Streptomyces: Characteristics and Their Antimicrobial Activities

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Abstract

The Streptomyces are gram positive bacteria with a filamentous form that present in a wide variety of soil including composts, water and plants. The most characteristic of Streptomyces is the ability to produce secondary metabolites such as antibiotics. They produce over two-thirds of the clinically useful antibiotics of natural origin (e.g., neomycin and chloramphenicol). Another characteristic of Streptomyces is making of an extensive branching substrate and aerial mycelium. Carbon and nitrogen sources, oxygen, pH, temperature, ions and some precursors can affect production of antibiotics. This review also addresses the different methods to study the antimicrobial activity of Streptomyces sp. Because of increasing microbial resistance to general antibiotics and inability to control infectious disease has given an impetus for continuous search of novel antibiotics all the world.

Key words: Streptomyces, soil, PH, Antibiotics

Introduction

First time the genus Streptomyces was introduced by Waksman and Henrici in 1943 (Williams et al., 1983). Genus Streptomyces belongs to the Streptomycetaceae family (Arai, 1997). In general Streptomycetaceae family can be distinguished by physiological and morphological characteristics, chemical composition of cell walls, type of peptidoglycan, phospholipids, fatty acids chains, percentage of GC content, 16 SrRNA analysis and DNA-DNA hybridization (Korn-Wendisch & Kutzner, 1992). Streptomycetaceae family are in Actionobacteria phylum and Actinomycetales order within the classis Actinobacteria and the genus Streptomyces is the sole member of this family (Anderson & Wellington 2001). In terms of number and variety of identified species, Streptomyces represents one of the largest taxonomic items of recognized Actinomycetes (Bhattacharyya, pal, & Sen, 1998). They are distinguished as gram-positive bacteria, aerobic, non-Acid- Fast and with a high GC content more than 70% (Dehnad, Parsa Yeganeh, Bakhshi, & Mokhtarzadeh, 2010). Streptomyces can grow in different environments (Maleki, Dehnad, Hanifian, & Khani, 2013). They produce layer of aerial hyphae that can differentiate into a chain spores (Korn-Wendisch & Kutzner, 1992). More than 500 species of the genus Streptomyces have been described and nearly two third of the naturally occurring antibiotics are produced by Streptomyces (Mohanraj & Sekar, 2013).
Characteristics of Streptomycetes

*Streptomyces* is the largest genus of Actinobacteria and the type genus of the family Streptomycetaceae (Hong et al., 2009). Over 500 species of *Streptomyces* bacteria have been described (Lee, Jung, & Hwang, 2005). *Streptomyces* species are chemoorganotrophic, filamentous, gram-positive bacteria but not acid-alcohol fast, not fungi and occur in the same habitats as fungi and are superficially similar (Ikeda et al., 2003). They have genomes with high GC content 69-78% (Kavitha, Vijayalakshmi, Sudhakar, & Narasimha, 2010). The filaments and spores are very small usually 1 µm or less in diameter (Willemse, Borst, Waal, Bisseling, & Wezel, 2011). The spores are formed by the fragmentation of the filaments and are borne in straight, wavy, or helical chains (Chater, 1993). The colonies are slow-growing and often have a soil-like odour because of production of a volatile metabolite, geosmin (Jüttner & Watson, 2007). Firstly colonies are relatively smooth surfaced but later they develop a weft of aerial mycelium that may appear floccose, granular, powdery, or velvety (Ambarwati, Sembiring, & Soegihardjo, 2012). They produce a wide variety of pigments responsible for the colour of the vegetative and aeral mycelia (Flärhd & Buttner, 2009). *Streptomyces* species are nonmotile, catalase positive, reduce nitrates to nitrites and degrade adenine, esculin, casein, gelatin, hypoxanthine, starch, and L-tyrosine (Smaoui, Mathieu, Fguira, Merlina, & Mellouli, 2011). The cell wall peptidoglycan contains major amounts of L-diaminopimelic acid (L-DAP). They have no mycolic acids, contain major amounts of saturated, iso- and anteiso-fatty acids, possess either hexa- or octahydrogenated menaquinones with nine isoprene units as the major isoprenolog, and have complex polar lipid patterns that typically contain diphosphatidyl glycerol, phosphatidyl ethanolamine, phosphatidyl inositol, and phosphatidyl inositol mannosides (Cummins & Harris, 1958).

The life cycle of Streptomyces

The life cycle of Streptomyces starts when a spore settles in a nutrient rich medium. This stimulates the spore to exist its dormant state and undergo germination and form germ tubes. Germ tubes grow by tip elongation and the cells don’t undergo binary fission. Through extension and branching the germ tubes give rise to a network of filaments which grow into and across the surface of an agar plate. This network is called the substrate mycelium (Flärhd & Buttner, 2009). As the colony continues to grow, the mycelium in the center of the colony starts to differentiate. Differentiation results in the formation of a new cell type, the aerial hyphae. When heavy word growth of the spiraling, multi-gonomic aerial hyphae stops, the aerial hyphae undergo synchronous cell division giving rise to monoploid compartments each of which will develop in to a resistant spore (Kieser, Bibb, Buttner, Chater, & Hopwood, 2000). The life cycle of a *Streptomyces* sp. has been studied by the Robinow HC1-Giemsa method of nuclear staining, and is described in the subsequent manner: (1) initial nuclear division phase; (2) primary mycelium; (3) secondary mycelium (Including aerial); (4) the formation of spores. The primary mycelium develop after the initial nuclear division phase and produce side divisions, then later gave rise to single swellings in the hyphae. These swellings grow to form large round cells, each of which contain many nuclei. The secondary mycelium develops, a part of which become aerial and terminates in the form of chains of spores (Mc Gregor 1954).

Streptomyces habitats

Although streptomycetes widely distributed in soil, water and other natural environments (Seong, Choi, & Baik, 2001), (Singh, Baruah, & Bora 2006). The population of them in an ecosystem are determined by
numerous physical, chemical and biological factors (Kharat, Kharat, & Hardikar, 2009). Identification of novel ecological systems is therefore crucial for the discovery of novel Streptomyces (Wang, Zhang, Ruan, Wang, & Ali, 1999).

Streptomyces make 40% of soil bacteria (Boone, Castenholtz, & Garrity, 2001). Under dry and alkaline conditions Streptomyces sp. are the numerous microbial population in soil and because of their filamentous form, they cause the strength of soil texture and protect it from wind and rained eradication (Vetsigian & Roy Kishony, 2011). The percentage of Streptomyces sp. in total microbial population has positive correlation with the depth of soil and they can even be obtained from the horizon C of the soil (Kim, Seong, Jeon, Bae, & Goodfellow, 2004). Distribution of Streptomyces in water and soil, depends on food stress, temperature, pH, moisture, salinity, soil texture and climate (Locci, 1989). Although soil is the most important Streptomyces habitat (Mokrane, Bouras, Sabaou, & Mathieu, 2013), other habitats are:

**Hay and other organic material:** Mesophilic and Thermophilic Streptomyces sp. can degrade many natural substrates, cotton textiles, plastics, rubber and paper (Subbarao 1999). Most of streptomycetes subjected as indigenous microbes isolated from soil and had successfully capability to growth remove and use different organic compound (Rahmansyah, Agustiyani, Julistiono, & Dewi, 2012). Streptomyces are capable to produce degrade Cellulose, lignocellulose, chitin and different organic compounds in biogeochemical cycles (Horn, Vaaje-Kolstad, Westereng, & Eijsink, 2012).

**Fresh water and marine habitats:** In addition to drinking water systems with drainage after heavy rainfall (Rowbotham & Cross, 1977). The isolation of Streptomectes from marine environments has been an abundant area of investigation in the past decade (Remya & Vijayakumar, 2008). Of the marine inhabitants studied, marine invertebrates, particularly sponges, are of excessive interest for discovering novel Streptomyces (Selvakumar, Arun, Suguna, Kumar, & Dhevendaran 2010). In recent times, the marine derived actinomycetes have become documented as a source of novel antibiotic and anticancer agent (Baskaran, Vijayakumar, & Mohan, 2011).

**Plants:** Streptomyces play a minor role as plant pathogens such as Streptomyces scabies, S.ipomoea, S.turgidiscabies, S.aureofaciens, S.acidiscabies and S.tumescans causing gall potato, soil rot or pox, pitted scab, netted scab, gall potato in acidic soil and root gall respectively (Fatope, Al-kindii, & Abdulnour 2000). In the other hand some species of Streptomyces act as biological control (Rugthaworn 2007). Biological control is an environmentally sound and effective means of reducing or mitigating pests and pest effects through the use of microorganisms. For example biological control of sunflower stem rot (Baniasadi 2009 ), (Aghighi, Shahidi Bonjar, & Saadoun, 2004) and potato common scab (Kalantarzadeh 2006 ).

**Animal and humans:** Although a small number of clinical Streptomyces have been isolated so far, their role as pathogens and infectious disease cannot be ignored (Korn-Wendisch & Kutzner, 1992). Streptomyces are uncommon pathogens, though infections in humans, such as mycetoma, can be caused by Streptomyces somaliensis and Streptomyces sudanensis (Quintana et al., 2008).

**Streptomyces requirements**

Streptomyces are aerobes, chemoorganotrophic bacteria and they need organic carbon source, inorganic nitrogen sources, and mineral salts and don’t need vitamins and growth factors (Lee & Demain 1997). Streptomyces requirements has been investigated by kutzner (Kuster & Williams, 1964).
Most of *Streptomyces* sp. are mesophile and grow in temperatures 10-37°C (Deebel, Fazeli, Cove, & Baumberg 2005) but three species *Streptomyces thermonitrificans, S.thermovulgaris* and *S.thermoavulans* are thermophile and grow in temperature 45-55°C (Srivibool, Kurakami, Sukchorotiratanac, & Tokuyamab, 2004). Streptomycetes grow in pH 6.5-8.0 (Cabello, Gonzalez, & Genilloud 2003). Streptomycetes are not only more resistant to drought and form arthrospore but also require less moisture than other bacterial and are very sensitive to water logged conditions(Subbarao 1999). Some of reports described that drained soils (sandy loam, calcareous) have more *Streptomyces* than heavy clay soils (Sujatha, RajuB, & Ramana, 2005).

### Streptomycetes metabolites

A primary metabolite is a kind of metabolite that is directly involved in normal growth, development, and reproduction. It usually performs a physiological function in the organism. A primary metabolite is typically present in many organism or cell (Demain 1980). Secondary metabolites are organic compounds that are not directly involved in the normal growth, development, or reproduction of an organism (Shomurat, Yoshida, Amano, Kojina, & Niida, 1979). Therefore secondary metabolites differ from primary metabolites (e.g. amino acids and nucleotides) in four manner: 1) they are not essential for growth, 2) their production is dependent on growth conditions liked type of culture media, 3) they are often produced as groups of closely related molecules, and 4) it is often possible to overproduce these components (Drew & Demain, 1977).The most important characteristic of Streptomycetes is the ability to produce secondary metabolites with antibacterial, antifungal, antiviral and antitumoral properties. Two species of *Streptomyces* by the name of *Streptomyces griseus* and *Streptomyces coelicolor* are used for industrial production of Streptomycin and novel antibiotics such as dihydrogranticin respectively. Doxorubicin as anticancer agents (Mukhtar, Ijaz, & Ul-Haq, 2012) and Rapamycin as immunomodulatory agents are Secondary metabolites produced by *Streptomyces* (Ying & Marta, 2001).Another metabolite of *Streptomyces* known as the ‘Geosmin’ and sidrophore are responsible for the earthy odor (Sanglier, Haag, Huck, & Fehr, 1993). However volatile product secreted by *Streptomyces* may also be responsible for the specific smell (Bais, Nimbekar, Wanjaril, & Timande, 2012).

### Streptomycetes and antibiotics

Streptomycetes have high potential to produce secondary metabolite such as antibiotics (MCIntyre, 2002), anthelminthic enzymes, herbicides (Kariminik & Baniasadi, 2010), anti-cancer drugs (Berdy, 2005), growth factors like vitamin B12 (Bibb, 2005) and immune- modulators (Mann, 2001). Louis Pasteur was one of the pioneers of modern antibiotics knowledge, in 19th century (Gray & Jacobs 2003). He found that, some of microorganisms are able to kill other microorganisms. Penicillin was the first antibiotic that discovered by Alexander Fleming from *Penicillium notatum* in 1929 (Silva & Anne, 2004). The history of antibiotics obtained from *Streptomyces* sp. began with the discovery of Streptothricin in 1942(Sanglier et al., 1993), and the discovery of Streptomycin in 1943 (Schats, Bugie, & Waksman, 1994), and then scientists intensified the search for other antibiotics within the genus(Wave, Tichoo, MM, & Bhole, 2001). The most productive years of antibiotic discovery were between 1945 and 1960 (Wave et al., 2001). In 1949 Rachel Brown extracted the first antibiotic for treatment of fungal disease called Nystatin from *Streptomyces noursei* (Orna, 2001). They produce over two-thirds of the clinically useful antibiotics of natural origin (e.g., neomycin and chloramphenicol). Today, 80% of the antibiotics are derived from
Streptomyces sp. (Kharat et al., 2009). Some of antibiotics has listed in table 1. Of the clinically useful antibiotics, more than 50% are derived from Streptomyces (Keiser et al., 2000).

Because of microbial resistance, despite the success of the discovery and production of antibiotics, infectious diseases still seem the second agent of death in the world (Nikaido, 2009). The resistance may be due to genetic changes such as mutation or acquisition of resistance factors such as plasmids through horizontal genetic transfer (Wright 2010). In the other hand some physiological state of the bacteria such as biofilm formation can induce antibiotic resistance and also microorganisms related to chronic and recurrent infections usually are resistant to antibiotics (Hassan et al., 2011). As a result, scientists are trying to change to existing antibiotics or discover new agents that are more effective and stable to inactivating enzyme produced by resistant bacteria (Garza-Ramos, Silva-Sánchez , & Martínez-Romero, 2009).Granaticin is a pigmented and pH sensitive antibiotic which produce by Streptomyces thermoviolaceus. Granaticin is specially synthesized at temperatures as high as 55 °C but optimally at 45 °C. Streptomyces thermoviolaceus possesses a termotolerant polypeptide pathway. Although the rate of production of the secondary metabolite is fastest at 37 °C the yield is not as high as that seen at 45 °C. We attribute this to a preference for producing biomass at 37 °C and there is an inverse relationship between cell yield and titre of antibiotic over the temperature range 30 to 50°C (James & Edwards, 1989),(Procópioa, Silvaa, Martinsa, Azevedoa, & Araújob, 2012).

Table 1. List of some antibiotics produced by Streptomyces sp.

<table>
<thead>
<tr>
<th>Streptomyces sp.</th>
<th>Antibiotic</th>
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<th>Antibiotic</th>
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<tbody>
<tr>
<td>S. orchidaccus</td>
<td>Cycloserin</td>
<td>S.erythraeus</td>
<td>Erythromycin</td>
</tr>
<tr>
<td>S.orientalis</td>
<td>Vancomycin</td>
<td>S.vensuella</td>
<td>Chloramphenicol</td>
</tr>
<tr>
<td>S.fradiae</td>
<td>Neomycin, Actinomycin, Fosfomycin, Dekamycin</td>
<td>S.aureofaciens</td>
<td>Chlortetracycline, Dimethylchlor tetracycline</td>
</tr>
<tr>
<td>S.nodosus</td>
<td>Amphotericin B</td>
<td>S.ambofaciens</td>
<td>Spiramycin</td>
</tr>
<tr>
<td>S.noursei</td>
<td>Nistatin</td>
<td>S.avermitilis</td>
<td>Avermicin</td>
</tr>
<tr>
<td>S.mediterranei</td>
<td>Rifampin</td>
<td>S.alboniger</td>
<td>Puromycin</td>
</tr>
<tr>
<td>S.griseus</td>
<td>Streptomycin</td>
<td>S.niveus</td>
<td>Novobicin</td>
</tr>
<tr>
<td>S.knanamyceticus</td>
<td>Kanamycin</td>
<td>S.platensis</td>
<td>Platenmycin</td>
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<tr>
<td>S.tenebrarius</td>
<td>Tobramycin</td>
<td>S.roseosporus</td>
<td>Daptomycin</td>
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<tr>
<td>S.spectabilis</td>
<td>Spectinomycin</td>
<td>S.ribofusidicus</td>
<td>Ribostamycin</td>
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<tr>
<td>S.viridifaciens</td>
<td>Tetracycline</td>
<td>S.garyphalus</td>
<td>Cycloserine</td>
</tr>
<tr>
<td>S.lincolensis</td>
<td>Lincomycin, Clindamycin</td>
<td>S.vinaceus</td>
<td>Viomycin</td>
</tr>
<tr>
<td>S.rimosus</td>
<td>Oxytetracyclin</td>
<td>S.clavuligerus</td>
<td>Cephalosporin</td>
</tr>
</tbody>
</table>

**Antibacterial activity of Streptomycetes**

**Sampling**

According to the many investigations, it is better to do the sampling from various parts of the soil from depth of 5 to 10 cm (Shahidi Bonjar, 2004). Streptomycetes exist in different types of soil and the surface
layer of soil is more numerous because of their strict aerobic metabolism. Streptomyces population is more in alkaline soils, compost, river's mud and riverbeds (Kariminik & Baniasadi, 2010). Their concentration in soil depends on physical properties, organic matter content, pH, moisture, soil reactions and soil texture (Nonoh et al., 2010). Streptomycetes are highly tolerant to salinity and that's why many species are found in salty soil and marine (Selvakumar et al., 2010).

Nutrient requirements for antibiotic production

Streptomycetes can grow on Muller-Hinton agar, Trypticase soy agar, and Nutrient agar with calcium chloride (Busti & Yushi, 2006). Carbon and nitrogen sources, oxygen, pH, temperature, ions and some precursors can affect production of antibiotics (Rafieenia, 2013).

Carbon: Some investigators reported that glucose decreases antibiotic production because of suppressing the enzymes involved in antibiotic biosynthesis and can be related to effect of growth rate on antibiotic biosynthesis (Lounes, Lebrihi, Benslimane, Lefebvre, & Germain, 1996), (Lounes et al., 1996). Polysaccharides such as starch and glycerol are commonly the best carbon sources as they support a slow growth rate that is appropriate for antibiotic production (Jonsbu, McIntyre, & Neilson, 2002). Slavica Ilić et al showed the use of new carbon and can lead to the best microbial growth and antibiotic production. The maximum production of some antibiotics was in the culture media with glucose and lactose, while the minimum antibiotic production was observed in the medium with ribose (Ilić, Konstantinović, Veljković, Savić, & Gojić-Cvijović, 2010). (Jonsbu et al., 2002)

Nitrogen: Several studies have showed that antibiotic synthesis related to type and concentration of nitrogen source in culture media (Rafieenia, 2013). Simple and inorganic nitrogen sources usually decrease antibiotic production (Young & Kempe, 1985). Complex nitrogen sources such as soybean meal, corn steep liquor and yeast extract can increase antibiotic production which can be attributed to slow breakdown of these compounds in the medium. Slavica Ilić et al found that replacement of soybean as nitrogen source with isatin-Schiff bases (isatin-3-thiosemicarbazone – ITC, isatin-3-semicarbazone – ISC and isatin-3-phenylhydrazone – IPH;) increased the level of antibiotic production (Ilić et al., 2010).

Rate of growth: Bacterial growth in exponential phase, which has the optimum growth rate, can effect on more antibiotic production (Sejiny, 1991). The biosynthesis of antibiotics is a particular possessions of microorganisms and depends on growth conditions (Young & Kempe, 1985). These microorganisms use a wide range of substrates for growth; however, many of these substrates can have a negative effect on the production of secondary metabolites. Under several nutrient limitations, the production of secondary metabolites will be higher than non-limiting nutrient conditions (Sejiny, 1991).

Minerals: Minerals such as phosphorus (Martin, 2004), potassium, iron, zinc and manganese in trace increase level of antibiotic production (Gesheva, Ivanova, & Genava, 2005). Many secondary metabolites are produced only when phosphate is the growth limiting nutrient. It has been shown that antibiotic synthesis starts after decrease of phosphate source (Martin, 2004).

Oxygen: Streptomycetes are aerobic bacteria. Thus appropriate oxygen has a great influence on their growth and antibiotic production (Wang et al., 1999).

pH: Most antibiotics are optimally produced in pH close to 7.0 (Saadoun, Momani, Malkawi, & Mohammad, 1999).
Precursors: Precursors such as amino acids and Short-chain fatty acids are the precursors of some antibiotics and added to media in industrial processes (Tang, Zhang, & Hutchinson, 1994).

Ions: Divalent ions as Mn2+, Cu2+, Fe2+ usually stimulate antibiotic biosynthesis (Gesheva et al., 2005).

Antibacterial activity protocols.

There are many different techniques for investigation of antimicrobial properties.

Cross streak method

Agar plates are prepared and inoculated with Streptomyces isolates by a single streak in the center of petridishes and incubated at 30 °C for 7 days. The plates were then inoculated with the test bacteria by a single streak at 90° angles to the Streptomyces isolates and incubated at 37°C for 24 hr. Antibacterial activity is recorded by zone of inhibition (Kumar, Preetam Raj, Duraipandiyan, & Ignacimuthu, 2012).

Agar overlay method

In this method, Streptomyces isolates are spot inoculated in medium for 7 days in 30°C. Following incubation, one ml of chloroform is added to arrest the growth of inoculated isolates and after 40 minutes they are overlaid with 7 ml of semisolid nutrient agar (0.7%) inoculated with 0.1 ml of overnight culture of tested bacteria. Plates are incubated for 24 hours at 37 °C and zone of inhibition is measured in millimeters (Sahin & Ugur, 2003).

Disc Diffusion assay

The Streptomyces isolates are inoculated as submerged culture in 500 ml Erlenmeyer flasks containing 100 ml of broth Streptomyces medium and incubated in shake incubator at 200 rpm in 30 °C for 5 days. The cultures are centrifuged for 15 minutes in 1500 rpm. The culture filtrates are extracted with ethyl acetate solvent (1:1 v/v) and shaken for 20 minutes. The supernatant organic layer are collected and evaporated in Rotary evaporator system at 40°C. A crude and dry extract obtained, are suspended in ethyl acetate at a concentration of 1mg/ml for antibacterial studies. Sterile blank discs - 6mm in diameter- are saturated with 50 ml suspension, dried and placed on to the plates previously inoculated with tested bacterial. The plates are incubated for 24 hours in optimum temperature of bacterial and zone of inhibition are measured. The inhibitory effects are evaluated by the measurement of inhibition zone diameter(Gebreyohannes, Moges, Sahile, & Raja, 2013) and (Manivasagan et al., 2009).

Agar well diffusion assay

250 ml Erlenmeyer flasks containing 150 ml sterile starch casein nitrate broth are aseptically inoculated with the spore suspension of Streptomyces isolates and incubated aerobically at 30 °C for 10 days. After incubation, the contents in the flasks are filtered through sterile what man paper No.1 the culture filtrates are centrifuged and supernatant and ethyl acetate (1:1 v/v) is used for solvent extraction, and agitated for 30 minutes. Solvent layer is removed and the supernatant is twice extracted with ethyl acetate and then the ethyl acetate layer evaporated by Rotary evaporator at 40 °C to dryness. The bacterial isolates are grown on with 1.5×10³/ ml equal 0.5 McFarland solvent, Nutrient agar plate using sterile cotton swabs.
Wells of 6 mm diameter are punched in the inoculated plates. Ethyl acetate extracts (5 mg/ml of 25% dimethyl sulfoxide) are filled in wells. The plates are incubated at optimum temperature of bacteria for 24 hours. The inhibition zones formed around the wells are measured in millimeters (Pallavi et al., 2013).

References


