REVIEW

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Isolation of actinomycetes from natural habitat.

Williams* and *Cross, (1971) described the methods for the isolation of streptomycets. ***Ahmad, (1990)*** isolated 87 actinomycetes isolates, 34 of them exhibited anti-microbial activities. He illustrated the method and media used for such purpose. ***Daqun et al., (1996)*** isolated 93 *Streptomyces* isolates, twenty-two strains showed more antibiotic activity against virulent *Streptomyces scabies* RB 311 than the standard pathogen-suppressive strains.

Causal organism of chocolate spot diseases.

Naguib, (1948) demonstrated that, chocolate spot disease of faba bean in Egypt can be caused by either *Botrytis fabae* or *B.cinerea*, while spots caused by the *B.cinerea* were inconspicuous compared with those by *B.fabae*.

Leach, (1955) illustrated that, leaf spots caused by *B.cinerea* effect only on epidermal cells where as *B.fabae* nearly always causes necrosis of the mesophyl.

Myra* and *preece, (1968) showed that, the addition of pollen grains or orange juice to the spores on the leaf surface improved spore germination and germ tube elongation and induced the development of the aggressive phase of chocolate spot, which is normally caused by *B.fabae*.

Mansfield* and *Deverall, (1974) found that Conidia of *Botrytis fabae* produced spreading lesions at nearly all inoculation sites on leaves

grown in green houses. The development of lesions produced by *Botrytis cinerae* was more variable.

Harrison, (1980) found that, under conditions of continuous high humidity, limited lesions become aggressive darkening and rapidly increasing in size, often causing defoliation and eventually killing the entire shoot system.

Abou-Zeid* and *Le Normand ,(1981) mentioned that, the most effective inoculum concentration of *B.fabae* suspension was $250x10^3$ spore / ml which used for artificial inoculation.

Mohamed et al., (1981) studied the range of variability within the fungus *B.fabae*, they tested five isolates from different area, Sakha, Nubaria, Ismalia, Gemiza and Alexandria, on different cultivars from faba bean, they found that, isolate from Nubaria is the most virulent than the rest.

Abau-Zeid* and *Mohamed (1987) demonstrated that, the optimum concentration for inoculation of faba bean differed according to *B.fabae* isolate, the faba bean cultivar and the method of inoculation used. The investigators found that, the reaction of the detached leaflets from different nodes of Giza 3 and Rebaya 40 faba bean plants to *B.fabae* isolated from Nubaria differed significantly, the newest node (5^{th}) were more resistant than the older one (1^{st}).

Harrison, (1988) reported that, both *Botrytis* sp. could cause chocolate spot disease on faba bean in the field, but *Botrytis fabae* was the more important pathogen because it was more aggressive than

B.cinerae. in the laboratory, however, the pathogenicity of *B.cinerea* could be manipulated by the addition of certain compounds to the inoculum droplets, or by using very young conidia, to be similar to *B.fabae* in inducing infection and development.

Hassanein et al., (1990) reported that, *B. fabae* isolates which showed the least amount of growth and spore production were the most virulent on the twice tested faba bean entries while isolates of vigorous growth and high spore production (*B. cinerea*) were less virulent

Abou-Zeid et al., (1998) isolated 40 isolates of *Botrytis* sp. from different growing area of faba been in Egypt during 1994 / 1995 season and they identified 40 isolates to the species level based up on the key of ***Ellis (1976)*** as *B.cinerea* (16 isolate) and *B.fabae* (24 isolate).

Kuti* and *Nawer, (1999) demonstrated that, in pathogenicity studies of five isolates of chocolate spot fungi (3 *B.cinerea* and 2 *B.fabae*), one isolate of *B.cinerea* originally isolated from faba bean was more pathogenic to faba bean than the two *B.fabae*, also originally isolated from faba bean, while the other two isolates of *B.cinerea* isolated from graps and eggplant respectively were moderately pathogenic to faba bean.

Biological control of chocolate spot disease.

Forbes* and *Bretag, (1991) reported that, a streptomycin was applied for control of bacterial blight of peas. The crop losses by the diseased were estimated in field trials by using streptomycin sprays, which decrease the disease by 62%.

Jackson et al., (1991) have been studied the potential of both bacterial and fungal isolates, isolated from soil and leaves to using

significant control leaves against downy mildew of lettuce (*Bremia lactucae*), powdery mildew (*Uncinula necator*) and *Botrytis cinerea* of grape, late blight (*phytophthora infestans*) and several other host pathogen combinations. The resulting product is long shelf life, efficacious and easy to use.

Abou-Zeid* and *Hassanein, (2000) reported that, some isolates of *Bacillus* spp. isolated from phylloplane had an antagonistic effect against *B.fabae* on PDA medium. *In vivo*, data indicated that, the isolates No. 1,2,3, and 4 were more effective than the other isolates, till the end of experiment, while the isolates No. 5,6,and 7 were less effective after 7 days from incubation. This indicates the inhibitory effect is not persistent with the same efficacy or otherwise the pathogen growth overcomes the activity of the antagonist under the experimental conditions. Also, it may indicate that the operating inhibitory agent is not always the same as that operating on leaf surface. Other factors such as the inoculum potential of the antagonistic bacteria, the nature of interaction of antagonist with others and/or the variability within the tested isolates of the pathogen can also play a role in such situation.

Identification of streptomycetes and their anti-microbial activities.

Goodfellow & Board, (1980)* and *Goodfellow & Cross, (1984a&b) reported that, the, application of new and reliable biochemical, chemical, genetical numerical and molecular biological techniques is the resason of the revolutionary change in bacterial systematic in the last 20 years and the rapidly changing view on having

The actinomycetes as antibiotic producing microorganisms.

Actinomycetes are a successful and widely distributed group of bacteria which have number of properties that favor them in competition with other saprophytic microorganisms. Most of the known antibiotics were isolated from various species belonging to actinomycetes.

What's antibiotic?

Antibiotic was first used by Waksman in 1942 to describe a chemical substance produced by a microorganism and inhibit other microorganisms Waksman in 1947 have been define antibiotic as a chemical substance produced by microorganism which has the ability to inhibit the growth and even to destroy bacteria and other microorganisms ***(Betina, 1983).*** Also, the problem of bacterial resistance to antibiotics had evolved, and new compounds or derivatives of known antibiotics had to be found to replace existing ones.

The isolation of (6-APA) 6-amino-penicillin acid in the late of 1950s opened the filed to the semi-synthetic penicillin and provided an example for development of semi-synthetic antibiotics in general, ***(Rolinson, 1979* and *Vandame, 1980).***

At the end of 1950s, arrival of antibiotic discovery occurred owing to the application of novel screening programs, supersensitive test organisms, new antibiotics sources and the broading of the search for novel microbial products to include agents with pesticidial, anti tumor, insticidial, herbicidal, anticoccidal, cytotaxic, anthelminthic hormonal immunoregulatory, food preserving, growth promoting and enzyme inhibiting activities, as well as products with pharmacological activity ***(Lwai & Omura, 1982; Miller et al., 1983* and *Demain, 1983).***

Most of the antibiotics were isolated from species that belong to genus *Streptomyces*. The best known antibiotics produced by the genus *Streptomyces* include **aminoglycosidies** (streptomycins, olendomycins, spirmaycines, *etc.*) **polyenemarcolides** (nystatin, Amphatracin, B.filipin, etc.) **Tetracyclines, anthracyclines** (chloramphenicol) **heterocylic** (polymixin), **alicyclic** (cyclohexi-mide), polypeptides (viamycin and actinomycins).

Micromonospora is anther important genus show the ability to produce several antibiotics as **gentamycins** and other antibacteria, the most recent description of antibiotics from *Micromonospora* was reported by ***Wagman* and *Weinstein, (1980).***

The antibiotics functions in the nature.

Antibiotic function in the metabolism of the producing organisms has been the subject of considerable speculation and discussion. However, we still in need to understand more about the role they actually do. Some ideas were discarded which involved antibiotics as evolutionary relics, waste products of cellular metabolism, reserve food materials, spore coat components, or break down produces derived from cellular macromolecules. Other classical hypothesis are,

(1) Secondary metabolism serves to maintain the enzymatic machinery of the cell in working order until conditions favorable for growth are found.

(2) Antibiotics are detoxification products one possibility still being considered that, antibiotic function is to kill or inhibit the growth of other organisms in nature by providing a competitive advantage to the producing species ***(Demain 1980 a).***

Makato et al., (1995 I&II) they isolated three new macrolide antibiotics from *S.violaceusninger* which isolated from a soil sample. These antibiotics had anti-microbial activities against fungi, and Gram positive bacteria. They described the taxonomy, production, isolation ,biological activities, physicochemical properties and their structure.

Sugata et al., 1995 reported that, the *Streptomyces* spp. produce phencomycin which was active against Gram positive bacteria..

Ogawa et al., (1995) during their screening of endo-thelin antagonists they have isolated a novel cyclic peptide RES-701-1 from the fermentation broth of *Streptomyces* sp. RE-701. They found that many strains of *Streptomyces* produce RES-701-1 related compounds and isolated three novel compounds from the culture broth. In their work they, describe taxonomy of the producing strains, fermentation, isolation and biological properties of RES-701, 1, 2, 3 and 4.

Strain HILY-9120362 isolated from soil, produced a new members of a Zalomycin class, 2-demethylazalomycin F4a 1 and 2-demethyl-azalomycin F5a (2). Both the compounds 1 and 2 exhibited *in vitro* and *in vivo* activity against a wide range of fungal strains, but they didn't exhibited any antibacterial activity, ***(Mukhopadhay et al., 1995).***

Baker et al., (1996) they discovered the potent anthelmintic activity exhibited by the milbemycins and reported the isolation and structure elucidation of the milbemycins, also they have been describe the fermentation and isolation.

Burkhardt **and** ***Hans-Peter,(1996)*** demonstrated the taxonomical characterization, fermentation as well as the isolation and purification procedures studies of *Streptomyces griseoviridis* (FH-S 1832). ***Hanafi et***

Although microorganisms perform most of their growth processes before they produce antibiotics they might be killed by their own antibiotic during production, we known however that, industrial fermentions are usually conducted for many days after the onset of antibiotic production. Thus the synthesizing organism develops resistance during production ***(Demain, 1974).***

The resistance mechanisms developed by antibiotic producing microorganisms against their own antibiotic are not different from those in clinically resistant bacteria. Permeability modifications are involved in many cases, antibiotics are pumped out of the cells against it's concentration gradient, permeability, during the idiophase, protects the organisms from high extracellular concentration of its own antibiotic. Additional mechanisms exist to protect the cells from internal antibiotic that is not excreted, or from antibiotic that escapes from an antibiotic production compartment ***(Krassilnikov, 1960).***

One of such mechanisms is the synthesis of enzyme that modify the antibiotic and many antibiotic producers possess enzyme capable of converting their antibiotic into inactive or less active derivatives. Another mechanism involves a modification in the machinery of the producer, such as, in ribosome which serve as targets of the particular antibiotic. Another means by which antibiotic producers protect them selves is by feed back inhibition of repression of antibiotic production ***(Vining, 1979)***

Factors affecting on antibiotic Biosynthesis.

Effect of different incubation periods.

The determination of incubation period on the production of antibiotic is quite important, there's a relation between productivity of antibiotic and the time of incubation. Time required for high yield

production of antibiotic by microorganisms varied greatly from one to another. For example corminomicin biosynthesis depends on the culture growth time, the maximum antibiotic production was obtained on the 7th to 8th day. With an increased incubation time a relative content of long chained components of phytobacteriomycin increased in *Actinomyces lavendulae* culture (***Samoilov et al., 1967).***

Juslen et al., (1982) reported that, the maximum yield of tetracycline, isolated from a strain of *Streptomyces* spp. was obtained after 4 days incubation at 27°C. ***Evans et al, (1983)*** mentioned that, a good result was obtained with incubation time from two to four days for the production of a new clavamantibiotic, produced by *Streptomyces clavuligerous.*

Ahmad, (1990) found that the maximum AZ- B_{316} biosynthesis and mycelial formation were obtained after 5 days. Also ***Baker et al., (1996)*** isolated novel milbemycin from *Streplomyces* sp. after incubation time of 48 hours. ***Nabuo et al., (1996)*** reported that , a new ansomycins designated hydroxymoctirenins A and B were isolated from *Bacillus* spp. fermentation broth after 2 days incubation.

Effect of different hydrogen ion concentrations (pHvalues).

Ketoki **and** ***Majumdar, (1973)*** found that, the pH value of medium might be an important factor for kanamycin formation by *Streptomyces kamamyceticus,* which give high production at alkaline pH.

Takiguchi et al., (1981) reported that, milbemycin biosynthesis produced by *Streptomyces hydrosopius* was detected at pH 7.2.

The initial pH value of the growth medium is important for antibiotics production by certain actinomycetes. The optimum pH values for antibiotics biosynthesis varied, an acidic, neutral and alkaline environments were reported to have a great effect on both growth and metabolic activities, some bacteria *e.g* lactic acid *Streptococci* grow and produce Nisin at pH 5.5-6.0 ***(Egorov,1985).*** He added that most antagonistic *Streptomyces* grew well at pH values 6.7 to 7.8 but not at pH below 4.5-4.0 However acidophilus streptomycetes have been isolated at pH 3.5 to 6.5. ***Razak et al., (1994)*** found that ,pH 5.0 was the best initial medium pH for the protease production.

Effect of different incubation temperature.

This can be explained by substantial effect of temperature on the activity of the enzymes, the activity of the transport systems, and other important physiological and biochemical function of the microbial cell. When a penicillin producing strain was grown at 30°C and then shifted to 20°C for the antibiotic production, a highly effective process was obtained ***(Owen & Johnson, 1955).*** Streptomycetes produced antibiotics when cultivated at temperature ranges from 26 °C to 30°C, although some streptomycetes grow at lower temperature from (0 to 18°C) and also elevated temperature (55 °C to 60 °C)(***Johnson, 1955).***

Streptomyces spp. No.81 strain, produce the antibiotic M-81 at 27°C and forms of Gryomycin at low cultivating temperature of 12°C ***(Yashida et al., 1974). Sidler and Zuber ,(1980)*** reported that, the optimum cultivation temperature for *B.stearothermophilus* strains NCI B – 8924 and NRRL-3880 was at 55°C and 37°C for strain A TCC-7954.

The growth temperature has an important effect on both growth and metabolic activities of microorganisms. The optimum temperature

differs for different groups of microorganisms. Most bacteria develop at incubation temperature ranges from 30 to 37°C. The optimum temperature for culture producing gramicidin (*Bacillus brevis*) is 40°C and the some microorganisms can also develop normally and synthesize the antibiotic at 28°C, but the maximum amounts of gramicidin was produced at incubation temperature 40°C ***(Egorov,1985).***

Razak et al., (1994) reported that, optimum protease production by *Bacillus stearothermophilus* was occurred at 60°C. New ansomycins designated hydroxymcotrienins A and B were isolated from culture broth of *Bacillus* sp. BMJ958 –624 at 27°C. ***(Nobuo et al., 1996).***

Extraction and purification of the anti-microbial product.

El-Gamal, (1985) reported that, *S.violochromagens* produce deep yellow antibiotic in starch nitrate media which was moved as one single clear zone eluted and precipitated by petroleum ether.

Ahmad, (1990) studied the extraction and purification of the anti-microbial product obtained from *S.nogalater Az-B*$_{316}$. The product showed only yellow fluorescence band at Rf 0.8. ***Hussein et al., (1998)*** found that, n-butanol succeeded to extract the antibiotic from the broth. The extract resulted in a yellow substance with high Rf value with butanol pyridine water.

Yassin, (1998) reported that, *Streptomyces violaceus* produce a yellow cryslalline antibiotic which was extracted from broth using n.butanol at pH 2.0 ***Yuji et al., (1998)***. mentioned that, the crude powder obtained from strain ku-4. 486 was chromatographed on silica gel column (ϕ 29 x 100 mm, Wako gel C. 200) resulted in three products I, II and III with Rf 0.71, 0.45 and 0.12 respectively.