ACTINOMYCETES- A NEW POTENTIAL BIOCONTROL AGENT FOR BLACK PEPPER PATHOGENS

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Actinomycetes are prokaryotic organisms. They are transitional forms between bacteria and fungi and called as the "higher bacteria," or filamentous bacteria. They are also known as Ray fungi. Like bacteria , they have prokaryotic nuclei and are *Gram-positive* having a thicker layer of peptidoglycan in their cell wall. Like fungi they form filaments called hyphae (Subst4 rate mycelium) and also form aerial mycelium that produces spores similar to fungi. But they are not true fungi because they do not have chitin or cellulose in their cell wall but containing muramic acid. Under a light microscope, the actinomyces appear fungus-like, thin and joined together to form branching networks.

They are the premier group of secondary metabolite producers and are the major sources of antibiotics. The most beautiful activity of soil Actinomycetes is that it gives the "earth smell" due to the emission of a volatile compound called **"geosmin" the earth odour** that arose in the air when rain falls after a dry spell of weather. The biological properties of Actinomycetes include antibacterial, antifungal, antiprotozoal, antihelmintic, antiviral, insecticidal, antioxidant, cytotoxic and anti-inflammatory. The beneficial activities of the Actinomycetes also include their biocontrol potential against phytopathogens. Many actinomycetes produce enzymes like cellulases, xylanases, amylases, lipases, collagenases, proteases, chitinases, lignases, and etc.The first antibiotic like substance isolated from Actinomycetes was actually an enzyme called Actinozyme. Among the genera of Actinomycetes, species of *Streptomyces* are the major producers of antibiotics and out of 10,000 known antibiotics, more than 50 % are produced by Streptomyces.

Categorization

Actinomycetes belonging to the order Actinomycetales of the subclass class-Actinobacteridae of Phylum Actinobacteriae. According to Burgey's Manuel, the order Actinomycetales comprises of four families namely *Mycobacteriaceae , Actinomycetaceae, Streptomycetaceae* and *Actinoplanaceae*. But in the current systematic classification, the order actinomycetales comprises 13 suborders *viz. Actinomycineae, micrococciniae, Catenulisporiniae, corynebacterinae, micromonosporineae, Propionibacterineae, Actinopolysporineae, Pseudonocardinae, Streptomycineae, streptosporanginae, Frankineae, Kineosporiineae* and *Glycomycineae*

Habitation

Actinomycetes are inhabitants of soil, fresh water, marine, organic matter and also habituated as endophytes in plants, humans and animals. Some actinomycetes form symbiotic nitrogen fixing association with plants. Around 200 species of plants are found to have symbiotic association with Actinomycetes mainly for nitrogen fixation. A number of Actinomycetes also

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form close association with plants and act as plant growth promoters and biocontrol agents. They also form lichen-like associations with green algae called actinolichens. Most actinomycetes live as saprophytes on soil organic matter. There are also reports that some actinomycetes are pathogenic in plants (causing diseases like potato scab, gall, and wilt) and also in animals. Members of the genus Actinomyces are normal residents of the mouth, throat, and intestinal tract, but they are capable of causing infections both in humans and in cattle. Based on the habitat Actinomycetes are categorized into Aquatic, endophytic, rhizospheric etc.

Biological Potential

Streptomyces sp. is the major component of the total actinomycetes population and they are the major producers of antibiotics. Out of 10,000 known antibiotics, 45-55% is produced by *Streptomyces* sps. Their biological activities include antibacterial, antifungal, antiprotozoal, antihelmintic, antiviral, insecticidal, antioxidant, cytotoxic and anti-inflammatory (Prashith *et al.*, 2010). The beneficial activities of the Actinomycetes also include their biocontrol potential against pathogens of crop plants.

Among the actinomycetes, the *Streptomyces* family, provide many of the important antibiotics of common in medicine while the Frankia family, form a symbiotic relationship with many non leguminous plants as nitrogen fixing bacteria. Polyene antimycotics are a class of antimicrobial Polyene compounds obtained from some species of *Streptomyces*. Amphotericin B, nystatin, and natamycin are examples of Polyene antimycotics.So also some actinomycetes are decomposers by digesting hard tissues of cellulose and lignin in bark, paper and stems and the chitin or hard exoskeletons of insects. They decompose most of the organic matter on the Earth. It is reported that, if actinomycetes do not exist in the earth the whole food chain will be disrupted. The organic matter especially cellulose and chitinous materials would drastically increase, and there will not be any composting which in turn leads a drastic reduction in humus on the earth's surface and due to the lack of humus there would be a 'domino effect' in the entire food web. Without producers, herbivores would die and without herbivores, there would be no omnivores or carnivores. Plants would also be malnourished. Hence Actinomycetes are such an unavoidable component of the soil. It is estimated that one kilogram of soil contains over 10 billion actinomycetes. Besides, actimycetes are factories of essential life saving medicines like antibiotics curing diseases like typhoid fever and tuberculosis as described elsewhere, but also one path to a future of cancer treatment .Recently Anti-cancer agents and some other bioactive compounds have also been detected in actinomycetes.

The metabolites produced by various Actinomycetes includes a microcline antibiotic Brasilinolide A, produced by *Nocardia brasilliensis* active against *Aspergillus niger,* Polyene antibiotic produced by *Streptomyces* species active against Botrytis cinerea, Oligomycin A from *Streptomyces libani* active against pathogenic fungi, Bifilamycin B1 and C1 produced by *S. halsteadii* K122 etc. Similarly isochainin from an actinomycetes strain Ap1 showed inhibitory effect on F. *oxysporum* f. sp *albedenis* and *Verticillium dahliae*). In Korea Streptomyces strains A1022 was found as better biocontrol agent against anthracnose of pepper (*Capsicum*

annuum) caused by Colletotrichum gloeosporioides.

A number of institutes and companies are currently devoted to the commercialization of bioactive compounds from actinomycetes. The fields of application for these bioactive compounds of the actinomycetes range from human and veterinary antibiotics through to bio remedial processes. Recently with the advent o antibiotic resistance to presently available antibiotics, there is resurgence in the search fo new antibiotics in Actinomycetes. A list is giver below showing the different antibiotics produced by actinomycetes (Table 1).

Table 1. List of antibiotics produced from certain Streptomyces species

Antibiotics produced	Actinomycetes	Antibiotics produced	Actinomycetes
Actithiazic acid/Mycobacidin	*S.virgineae*	Neamine	*S.fradiae*
Amicetin	*S.fasciculatus*	Nystatin/Fungicidin	*S. noursei*
Antimycoin	*S.aureus*	Neomycin	*S.sp*
Ascosin	*S. canescus*	Neonocardin	*N.kuroishi*
Borellidin	*S. rochei*	Neomycin	*S. fradiae*
Cacaomycin	*S.cacaoi*	Netropsin	*S. netropsis*
Camphomycin	*Streptomyces.* Sp.	Nigericin,	*S.*sp
Candicidin	*S. greseus*	Nitrosporin	*S.fasciculus*
Carbomycin	*S. halstedii*	Nocardamicin	
Cardicin	*Nocardia* sp.	Nocardamin	*N. sp--flavescens*
Catenulin	*S.*sp	Nocardianin	*N.* sp
Cefoxitin	*S. lactamdurans*	Nocardin	*N. coeliaca*
Chloramphenicol/Chlormycetin	*S. venezuelae*	Proactinomycins	*Nocrdia gardneri*
Chlortetracyline/Duomycin/ aureomycin	*S.aureofaciens*	*Oxyteracycline/* Terramycin	*S.rimosus*
Chromin	*S.antibioticus*	Rifamycin	*S. lediterranei*
Cinnamycin	*S.cinnamoneus*		
Coelicolorin	*S.coelocolor*	Picromycin	*S.felleus*

Antibiotics produced	Actinomycetes	Antibiotics produced	Actinomycetes
Cycloheximide/Actidione	*S. griseus*	Pleocidins	*S.lavendulae*
Daptomycin	*S. roseosporus*	Roseomycin	*S.roseoch-romogenus*
Dextromycin	*S.*sp	Rotaventin	*S. reticuli*
Dihydrostreptomycin-derivate of streptomycin	*S.sp*	Rimocidin	
Ehrlichin	*S.lavendulae*	Rhodocidin	*S.phoenix*
Endomycin	*S.sp--albus*	Rhodomycetin	*S. griseus (red mutant)*
Erythromycin/Erythricin/Ilotycin	*S.erythreus*	Puromycin/ Achromycin	*S. alboniger*
Exfoliatin	*S.foliatus*	Rhodomycin	*S.sp*
Fosfomycin	*S. fradiae*	Phaeofacin	*S.phaseofaciens*
Flavomycin/Neomycin	*S.roseoflavus*	Phagolessin	*S.sp*
Fradicin	*S.fradiae*	Phalamycin	*S.noursei*
Framycetin/Soframycine	*S.sp*	Puromycin	*S. llboniger*
Grisein	*S.griseus*	Resistomycin	*S.resistomycificus*
Griseoflavin	*S.griseoflavus*	Sarcidin	*S. achromogenes*
Griseolutin	*S.griseoluteus*	Sarkomycin	*S.sp. --erythro-chromogens*
Helixin /endomycin	*S.sp*	Streptin	*S.sp.--lavendulae*
Hydroxystreptomycin/Reticulin	*S. griseocarneus S.reticuli*	Streptocin	*S. griseus*
Lavendulin	*S. lavendulae*	Streptolin	*S.sp.*
Leucomycin	*S.kitasatoensis*	Streptomycin	*S.sp. S.griseus S.bikiniensis*
Lincomycin	*S. Incolnensis*	Streptothricin	*S. lavendulae*

Antibiotics produced	Actinomycetes	Antibiotics produced	Actinomycetes
Litmocidin	*N.cyanea*	Sulfactin	*S. roseus*
Luteomycin	*S. tanashiensis*	Tetracycline	*S. rimosus*
Manosidostreptomycin/ Streptomycin B	*S. griseus*	Thermomycin	*S. thermophilus*
Microcin	M.sp	Thioaurin	S.sp.
Micromonosporin	*Micromonospora* sp.	Thiolutin	*S.albus/ S.celluloflavus*
Miramycin	*S.mirabilis*	Trichomycin	*S. hachijoesnsis*
Moldin	*S.phaeochromogenus*	Vancomycin	S. orientalis
Musarin	*S.*sp.	Vinactin/ Viomycin	S. vinaceus
Mycelin	*S. roseoflavus*	Viomycin/viocin/ vinactin	*S. floridae, S.juniceus S. vinaceus*
Mycetin	*S.violaceous*	Vinacetin	*Streptomyces* sp.
Mycomycin	*N. acidophilus*	Xanthomycins	*Streptomyces* sp

Actinogen Limited is a Western Australasian company dedicated to the discovery and isolation actinomycetes. The company has isolated two streptomycetes isolates that produce bioactive compounds which show significant antibiotic activity against "Superbug" Methicillin Resistance Staphylococcus Aureus (MRSA) known as Golden Staph and Vancomycin Resistant Enterococci (VRE) bacteria. They also discovered a series of actinomycetes that can produce Shikimic acid which is a main component in the production of Tamiflu , the oral antiviral treatment . The production of Tamiflu is complex and one of the most expensive components is Shikimic acid. The Company is also having low cost production technology for producing cellulase(s) from actinomycetes in an aerobic environment at room temperature. Cellulase(s) are an important step in the production of second generation bio-ethanol. Actinogen also designed to discover new bioactive anti cancer molecules produced by actinomycetes from soil in Western Australasia. They used to screen liquid cultures of the actinomycetes for the presence of bioactive molecules that are either cytotoxic or cytostatic for a series of cell lines derived from various human and animal cancers. There are few commercial formulations available in the market like Mycostop and spinosard. Mycostop is a commercial biofungicide formulation developed from dried spores and mycelium of

Streptomyces griseoviridis Strain K61for the control of seed rot, root and stem rot and wilt caused by *Fusarium, Alternaria* and *Phomopsis* of container grown ornamentals, vegetables and tree and forest seedlings whereas **Spinosad** is a commercial organic insecticide prepared from a soil actinomycete called *Saccharopolyspora spinosa*. Similarly actinomycetes are reported to have Nematicidal activity also. Fervenulin isolated from a nematicidal actinomycetes, *Streptomyces roseoverticillatus* produced secondary metabolites that inhibited egg hatch and increased juvenile mortality of *Meloidogyne incognita in vitro*. There are several attempts to exploit the antifungal activity of Actinomycetes against fungal pathogens such as *Fusarium, Verticillium ,Botrytis* and even *Phytophthora*.

At Indian Institute of Spices Research, experiments were conducted to exploit the potential of Actinomycetes against phytopathogens of black pepper and ginger. Actinomycetes were isolated from the rhizosphere of black pepper and ginger from different tracts of Kerala, Karnataka and Tamil Nadu and screened against pathogens like different species of *Phytophthora* such as *P. capsici, P. palmivora, P. nicotianae, Fusarium oxysporum, Colletotrichum gloeosporioides, Sclerotium rolfsii Pythium myriotylum and Ralstonia solanacearum.* Certain isolates showed cent percent inhibition of the pathogens invitro . Among the isolates certain isolates showed inhibition to all the pathogens tested. The potential isolates were shortlisted and conducted inplanta studies. The isolates also showed positive effect inplanta. The potential isolates were characterized morphologically and molecularly and identified as strains of *Streptomyces* sp. and *Kitasatospora* sp, besides they were also found as growth promoters in black pepper. Growth parameters of the plants were highly promising in consortia containing two different Streptomyces strains viz. *S.tauricus* and another *Streptomyces* sp. They showed significantly superior growth characters like increased number of total roots, leaves, nodes, root length, plant height, fresh weight and dry wt. The dehydrogenase activity was also found to be higher in the consortia. The study conducted at IISR is a prelude to the biological control of black pepper pathogens using the potential of Streptomyces species.

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