

BIOCIDAL ACTIVITIES OF SOME STREPTOMYCES SPP.
ON RICE LEAF SPOT DISEASE

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ABSTRACT

Fourty seven strains of actinomycetes were isolated from infected rice fields. Only twenty four strains were capable of producing substance(s) antagonistic to rice leaf spot disease caused by Helminthosporium oryzae Breda de Hean. The antagonistic organisms were identified as strains of the following species: Streptomyces ramulosus, S. catenulae, S. clavifer and S. griseus. Their culture filtrates could inhibit the disease when sprayed on the diseased leaves, or when added to the soil contains the spores of the pathogenic fungus.

INTRODUCTION

Rice plant is subjected to various pests which affect its growth and productivity. Among these pests is Helminthosporium oryzae Breda de Hean which causes the leaf spot disease of rice. This disease is wide-spread in Egypt (El-Helaly et al, 1966; Abdel-Mohty, 1969; Ibrahim, 1971; Ashour et al., 1973; El-Kazzaz, 1973; Sirry et al, 1974; El-Bigawi, 1976; Aidy, 1977; Othman, 1978; and Agwa, 1985).

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as well as other rice-growing countries (Nisikada and Miyake, 1922; Padwic, 1950; Iman and Schroeder, 1966; Heaten, 1969 and Ou, 1972). It caused the highest percentage of pre- and post-emergence damping off to seedling and gave the lowest yield as a result of artificial inoculation of rice plants in flowering stage (Bedi and Hill, 1960; Singh et al, 1971; Ashour et al, 1973; Awoderu, 1974; El- Bigawi , 1976 and Othman, 1978).

The aim of this investigation is to isolate soil actinomycetes which can antagonize H. oryzae and study of the biological control of the disease in infected plants with them.

MATERIALS AND METHODS

Rice plant leaves suffering from spot disease as well as the rhizospheric soil of the plants infected by the same disease were collected from rice fields of Kafr El-Sheikh Governorate.

The following media were used: Czapek-Dox, Potato dextrose agar, rice polish and Corn meal (Ezzat, 1982). The inorganic salt solution was prepared according to Kuster (1959) and Shirling and Gottlieb (1966). The trace salt solution was prepared after Pridham et al (1958)

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The pathogenicity of the isolated fungus was tested by inoculating 3 - 4 leaf stage plants with its spore suspension (60 spores / microscopic field [x 60]), Disease symptoms were observed at constant time intervals.

The antagonistic activities of the isolated actinomycetes were carried out using the cut plug technique and paper disc assay method (Pridham et al, 1956).

The identification of the isolated actinomycetes was carried out using morphological studies, cultural and physiological properties as described in Bergey's manual of determinative bacteriology (1974).

The biological control of the disease by the isolated actinomycetes filtrates was tested by the following techniques:

- (a) Spraying the diseased leaves, and
- (b) Addition to the grains containing the fungal spores following by cultivating these grains and observing the symptoms.

Addition of the antagonistic organism to the soil containing the fungal spores was carried out, cultivating the rice grains and then observing the symptoms.

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RESULTS

Isolation of the pathogen

The isolated fungus grow rapidly on potato dextrose agar medium and sporulated excellently (Table 1). Czapek-Dox agar, rice polish and corn meal media gave lower sporulation ratios.

Isolation of the actinomycetes

From different localities at Kafr El-Sheikh Governorate, it was possible to isolate 47 different actinomycetal organisms, divided into 14 groups depending on the reverse colour (Table 2).

The antagonistic activities of the isolated actinomycetes

The antagonistic activities of the isolated actinomycetes were carried out using both the plug technique and paper disc assay method. The results showed that upon using the plug technique, only 24 organisms were able to antagonize the pathogen. However, upon using paper disc assay method, 33 organisms were found to be antagonistic (Table 3). Organisms numbered 17, 21, 33 and 41 conducted the highest antagonistic values in both methods; thus these organisms subjected to further investigations aiming at characterizing and identifying them.

Identification of the most active isolated actinomycetes

(a) Morphology of spore chain and sporophores

The direct microscopic observation of the four isolates

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on either starch nitrate agar, glycerol asparagine or malt extract-yeast extract agar medium revealed that all of them produce long spore chain and the organism are probably belong to the family Streptomycetaceae (Fig. 1).

(b) Spore morphology and surface

Electron micrographs of the spores (Fig. 1) showed that all of them produced rod to ovoid spores. The surface of the spores are plain and smooth without any undulations or outgrowths. The spore chains are straight or flexuous.

(c) Cultural characteristics

Table 4 shows the cultural characteristics of the isolates. Thus, the isolated species were all belong to the genus Streptomyces. The isolates numbered 17,21,33 and 41 were identified as: S. ramulosus , S. catenulae, S. clavifer and S. griseus.

The biological control of the disease

The results of this test are found in Tables 5 and 6. It was evident that S. griseus produced the more powerful antibiotic properties against the disease. The three other actinomycetes showed more or less the same degree of disease inhibition as indicated by the methods applied.

Discussion

Many of the isolated actinomycetes were capable for producing antagohistic substance(s) at various levels.

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The four isolates tested in this work were screened out from 47 actinomycetal organisms isolated from the rhizosphere of rice plants cultivated in Kafr El-Sheikh Governorate (Egypt). The selected four organisms exhibited the most antagonistic characteristics. Streptomyces ramulosus, S. catenulae, S. clavifer and S. griseus were the most active isolates since they produced the largest inhibition zones both in solid or in liquid culture. It was found by Dixit and Gupta (1980) that the cultural filtrates of S. olivaceous were antagonistic against leaf blotch disease of barley.

As a recommendation for the control of the leaf spot disease of rice, one of the following may be followed:

- (a) Soaking the rice grains in the filtrates of the Streptomyces spp.
- (b) Adding the filtrate of the isolated Streptomyces spp. to the soil.
- (c) Spraying the infected leaves with the filtrates of the Streptomyces spp.

The most effective procedure was the spraying of the infected leaves, followed by soaking the grains in the filtrate containing the antagonistic substance(s). The produced antagonistic substance(s) may be absorbed by the leaves (Reed, 1956).

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Table 1. Spore formation by Helminthosporium oryzae when cultivated on different culture media.

Medium	Sporulation
Czapek-Dox's agar	+
Potato dextrose agar	+++
Rice polsish	++
Corn meal	++
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+	weak sporulation
++	moderate sporulation
+++	heavy sporulation

Table 2. The reverse colour groups of the isolated actinomycetes.

Colour	Number of isolates
Pink	1 - 3
Yellow brown	4 , 5
Pale yellow	6 - 12
Yellow green	13 - 16
Grey	17 - 20
White to grey white	21
Green	22 , 23
Grey blue	24 - 26
Green blue	27 - 29
White	30 - 32
White to yellow white	33 , 34
Pale red	35
Yellow cream	36 - 42
Grey to violet	43 - 47

Table 3. The potentiality of the isolated actinomycetes to antagonize H. oryzae using plug technique and paper disc assay method. (\pm standard error)

Isolate number	Inhibition zone (mm)	
	Plug technique	Paper disc assay
7	5.0 \pm 0.4	5.0 \pm 0.5
8	4.5 \pm 0.4	6.0 \pm 0.7
13	4.0 \pm 0.4	5.0 \pm 0.6
16	4.0 \pm 0.4	5.0 \pm 0.4
17	10.0 \pm 0.8	12.0 \pm 1.1
21	13.0 \pm 1.0	21.0 \pm 1.8
33	12.0 \pm 1.0	14.0 \pm 1.4
34	5.0 \pm 0.3	3.5 \pm 0.2
41	13.0 \pm 0.8	16.0 \pm 0.9
43	6.0 \pm 0.5	7.5 \pm 0.6
45	5.0 \pm 0.3	6.0 \pm 0.4

Table 4. Cultural characteristics of the different actinomycetes isolates.

Test	Isolate 17	Isolate 21	Isolate 33	Isolate 41
Spore surface morphology	smooth	smooth	smooth	smooth
Colour of aerial mycelium	grey	faint grey	white	creamy yellow
Reverse side colour	creamy	creamy	creamy	creamy
Diffusible pigment	-ve	-ve	-ve,	-ve
Melanin pigment	-ve	-ve	-ve	-ve
Carbon source utilization				
Glucose	+	+	+	+
Xylose	+	-	+	+
Arabinose	-	-	-	-
Rhamnose	-	-	+	+
D-Fructose	+	+	+	+
Raffinose	+	-	-	-
D-Mannitol	+	+	+	+
i-Inositol	-	-	-	-
Sucrose	-	-	-	-
Pectin degradation	+ve	+ve	-ve	-ve
Growth at 45°C	-ve	-ve	-ve	-ve

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Table 5. The percentage of recovered diseased rice plants sprayed with filtrates of S. ramulosus, S. catenulae, S. clavifer and S. griseus.

Control	14
<u>S. ramulosus</u>	60
<u>S. catenulae</u>	65
<u>S. clavifer</u>	70
<u>S. griseus</u>	83

Table 6. The percentage of diseased rice plants when sprayed the diseased grains or the soil contained the pathogen with filtrates of S. ramulosus, S. catenulae, S. clavifer and S. griseus.

	Grain spray	Soil spray
Control	50	32
<u>S. ramulosus</u>	10	12
<u>S. catenulae</u>	9	13
<u>S. clavifer</u>	8	12
<u>S. griseus</u>	3	8

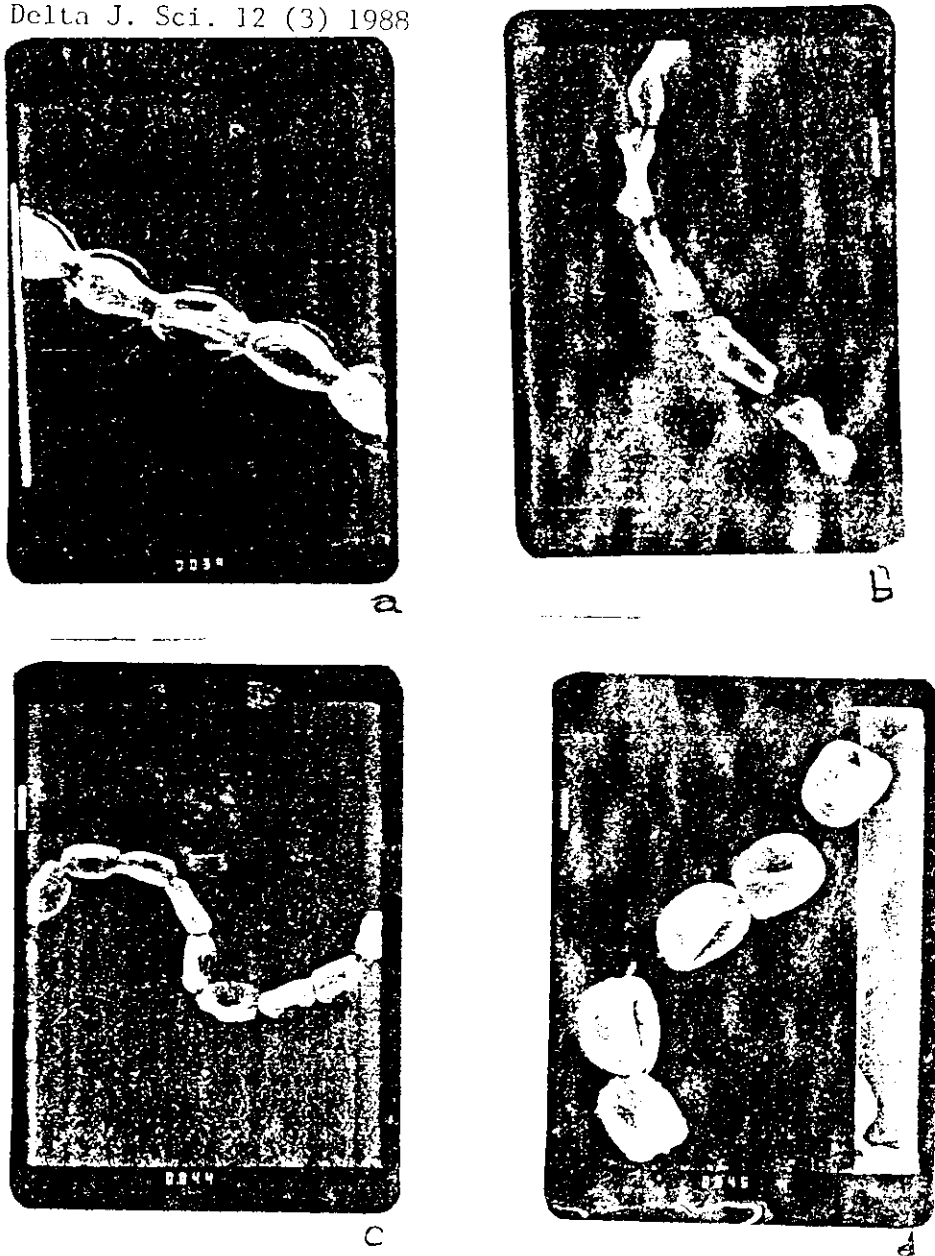


Figure 1. Electron micrograph of 14-day-old isolates number 17, 21, 33 and 41 (a, b, c and d) cultivated on starch mineral salt medium at 30°C . a x 4,000. b x 8,000 c and d x 5,000 .

نشاطات بعض أنواع استربتومايسس المضادة
لمرض تبقع أوراق الارز

مصطفى أحمد السيد ، محمد السيد عثمان ، يوسف عابد حسن محمد
حمى الرفاعى عجوة

قسم النبات ، كلية العلوم ، جامعة طنطا ، طنطا ، مصر

من بين ٤٧ نوعا من الاكتينومييسيتات التى تم عزلها من حقول الارز
المصابة وجد أن ٢٤ نوعا لهم المقدرة على تكوين مواد مضادة لفطرة هلمنثيو-
سيوريم أوريلى ، وتم تعريف أربعة أنواع من جنس استربتومايسس هم: استربتومايسس
رامبولوزس ، استربتومايسس كاتنولس ، استربتومايسس كلايفير ، استربتومايسس جريشيوس*
وكان راشح مزارعهم له المقدرة على ازالة المرض اذا نشر فوق الاوراق المصابة أو اذا
اضيف الى التربة المحتوية على أبواغ الفطر المرض*.