

## A Strain of *Streptomyces nigrifaciens* Wakman antagonistic to *Colletotrichum capsici*

By S. Gupta and K. C. Basu Chaudhary\*)

Botany Department, Agra College, Agra, India

Actinomycetes comprise a large and important group of microorganisms capable of inhibiting growth of other organisms. Voluminous literature has accumulated to demonstrate production of antibiotics by species of Actinomycetes. This paper deals with the isolation of a new strain of *Streptomyces nigrifaciens* Waksman which is found to produce antibiotic substance active against *Colletotrichum capsici* (Syd.) Butler & Bisby, its biological activity and certain physical properties of the active substance in culture filtrate.

### Morphology and cultural characteristics:

The actinomycetes were isolated from soil samples collected from Black Wattle Forest at Yercud (Salem South Forest Division) in Tamil Nadu on Thornton's medium (Allen, 1951) using dilution plate technique (Waksman, 1927) and incubated at 28–30° C for 10 days.

The Actinomycete (Isolate No. A 22) forms both submerged and aerial mycelium on agar media. Vegetative mycelium is thin, hyaline and branched irregularly. Sporophores are sympodially sometimes monopodially branched with terminal hooks, loops and primitive open spirals. Spores are smooth walled, oval and short cylindrical measuring 1.4–1.6  $\mu$  by 1.8–2  $\mu$ . According to Pridham, Hesseltine and Benedict (1958) it can be placed under section „Retinaculum apertum -Grey series“.

The isolate was grown on different media that are commonly used in the study of the members of the genus *Streptomyces*. The type of growth, pigment production and other characteristics were noted. Observations were recorded after incubation period for ten days at 28° C. The colour terminology refers to those of Ridgway (1912).

1. Čapek agar: Growth fair, thin spreading. Aerial mycelium Dusty velvety, Palegrey. Submerged mycelium Pale Olive- Buff. Reserve-Pale. Buff. No soluble pigment.

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\*) Present address: Department of Mycology and Plant Pathology Faculty of Agriculture, B. H. U., Varanasi-5.

2. Oatmeal agar: Growth good, spreading. Aerial mycelium abundant, smoke grey, narrow, white margin. Submerged mycelium — Grey or yellow. Soluble pigment brown. Extrusion of many minute colourless droplets.
3. Starch agar: Growth good, raised. Aerial mycelium very abundant, dusty, pale mouse grey, narrow white margin. Submerged mycelium not visible from above. Reverse dark olive brown with yellowish margin, Formation of halo around colony surrounded by pale pinkish brown ring. Hydrolysis moderate.
4. Cellulose agar: Growth good, not or only slightly raised in the centre. Aerial mycelium thick, dusty, powdery, mouse grey. Submerged mycelium not visible from above. Reverse olivaceous brown.
5. Calcium malate agar: Growth good, spreading. Aerial mycelium very abundant, velvety, pale olive grey. Submerged mycelium not visible from above. Reverse dark brown. Soluble pigment dark brown.
6. Glucose-asparagine agar: Growth good, spreading. Aerial mycelium very abundant, light mouse grey. Submerged mycelium not visible. Reverse brownish yellow. Soluble pigment brown.
7. Glycerin-asparagine agar: Growth good, spreading. Aerial mycelium dusty velvety, drab grey. Submerged mycelium not visible. Reverse brown. Soluble pigment brown.
8. Glucose-peptone agar: Growth good, spreading, wrinkled at bottom. Aerial mycelium velvety, dirty white, submerged mycelium not visible. Reverse pale yellow. Soluble pigment brownish black.
9. Nutrient agar: Growth good, not raised, thin, Aerial mycelium abundantly very thin, velvety, at bottom end little thicker and dirty white. Submerged mycelium pale olive buff. Reverse brown. Soluble pigment brown.
10. Tyrosine agar: Growth good, slightly raised. Aerial mycelium white to yellowish white, velvety. Submerged mycelium not visible. Reverse black. Soluble pigment very dark brown almost black when seen from above. Extrusion of brownish yellow droplets.
11. Potato plug: Growth good, raised, wrinkled. Aerial mycelium abundant, dusty velvety, pale mouse grey with pinkish tinge in margin. Soluble pigment brown. Plugs brownish black.
12. Milk: Growth good, thick, convoluted, surface pellicle. Aerial mycelium scanty, thin, velvety, white. Submerged mycelium drab grey becoming dark almost brown to almost black at upper end of ring. Coagulation with subsequent peptonization change of pH to alkaline.
13. Gelatine broth: Growth good at surface. Aerial mycelium abundant, velvety, white at some places. Submerged mycelium not visible. Reverse yellowish brown. Soluble pigment brown. Extrusion of numerous brown droplets which stain the surrounding aerial mycelium pale brownish. Lignification slow.
14. Glucose-nitrate: Growth good. Aerial mycelium white velvety. Submerged mycelium not visible. Reverse straw yellow. Soluble pigment brown. Nitrate reaction positive.
15. Iron-peptone agar: Black pigment in agar.
16. Tryptone agar: Brown pigment in agar.

On the basis of its morphology and cultural characteristics, the isolate A 22 resemble very much *Streptomyces nigrifaciens* Waksman. But some peculiarities of this isolate are: the much slower growth, absence of aerial mycelium and soluble pigment in calcium malate agar and incomplete hydrolysis of starch. Slower growth is often asso-

ciated with the absence of aerial mycelium and the appearance of aerial mycelium in its turn is usually correlated with a better production of soluble pigment. Hence, the Isolate A 22 has been identified to be a strain of *Streptomyces nigrifaciens* Waksman. The identification has been confirmed by reference to the Centraalbureau voor Schimmelcultures, Baarn, the Netherlands.

#### Biological Activity

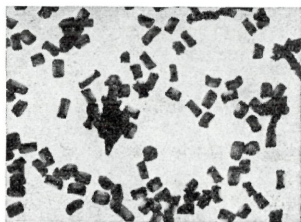
This strain of *S. nigrifaciens* was found to exhibit antagonistic property against *Colletotrichum capsici* (Syd.) Butler & Bisby. It was also active against some fungi and bacteria viz., *Thielavia terricola* (Gilman & Abbott) Emmons, *Chaetomium globosum* Kunze, *Phoma* sp., *Diplodia cajani* Raychaudhuri, *Aspergillus nidulans* (Eid.) Wint., *A. sydowi* (Bain. & Sart.) Thom & Church, *Penicillium oxalicum* Thom., *P. egyptiacum* van Beyma, *P. simplicissimum* (Oud.) Thom., *Curvularia verruculosa* Tandon & Bilgrami, *Alternaria brassicae* (Berk.) Sacc., *A. tenuis* Nees ex Pers., *Cladosporium* sp., *Helminthosporium sativum* Pammel, King & Bakke, *Sclerotium rolfsii* Sacc., *Macrophoma phaseoli* (Maulbl.) Ashby, *Escherichia coli* and *Shigella Flexery*.

#### Some Physical Properties

The active substance produced by this strain of *S. nigrifaciens* loses its activity when diluted with water or subjected to higher temperature. The observation reveals that the filtrate can be diluted 20 times but still, retaining the capacity to inhibit completely the germination of *C. capsici* spores. But on further dilution the inhibitory effect is impaired until in a dilution of 1:55 the activity is almost lost.

Thermal inactivation point was found to be related to the period to which the active substance was exposed to a particular temperature. With the rise in temperature from 50° C to 100° C the assay value decreased irrespective of the duration. Similarly there was a fall in the activity with increase in duration irrespective of temperature. The filtrate loses much of its activity at 100° C when kept for 20 minutes.

The active substance appears to be quite stable over a long period



of storage. At 50° C it lasts for 35 days and for 26 days at pH 7.5. Acidic pH range does not favour the stability of the active substance. At pH 4.5 & 5.5, the activity does not last even for 24 hours when stored at 28° C and for 2—3 days at 18° C. The most optimum condition for storage of the active substance was found to be at 50° C at pH 7.5.

### Summary

A strain of *Streptomyces nigrifaciens* Waksman has been isolated from soil which exhibits antagonistic property towards *Colletotrichum capsici* and a few other fungi and bacteria. The morphological and cultural characteristics of the organism and certain physical properties of the active substance has been described.

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Autor(en)/Author(s): Gupta Km. S., Chaudhary K. C. Basu

Artikel/Article: [A Strain of Streptomaxes nigrificiens Wakman antagonistic to Collectotrichum capsici. 130-133](#)