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REVIEW ON CURRENT TECHNIQUES IN ISOLATION AND CHARACTERIZATION OF *Streptomyces* FROM SOIL

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ABSTRACT

***Streptomyces* genus of actinomycetes is Gram-positive, filamentous bacteria are most potent source of antibiotic production. To achieve novel, safer and more effective antibiotics from *Streptomyces* necessitate systematic information about *Streptomyces* and use of high throughput techniques. Different chemical and physical pretreatment methods are available for selective isolation of *Streptomyces*. Morphological differentiation of *Streptomyces* based on morphological, physiological and biochemical characterization but conventional methods are not reliable at genus/ species/strain level therefore molecular characterization methods including Restriction fragment length (RFLP), Amplified ribosomal DNA restriction analysis (ARDRA), . Random amplified polymorphic DNA (RAPD), Pulse field gel electrophoresis (PFGE) and use of genus specific primers are applied. Production of antimicrobial compound greatly influenced by nutritional parameters: carbon and nitrogen source and physiological parameters: pH, temperature, oxygen availability. Maximum yield require formulation of culture media by means of carbon and nitrogen source at different concentration.**

KEYWORDS: *Streptomyces*, soil, characterization, Morphological differentiation

Streptomyces dominant genus of actinomycetes are mycelium producing, aerobic, gram positive, spore forming bacteria usually distributed in soil containing high G+C content (> 55%) in their DNA. The genus *Streptomyces* produce wide varieties of natural bioactive metabolites such as antibacterial, antifungal, antiviral, antitumor, anti-hypersensitivity and immunosuppressive (Omura *et al*., 2001; Bentley *et al*., 2002; Paradkar *et al*., 2003; Patzer *et al*., 2010; Khan 2011). *Streptomyces* produces 75 % of commercially and clinically valuable known bioactive compound which are broadly used in industries (Miyadoh 1993). Resistance of wide range of bacteria and fungi to many antibiotics and appearance of new diseases and effect of antimicrobial compound on immune system imposed the discovery of effective, new and safer and broad spectrum antimicrobial compound (Wise 2008; Demain and Sancheze 2009). Antimicrobial compound production greatly influenced or completely lost under different nutritional and environmental condition (Waksman *et al.,* 1961). High yield of bioactive compound depends on successfully formulation of culture medium with accurate carbon and nitrogen sources (Goodfellow and Fiedler 2010) and different cultural conditions, pH, temperature, oxygen availability, medium volume, incubation time and inoculum size (Srinivasan *et al*., 1991; Mehdi *et al*., 2006; Ruiz *et al*., 2010). *Streptomyces* are already known genus for production of bioactive metabolites although collection of broad information about *Streptomyces* and systematic use of methods and techniques are useful to produce even more profitable, safer, and effective bioactive compound (Nett *et al*., 2009).

BIOPROSPECTING FOR ANTIMICROBIAL COMPOUND

Antibiotic producing bacteria particularly *Streptomyces* extensively present in soil and through antibiotic production they inhibit their competitors and pathogens. Discovery of bioactive compound from soil *Streptomyces* decrease in past two decades because search of new compounds directed towards unexplored environments (Ramazani *et al*., 2013). Pharmaceutical industries uses product of soil *Streptomyces* since fifty years but merely a minuscule fraction of earth is exploited for isolation and production of bioactive secondary metabolite from this genus (Takahashi and omura 2003). Thus, there is a chance of finding new *Streptomyces* species and new bioactive compounds from this genus from unexplored region of earth. However, the frequency of rediscovery of known antimicrobial compounds from *Streptomyces* fairly high (Huck *et al*., 1991). *Streptomyces griseus* was first *Streptomyces* isolated from soil produced the first antibiotic streptomycin, after development of penicillin from *Penicillium chrysogenum*, this was first antibiotic used in treatment of tuberculosis (Procopio *et al.,* 2012).

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PRETREATMENT AND ISOLATION PROCESS

Pretreatment of soil samples implement by physical and chemical treatment to enhance selective isolation, liberate spores from vegetative cells and reduces growth of other microorganisms. Different types of Chemical treatment: Calcium carbonate treatment, calcium chloride treatment, chitin treatment, Sodium dodecyl and yeast extract, phenol and antibiotics (antifungal and antibacterial) treatment and physical method**:** Air dry, heat dry, moist heat, electromagnetic wave, UV treatment are applied as pretreatment for isolation of *Streptomyces* (Galatenko *et al*., 1990; Thumar *et al*., 2010; Khanna *et al*., 2011; janaki *et al*., 2014). Many types of culture medium formulation proposed for isolation of *Streptomyces* such as various ISP (International *Streptomyces* project) medium including Tryptone-yeast-extract-broth (ISP-1), Yeast extract-malt extract agar (ISP-2),Oat meal agar (ISP-3), Inorganic starch agar (ISP-4), Glycerol asparagine agar (ISP-5), Peptone yeast extract iron agar (ISP-6), Tyrosine agar (ISP-7). Tryptone soy agar medium, Glycerol arginine medium, Glucose-asparagine peptone, Czapek-Dox yeast extract medium, Norris medium, richard's medium , Starch casein nitrate agar, Sabouraud's, Nutrient agar medium, Starch casein glycerol medium.

TAXONOMIC FEATURES OF *Streptomyces*

Conventional Methods

Study of substrate mycelium features, colour of aerial and substrate mycelia, formation of soluble pigments, melanoid pigments, growth on standard media and utilization of carbon and nitrogen sources frequently applied to determine taxonomical features of *Streptomyces* (Pridham *et al*., 1948; Shirling *et al*., 1966). Acid-fast and Gram-stain properties were also used for taxonomical characterization (Taddei *et al*., 2006). Ability to produce different type of enzymes such as lecithinase, amylase, lipase, pectinase, gelatinase and catalase , degradation of xanthine, hypoxanthine, esculin, urea and citrate, tyrosine. Utilization of different carbon and nitrogen sources, growth observation in presence of inhibitors such as phenol, sodium azide, potassium tellurite and antibiotic sensitivity test and nitrate reduction, H_2S test, casein degradation, cell wall analysis can be used for biochemical characterization according to Bergey's manual (Holt *et al.*, 2000).

Molecular Characterization

However taxonomic characterization based on conventional method such as cultural, morphological and biochemical methods not always reliable therefore molecular methods are used for characterization up to genus, species and strain level. PCR based methods provided a rapid and accurate way to identify bacteria (Telenti *et al*., 1993; Mehling *et al*., 1995). Different techniques for molecular characterization; Restriction fragment length (RFLP) analysis using any one of the genomic DNA:16SrRNA, 23SrRNA, Particularly, partial 16S rRNA gene sequence compared with the sequences (reference species of bacteria) available in genomic data-base banks, Amplified ribosomal DNA restriction analysis (ARDRA) it is useful for strain/species level characterization and used for digestion of amplified rDNA with the help of different restriction enzyme for typing of microorganisms. Random amplified polymorphic DNA (RAPD), Pulse field gel electrophoresis (PFGE), Use of genus specific primers (khanna *et al*., 2011).

Screening

Screening allows detection and selective isolation of microorganisms which are able to produce bioactive compounds. Two types of screening applied for antimicrobial screening of *Streptomyces.* Primary screening allows recognition of potential microbes with different methods including spot inoculation method, Cross streak method, modified cross streak method. Microbes exhibited primary activity is subsequently selected for secondary screening which provide information about quantitative production of bioactive compound. Agar-disc diffusion method, Agar well diffusion and Minimum inhibitory concentration are secondary screening methods (Ball *et al*., 1957; Ilic *et al*., 2007; Thakur *et al*., 2007; Jemimah *et al*., 2012, Khanna *et al*., 2011; Tabrizi *et al*., 2013).

Optimization Process

Optimization is crucial for the Maximum antibiotic yield through different cultural condition including carbon and nitrogen source, phosphorous, metal ions, pH, temperature, oxygen availability, medium volume, incubation time and inoculum size (Srinivasan *et al*., 1991; Mehdi *et al*., 2006; Ruiz *et al*., 2010). Information on optimization of physical and nutritional parameter to increase yield of secondary metabolites

disclosed that different carbon source at different concentration such as Glucose ((Fguira *et al*., 2005; Valanarasu *et al*., 2009 Atta *et al*., 2011b), glycerol (Mellouli *et al*., 2004; Mehdi *et al*., 2006; Elleuch *et al*., 2010; Singh *et al*., 2012), mannitol (Thakur *et al*., 2009), maltose (Yu *et al*., 2008). KNO_3 (Saadoun *et al*., 2008; El-Nasser *et al*., 2010; kumar *et al*., 2012), Peptone (Cho *et al*., 2012; Singh *et al*., 2012), Yeast extract (Holkar *et al*., 2013; I-Son *et al*., 2014, Sanghvi *et al*., 2014), sodium nitrate (Hassan *et al*., 2001) as nitrogen sources are optimal nutritional parameters. Optimal temperature 28°C and 30°C and pH 6.0,7.0,7.2,7.5, 9.0 provide maximum yield of antimicrobial compound (Hassan *et al*., 2001; Yu *et al*., 2008; Elleuch *et al*., 2010; Atta *et al*., 2011b; kumar *et al*., 2012; Ramani *et al*., 2012; ,Kandula *et al*., 2013; Bhavana *et al*., 2014; Sanghvi *et al*., 2014; Souagui *et al*., 2015).

EXTRACTION AND PURIFICATION OF ANTIMICROBIAL COMPOUND

Different type of broth medium used for production of secondary metabolites such as Antibiotic production medium, Fermentation medium, Glucose yeast extract malt medium, Modified nutrient glucose (MNGA) medium, Tryptone soya broth, Yeast extract malt extract broth (YEMEB), Starch casein nitrate broth, Nutrient broth, Starch glycerol nitrate broth, *Streptomyces* antibiotic medium, Glucose soybean meal broth, Czapex-Dox broth (Singh *et al*., 2012; Sarvana kumar *et al*., 2014; Hamza *et al*., 2015).

After fermentation on suitable broth medium cell free filtrate extracted with different individually solvent such as alcohol, ethyl acetate, distill water, chloroform, methanol, dichloromethane, n-butanol at 1:1 (v/v) (Alapati *et al*., 2010; Afifi *et al*., 2014; Aouiche *et al*., 2014 ; sarvana kumar *et al*., 2014). The antimicrobial bioactive secondary metabolites are usually extracellular and their purification from the complex fermentation broth requires integrated method of various separation steps such as solvent extraction, chemical precipitation (Mellouli *et al*., 2003) and purification of bioactive metabolites through chromatography including ion exchange, High Performance Liquid chromatography, silica gel column chromatography , thin layer chromatography (Harindran *et al*., 1999; Mellouli *et al*., 2003; Roy *et al*., 2006; Ilic *et a*l 2007; Singh *et al*., Atta *et al*., 2011a).

Purified antimicrobial molecules characterized by using instrumental analysis including HPLC, GC-MS analysis, IR, UV, Mass spectrum, NMR spectrum, FTIR (Roy *et al*., 2006; Ilic *et al* 2007; *Terekhova et al*., 2007; Atta *et al*., 2011a; Singh *et al*., 2013; Kumar *et al*., 2014, Sanghvi *et al*., 2014).

CONCLUSION

Microbes provided natural bioactive compounds *Streptomyces* is one of them. Detection and identification of *Streptomyces* are valuable, provides medically important bioactive compounds. According to WHO emergence of drug resistant strain is quicker than discovery of new antimicrobial compounds. As the result of resistance of antimicrobial compound, now a days we are on the edge of medical disaster, that would put physician to the pre antibiotic days, where in apparently small infection could turn lethal due to lack of effective drugs therefore search of new antimicrobial compound necessary. Despite the continuous discovery of antimicrobial compound, infectious disease is still one of the most serious health threats due to rediscovery of known antimicrobial compounds. Novel bioactive compound can be achieved by exploration of new habitat by using more advance techniques.

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