, S. xanthochromogenes, S. varsoviensis, S. roseus, S. violaceaniger, S. thermorulgaris, S. albus* were isolated and all proved to produce antibiotics. Their inhibitory metabolic potency has also been assessed against *Bacillussubtilis, Escherichia coli, Pseudomonas fluorescens, Aspergillus* spp*, Candida albicans, Streptococcus pyogenes, Salmonella typhi* and *Trichophyton mentagrophytes.* The potency of these pathogens was tested for the ability to inhibit pathogenic micro-organisms of *S. albus, S. rimosus, S. pactum, S. violaceaniger, S. roseus*. The *S. violaceaniger* showed stronger inhibitory effect against most pathogenic micro-organisms. It is concluded that these species could possibly produce new powerful antibiotics against pathogenic micro-organisms which could be further used for clinical application.

Keywords: *Streptomyces*, inhibiting, metabolic potency, pathogenic micro-organisms.

INTRODUCTION

The bacteria members of the *Streptomyces* genus (Order: Actinomycetales) are similar to fungi in their branching pattern of filamentous structure which produce well developed vegetative hyphae. This genus involves more than 500 worldwide known species with soil as the habitat and sustain important in soil ecology (Euzéby, 2008). *Streptomyces* bacteria are Gram-positive and adapted to grow in various environments. The most interesting property of *Streptomyces* is the potency to produce bioactive secondary metabolites, such as antifungals, antivirals, anti-tumor, anti-hypertensive, immune-suppressants especially antibiotics (Hopwood, 2009). The production of most antibiotics is specific to species while the secondary metabolites deem important for *Streptomyces* species in order to compete to other micro-organisms that become in contact, even within the same gene (Chaudhary *et al*., 2013). There are several theories that explain antibiotic production; with the most widely accepted one is that antibiotics help the organism compete with other organisms in a relatively nutrient-depleted environment of the soil by reducing competition (Kieser *et al*., 2000).

The objectives of this study were to isolate and diagnose the *Streptomyces* spp. from different sectors of

Kirkuk province and to determine their ability to produce antibiotics using a spectrum assay technique for each.

MATERIALS AND METHODS

Fifty sample of soil (200-250 gm weight) as deep as 5-20 cm, after removal of 2cm from the top layer, were collected locally from different districts of Kirkuk. They were placed in polyethylene sterile soil bags and relevant necessary information were recorded and transferred to the laboratory using technique adopted by Kornwendisch and Kutzner (1992). Samples were dried out in a thermal oven 37°C for 4 days with the addition of calcium carbonate [$CaCO_3$]as (1:10 w/w), then decimally diluted gradually as (10^{-1}-10^{-5}) using sterilized Rinker solution (10.0gm of soil to 90mL of Rinkerin sterile Erlenmeyer flask). The final dilution, 0.2 mL was then used to cultivate the bacteria on yeast extract-Trypton agar media at temperature ranging between (28-30°C) for 7-14 days. Colonies were then isolated upon their similarity in terms of color of phenotypic traits of the bacteria *Streptomyces* and the colonies were purified by repeated culturing using the same media (Williams and Davies, 1995). Microscopic examination was conducted as soon as a growth pure isolates of the bacteria were completed followed by dying them by gram stain. Examination of the glass slides was carried out to identify the bacterial isolates compatible to *Streptomyces* as gram positive as filamentous. Followed by identifying the most important quality phenotypic bacterial isolates depending on both color substrata and aerial mycelia on using Trypton yeast extract agar.

Biochemical and physiological characterization tests involved the usage of various enzymes i.e. oxidase, catalase, lipase, lecithinase, citrate, urea, starch hydrolysis, casein hydrolysis, blood hemolysis, DNase, production of hydrogen sulfide and gelatin liquefaction were conducted in accordance with techniques adopted by Prescott *et al*., (2005).

The sensitivity of the antibiotic of the bacterial isolates with antibiotics technique adopted by Bauer and Kirby (1996) were used. The antibiotics discs, symbols, their concentrations and diameter inhibition zones were used according to National Committee for Clinical Laboratory Standards (NCCLS, 2004).

Those pathogenic micro-organism samples, from patients with bacterial or fungal injuries, referred by consultant doctors in Kirkuk Jumhuri Hospital, diagnosed were involved both the genus and species i.e. *Salmonella typhi* (*S.t*.), *Streptococcus pyogen* (*S.p*.), *Trichophyton mentagrophytes* (*Tmn*), *Bacillus subtilis* (*Bs*), *Pseudomonas fluorescens (Ps.f), Aspergillus spp (A.sp), Candida albicans (C.a)* were diagnosed according to (Prescott and Harley, 1996; Forobes *et al.,* 1998; Ellabib and Khalifa, 2001; Prescott *et al*., 2005; Szepietowsk and Schwartz, 2005).

The streaking cross agar overlay method was used to investigate the inhibitory potency of *Streptomyces* isolates against pathogenic micro-organism on a solid culture medium. The isolated culture of *Streptomyces,* as line-shaped longitudinal in medium central was incubated for 4-7 days at 30°C. The pathogenic organisms were cultured perpendicular on *Streptomyces* in *Petri* dishes for 24hrs at 37°C followed by measuring the amount of inhibition (Enefiok and Charles, 1978; Pandy *et al*., 2005).

RESULTS AND DISCUSSION

Morphological characterization based upon microscopic examination had confirmed that all the isolates are of filamentous shapes and Gram positive cells. Such description classifies them within the *Actinomycetes* family (Table 1). Only 13 species of *Streptomyces* isolated out of 50 the local soils collected from different regions at *Kirkuk* province. This finding confirms to be concomitant with those of (Nanjwade *et al*., 2010). These species of *Streptomyces* are tested to be strong antibiotics against other pathogenic bacteria and fungi. The latter indicates the fact that Kirkuk soil is rich in those bacteria which could well be the sources of antibiotic products to be explored for future industrial scale of pharmaceutical application.

The aerial mycelium isolates cultured on Trypton Yeast Extract Agar (TYEA) showed difference in colors i.e. varied between white to yellowish-white in contrast to isolates with gray and brown color, while the substrate mycelium expressed the rule of brown and light yellow. The results shown in table 2 are in consistent with other studies of Hong-Hui *et al*. (2007) and Nanjwade *et al.* (2010). Perhaps large variation observed of these bacteria during the isolation and diagnosis are related to many factors i.e. the nuclear material; as the most important magnitude while the natural changes could be relevant to the limits of the genome (10.5×10^3) Kbp and the percentage of Guanine and cytosine (GC) (69-78%). Most taxonomists have found difficulties in the development of major clear lines in diagnosing species to determine the formal qualities under the same conditions (Nanjwade *et al*., 2010). The latter has been due to variations in morphology and to genetic variations. Therefore, many other factors become necessary to encounter for the diagnosis purposes upon specie level. That is due to the great impact of the growth of conditions and the type of the media in changing recipes isolates (Houssam *et al*., 2011).

Table 1. The Phenotypic Characteristics of Streptomycin on Trypton yeast agar (TYEA).Spiral, Curvature, rectiflexbiles and serpenting

Samples	Spore chain ornamentation	Aerial spore mass Color	Substrate mycelium Color	Mycelium morphology Aerial	Substrate
S. albus	Warty smooth	Light yellowish	yellowish-Light	Spiral	Rectiflexibiles
S. violaceaniger	Spiny	Gray	Gray	Serpentine	Curvature
S. thermorulgaris	Warty coarse	White	Yellow white	Serpentine Curvature	Rectiflexibiles
S. roseus	Warty smooth	Brown light	Brown light	Rectiflexibiles	Rectiflexibiles
S. varsoviensis	Spiny coarse	White	Brown	Curvature	Rectiflexibiles
S. varsoviensis	Spiny coarse	White	Brown light	Curvature	Curvature
S. xanthochromogenes	Hairy coarse	White	Dark brown	Rectiflexibiles	Rectiflexibiles
S. chromogenus	Warty coarse	White	Dark brown	Rectiflexibiles	Rectiflexibiles
S. glaucescens	Hairy coarse	Gray	Dark gray	Serpentine	Serpentine
S. pactum	Hairy coarse	White gray	Dark brown	Serpentine	Rectiflexibiles
S. canus	Spiny	Milky	Brown	Serpentine	Serpentine
S. longisporoflavus	Warty smooth	Light yellowish	Light-yellowish	Serpentine curvature	Serpentine
Streptomycesrimosus	Spiny coarse	White	Dark brown	Rectiflexibiles	Rectiflexibiles
S. bambergiensis	Spiny coarse	White	Brown	Rectiflexibiles	Rectiflexibiles

Table 2. Biochemical Tests for Isolates of Streptomyces. AL/AL (alkaline/alkaline), AL/AC (Glucose fermentation only), AC/AC. (The fermentation of both Glucose and Lactose), β (beta hemolysis), α (alpha hemolysis), ¥(Non hemolysis).

Sample	Tyrosin	Egg-yolk agar	Blood agar	Casein hydrolysis Scam milk agar	DNAS	Gelatin hydrolysis	Kligler iron agar	Starch hydrolysis	citreat	urease	Catalase	Oxidase	Tyrosin
S. albus	+	+	¥	_	_	_	AL/AL	+	+	¥	_	_	_
S. violaceaniger	+	+	α	++	+	+	AL/AL	+	+	α	++	+	+
S. thermorulgaris	+	+	β	_	+	+	AL/AL	+	+	β	_	+	+
S. roseus	+	+	α	_	+	_	AL/AL	+	+	α	_	+	_
S. varsoviensis	+	_	¥	_	+	_	AL/AL	_	_	+	+	+	+
S. varsoviensis	+	+	α	++	+	_	AL/AL	+	+	+	+	+	_
S. xanthochromogenes	+	+	β	_	+	_	AL/AL	+	_	_	++	+	+
S. chromogenus	+	+	¥	_	+	+	AL/AL	+	_	_	++	+	_
S. glaucescens	+	+	β	++	+	_	AL/AL	+	++	+	++	++	+
S. pactum	+	+	α	++	+	+	AC/AC	+	+	_	+	+	_
S. canus	+	+	β	+	+	+	AL/AL	+	_	_	+	+	+
S. longisporoflavus	+	+	α	+	+	+	AC/AC	+	_	+	+	++	+
S. rimosus	+	+	α	++	+	+	AL/AL	+	++	+	+	++	_
S. bambergiensis	+	_	α	++	+	_	AL/AL	+	_	_	+	+	_

Table 3. Sugars fermentation tests to Streptomyces isolates

Sample	S/rose	Xylose	Mannitol	Trehalose	Raffinose	Galactos	Mainos	Fructose	Cellulose	Glucose	Maltose	Lactose
S. albus	+	-	+	+	+	+	+	_	_	+	+	_
S. violaceaniger	+	_	+	+	+	+	+	+	+	+	+	+
S. thermorulgaris	_	_	+	+	+	+	+	+	+	+	+	_
S. roseus	_	_	+	+	+	+	_	_	+	+	+	+
S. varsoviensis	_	_	+	+	+	+	+	+	+	+	+	+
S. varsoviensis	+	_	+	+	+	+	_	+	+	+	+	+
S. xanthochromogenes	_	_	+	+	+	+	+	+	+	+	+	+
S. chromogenus	_	+	+	+	+	+	+	+	+	+	+	+
S. glaucescens	+	_	+	+	+	+	+	+	+	+	+	+
S. pactum	_	_	+	+	+	+	+	+	+	_	+	+
S. canus	+	_	+	+	_	+	_	_	_	_	_	_
S. longisporoflavus	_	_	+	+	+	+	_	_	+	_	+	_
S. rimosus	+	_	+	+	+	+	+	+	+	+	+	+
S. bambergiensis	_	_	+	+	+	+	+	+	+	+	+	+

Table 4. L-Sugars fermentation tests to *Streptomyces isolates: Bacillassubtales, Escherichia coli, Pseudomonas Fluorescens, Aspergillus spp, Candida albicans, Streptococcus pyogen, Salmonella typhi, Tricophyton mentagrophytes*.

Sample	*B.subtillas*	*Ps.f.*	*E.coli*	*C. albicans*	*A.spp*	*S.t.*	*S.p.*	*T. m*
S. albus	+	_	++	+	+	_	+	+
S. violaceaniger	+	_	+	+	+	+	_	+
S. thermorulgaris	_	+	+	_	_	+	_	+
S. roseus	+	+	_	+	+	_	+	+
S. varsoviensis	+	+	+	_	+	+	+	+
S. varsoviensis	_	_	_	_	+	+	_	_
S. xanthochromogenes	+	+	_	_	+	_	+	+
S. chromogenus	_	_	_	+	_	_	_	+
S. glaucescens	+	+	+	_	+	_	+	_
S. pactum	_	+	+	+	+	_	+	+
S. canus	+	+	_	++	_	_	_	+
S. longisporoflavus	_	_	_	+	_	+	_	_
Streptomycesrimosus	+	+	+	_	+	_	+	+
S. bambergiensis	+	+	_	+	_	_	+	+

Biochemical and physiological characterization

The results displayed in Table 3 show that all isolates of *Streptomyces* do appear as catalase- and oxidase-positive. This result is in agreement with the study by (AL-Samiac, 2006; AL-Ajeeli, 2012). Sugar fermentation method has been used to diagnoses the *Streptomyces* isolates. Using the Methyl red as indicator the ability of the bacteria to ferment sugars is inferred to be a positive via changes in the color of media from red to yellow. The resultants were due to the fermentation of sugars as the latter lowered the *p*H of culture leading to change in the color of indicator (Kar, 2008). In Table 4, all isolates of *Streptomyces* were able to utilize mannitol, galactos and trehalose as a source of carbon. The majority of isolates displayed their inability to ferment Xylose, except *S. chromogenus*. These results were in consistent with those of (Dastager *et al.,* 2006). All these experiments had confirmed the genus of the *Streptomyces*.

The Sensitivity of *Streptomyces isolates* against antibiotics

Most isolates i.e. *S. albus, S. pactum, S. varsoviensis,* have appeared to be resistant to antibiotics *Pencillin*, *Cephalthin* and *Amoxicillin* due to their ability to produce *β*-Lactamase which works to break down the bonds in the *β*-Lactam ring, thus destroying the antibiotic. These results are in agreement with the studies of Mona *et al.,* (2007). The results also show that *S. albus* is resistant to the antibiotic Co-*trimoxazole*. The reason for this resistance is interpreted that *Streptomyces* genus possess linear genome which gives its expected characterization of biosynthetic pathways to produce formerly unknown product. Some of the latter may inhibit the action of the antibiotic through the formation of alternative products that are working on an antibiotic, or the ability of these products to analyze the antibiotic (Challis, 2008). These results are inconsistent with the study of (Imran *et al*., 2012).

The inhibitory effects of metabolism

Some of the isolates of *Streptomyces* species were inhibitory to the pathogenic micro-organism i.e. *S. canus* to *Candida albicans* while *S. albus* was strong inhibitory against *E coli*. The isolates of *Streptomyces* looked different in their ability to produce antibiotics according to the source of carbon and nitrogen that is used in the production of the antibiotic (Pandy *et al*., 2005). The results here do clearly confirm the accuracy of technique being used as with culture medium agar the solid allows the metabolic products of bacteria to spread uniformly during agar which gives a precise and clear results according to (Enefiok and Charles, 1978).

Taxonomy of *Streptomyces* isolates

The isolates of *Streptomyces* in the current study are classified according to international taxonomic keys recommended by Williams, *et al*. (1983); Williams, (1989) and Hensyl, (1994) that diagnose the bacterial isolates, based on morphological, physiological and biochemical characteristics, upto the species level. The results of isolates are comparable with the classification of *Streptomyces spp* on international level.

The fourteen bacterial isolates of *Streptomyces* species produced in this study were all inhibitors against the pathogenic micro-organisms which indicates the possibility of production of new and pure antibiotics on industrial scales for pharmaceutical purposes. This would open the floor widely for further studies in the line.

CONCLUSION

The thirteen species of Streptomyces isolated from 50 different regions of Kirkuk Iraq had proved potency as antibiotics and as inhibitors against some pathogenic micro-organisms. It is concluded that these species could possibly produce new powerful antibiotics both on commercial scales for clinical application.

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