



## Preliminary characterization of some *Streptomyces* species from four Tanzanian soils and their antimicrobial potential against selected plant and animal pathogenic bacteria

M.J.M. Ndonde and E. Semu\*

Department of Soil Science, Sokoine University of Agriculture, P.O. Box 3008, Chuo Kikuu, Morogoro, Tanzania

\*Author for correspondence: Tel.: +255 23 2 603999, Fax: +255 23 2 603259, E-mail: semu@suanet.ac.tz

Received 27 November 1998; accepted 2 May 2000

**Keywords:** Animal pathogens, antibiotics, antimicrobial activity, characterization, plant pathogens, *Streptomyces*

### Summary

This study was undertaken to characterize *Streptomyces* strains occurring in some soils of Tanzania as well as to evaluate their potential to synthesize antimicrobial compounds. Six main classes of isolates were observed according to the colour of aerial mycelium. These were gray, cream, blue, pink, red, and white. The gray colour class dominated. About 65% of the isolates produced soluble pigments of various colours while about 33% of the isolates did not produce any soluble pigments. Brown coloured soluble pigments dominated. About 57% of the isolates had spiral spore chains. Some *Streptomyces* isolates displayed strong (> 30 mm inhibition zone), moderate (20–30 mm), or weak (< 20 mm) antibiosis against the plant/animal pathogenic bacteria tested. Other isolates did not show any antibiosis against any of the test pathogens. The plant pathogens CMM IPO 542 (*Clavibacter michiganensis* ssp. *michiganensis*) and *Xanthomonas vasicatoria* were inhibited by most of the *Streptomyces* isolates. *Xanthomonas oryzae* pv. *oryzae* and *X. campestris* were inhibited by the least number of the *Streptomyces* isolates. Most of the animal pathogens tested seemed to show resistance to the antibiotics produced by some of the *Streptomyces* isolates which had shown high activity against the plant pathogens.

### Introduction

The ability of *Streptomyces* spp. to produce metabolites capable of inhibiting growth and development of pathogenic microorganisms has continued to manifest itself from the time members of this microbial group were first observed to produce these important compounds. Recent reports show that this group of microorganisms still remains an important source of antibiotics (Iwai & Omura 1982). Nearly 50% of all *Streptomyces* isolated have proved to be antibiotic producers (Brock *et al.* 1994). Alexander (1977) estimated that 75% of *Streptomyces* isolates produced antibiotics of one type or another. Thus, *Streptomyces* are prolific antibiotic producers (Korn-Wendisch & Kutzner 1992).

In some studies *Streptomyces* spp. have been observed to produce antibiotics active against plant pathogens like the *Pythium* root rot of sugarcane and corn (Johnson 1954) and *Fusarium vasiafectum*, the causal agent of cotton wilt (Arjunarao 1971). Other plant pathogens which were sensitive to antibiotics produced by *Streptomyces* were *Sclerotium rolfsii* and *Fusarium oxysporum*, pathogens of *Lens culinaris* (Mehrotra & Claudius 1972).

Yuan & Crawford (1995) observed that when spores or mycelia of a strain of *Streptomyces lydicus* were used

to coat pea seeds, the seeds were protected from invasion by *Pythium ultimum* in an oospore-enriched soil, implying possibilities for biological control. Dicklow *et al.* (1993) found that infection with a strain of *Streptomyces* resulted in a significant reduction of tomato root galling due to the nematode *Meloidogyne incognita*, thereby contributing to significant increases in yields as compared to yields in untreated controls. However, there are few reports of antibiotic production by *Streptomyces* in their natural soil habitat (Rothrock & Gottlieb 1984; Thomashow *et al.* 1990; Thomashow & Weller 1990).

Erwealor and Njoku-Obi (1990) in Nigeria isolated a strain of *Streptomyces* which had ability of producing an antibiotic(s) which inhibited a number of microorganisms, including *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Yersinia enterocolitica*, *Candida albicans*, and *Aspergillus fumigatus*.

Presently, there is little documented information on occurrence in Tanzania of *Streptomyces* spp. of potential to produce antimicrobial compounds. Yet, *Streptomyces* harboured by such unexplored environments may be looked upon as being wild types showing natural variability. This, in turn, may be a rich source of a diversity of useful metabolites. Lechevalier (1992) reiterated the seemingly endless

diversity of nature, and predicted that actinomycetes would continue to be a source of secondary metabolites with properties of interest in medicine and agriculture. Therefore, the study reported herein was undertaken to study the *Streptomyces* flora of some Tanzanian soils. The objectives were to isolate some *Streptomyces* occurring in some ecological locations of Tanzania, to undertake a preliminary characterization of them, and to evaluate them for ability to elaborate antimicrobial compounds.

## Materials and Methods

### Soils

The soil samples used in these studies were obtained from the Mkuyuni area of Morogoro region, the Kibaha area (Coast region), the Kitulo area (Iringa), and the Chimala area (Mbeya). These sites represented climatic conditions which were hot and sub-humid (Mkuyuni and Chimala), hot and humid (Kibaha), or cool (Kitulo). The pH of the soils ranged from 5.6 to 7.0. Organic carbon varied between 0.23 and 9.43%. Total N was between 0.08 and 0.73%. Clay content ranged from 5% (Kitulo) to 37% (Mkuyuni), making the textures of these soils to vary between sandy (Kibaha and Kitulo) and clay loam (Mkuyuni). Details of these properties are given elsewhere (Ndonde 1998).

### Isolation of *Streptomyces*

Tenfold serial dilutions of soil samples were made using sterile water. The soil suspensions were plated using starch-casein agar (Küster & Williams 1964), and the plates incubated at 25–28 °C for 14 days. These plates were used for isolation of some representative colonies of *Streptomyces*. The colonies were aseptically transferred to plates containing oatmeal agar (Küster & Williams 1964) or inorganic salts-starch agar (Küster 1959; Shirling & Gottlieb 1966). They were incubated for 14 days after which colours associated with the colonies, and morphology of spore chains, were determined.

### Determination of colour of the *Streptomyces* isolates

Colour of aerial mycelium was determined from mature, sporulating aerial mycelia of the *Streptomyces* colonies from both media. Colour was determined using the colour names lists compiled by Pridham (1964). Using these compilations, a colour name was chosen which most closely agreed with the colour of aerial mycelium of a particular colony/isolate. In this way, colour names were assigned to the various isolates.

Colour of the soluble pigments was determined visually by observing the colour changes in the medium due to the diffusing pigments produced by the *Streptomyces* isolates (Shirling & Gottlieb 1966).

### Determination of morphology of the isolates' spore chains

The morphology of the isolates' spore chains was determined by light microscopy. A small peripheral portion of a well grown mature part of a colony was picked using a sterile isolating loop, without disturbing the aerial mycelium of the part so picked. This was then transferred to a microscope slide and observed under the Nikon 98455 light microscope at the magnification of 600×. The observed morphology was assigned to one of the classes described by Pridham *et al.* (1958), Shirling & Gottlieb (1966), or Brock *et al.* (1994). Five fields were examined to confirm a morphological class.

### Testing of *Streptomyces* isolates for ability to inhibit growth of plant pathogenic and other bacteria

Using an inoculating loop, an inoculum of each *Streptomyces* isolate was transferred from an oatmeal agar plate to a nutrient agar plate. This was done by streaking a straight line of the *Streptomyces* inoculum across the surface of nutrient agar medium in the plate. The plate was incubated at 25–28 °C for 3 days when a visible colony was obtained. An inoculum of a test microorganism was then streaked at right angles to the *Streptomyces* straight-line colony as described by Prescott & Dunn (1959), Alexander (1977) or Brock *et al.* (1994).

The test organisms were the plant pathogenic bacteria *Acidovorax avenae*, *Clavibacter michiganensis* ssp. *michiganensis* (isolate CMM IPO 542), *Xanthomonas oryzae* pv. *oryzae*, *X. vasicatoria*, *X. campestris* and *X. phaseolicoli* var. *fuscoris*. Other test bacteria used were the animal/human pathogens *Streptococci agalactiae*, *E. coli*, *Salmonella gallinarum*, *Klebsiella pneumoniae* and *S. aureus*.

Inocula of these test bacteria were obtained from liquid cultures of the bacteria grown at 25 °C in nutrient broth (shaken at 100 rev min<sup>-1</sup>) for 3 days. These were the ones streaked at right angles to the *Streptomyces* (straight line) colonies as already described. The plates were incubated at 25–28 °C, observed for antibiosis on the third and sixth day, with final evaluation made on the ninth day. The extent of inhibition of growth of the test pathogens was recorded by measuring the length of the inhibition zone away from the *Streptomyces* colony.

## Results and Discussion

### Preliminary characterization of *Streptomyces* isolates according to colour of aerial mycelium and presence of soluble pigments

Table 1 shows the range of colours of the aerial mycelia and their frequency of occurrence in the *Streptomyces* isolated from the experimental soils. Six main classes of colour were observed, often with colour intergrades seen within a class. The main colours were gray, cream, blue,

Table 1. Colour classes of aerial mycelium and their frequency of occurrence in the *Streptomyces* isolates.

Colour of aerial mycelium	Number of isolates	% of all isolates
Cream	2	2.98
Blue	9	13.43
Gray	39	58.20
Pink	4	5.97
Red	2	2.98
White	11	16.41
Total	67	99.97

Table 2. Colours of soluble pigments produced by the *Streptomyces* isolates and their frequency of occurrence.

Colour of soluble pigment	Number of isolates	% of all isolates
Not clear	1	1.75
None	19	33.33
Brown	31	54.38
Violet	1	1.75
Yellow	5	8.77
Total	57	99.98

pink, red, and white. The gray colour class dominated. Table 2 shows the colours of the soluble pigments produced by the cultures and which diffused into the agar medium. Occurrence of these colours has been documented previously (Krasil'nikov 1958; Shirling & Gottlieb 1966).

From Table 2, about 65% of the isolates produced soluble pigments of various colours while about 33% of the isolates did not produce any soluble pigments. Brown coloured pigments were dominant in these isolates.

The variations in the colours of the aerial mycelia of the isolates as well as in those of the pigments they produced may be an indication contributing to diversity or variability of the isolated *Streptomyces*.

#### Characterization of the *Streptomyces* isolates by morphology of their spore chains

The different morphological types of spore chains of the *Streptomyces* isolates are presented in Table 3. Most (57%) of the isolates had spiral spore chains. However, the different morphological types generally occurred in all locations. This may imply that according to the

Table 3. Frequency of occurrence of the different morphological types in the *Streptomyces* isolates.

Morphological type	Number of isolates	% of all isolates
Flexuous/Flexibilis	7	11.11
Rectus	8	12.69
Retinaculum–Apertum	10	15.87
Spira	36	57.14
Monovercillus	2	3.17
Total	63	99.98

present study morphological characteristics did not have ecological limitations, but were generally adapted to the different (possibly all) ecological or environmental conditions of the study areas.

#### Evaluation of *Streptomyces* isolates for antimicrobial activity against bacterial plant pathogens

The ability of *Streptomyces* isolates to inhibit growth of some plant pathogenic bacteria is shown in Table 4. Some isolates showed strong (> 30 mm inhibition zone), some showed moderate (20–30 mm), while others showed weak (< 20 mm) antibiosis against some of the test organisms. Other isolates did not show any antibiosis activity against any of the plant pathogens. These results were observed as early as 3 days after streaking of the test organisms. The plant pathogens CMM IPO 542 (*Clavibacter michiganensis* ssp. *michiganensis*) and *X. vasicatoria* were inhibited (to various extents) by most of the *Streptomyces* isolates. On the other hand, *X. oryzae* pv. *oryzae* and *X. campestris* seemed to be inhibited by only a few of the *Streptomyces* isolates.

The sensitivity of CMM IPO 542 and *X. vasicatoria* to most of the *Streptomyces* isolates may suggest several things. First, it may suggest that the antimicrobial metabolites they produced were similar, or related, even if most of the *Streptomyces* isolates producing them were not related. Brock *et al.* (1994) have reported on ability of different species of *Streptomyces*, even from different regions of the world, to produce the same antibiotic.

Whether or not the *Streptomyces* isolates of the present studies were genetically related can only be speculated from the present observations. For example, five isolates shared some characteristics. All had the retinaculum–apertum spore chains. This may, perhaps, imply some resemblance. However, those isolates also differed, for example, in the colour of their soluble pigments. These are only a few observations. But if more detailed studies were undertaken it might be found that some isolates might, indeed, be related or similar. In that event, it is possible that the antimicrobial compounds they produce may sometimes be similar. Lechevalier

Table 4. Extent of antibiosis activity of *Streptomyces* isolates against plant pathogens.

Number of plant pathogens inhibited	Extent of inhibition (mm)	Number of <i>Streptomyces</i> isolates showing inhibition	% of all isolates
None	–	3	4.22
1	10–55	7	9.86
2	5–45	12	16.90
3	3–55	19	26.76
4	3–50	17	23.94
5	5–50	4	5.63
6	6–60	9	12.67
Total	–	71	99.98

*et al.* (1971) stated that similar actinomycetes tended to produce similar antibiotics.

The second explanation to the observed sensitivity of the test organisms may be that these pathogens were simply sensitive to different antimicrobial substances, and that the antibiotics as well as the *Streptomyces* isolates producing those antibiotics may not be necessarily similar. For example, another group of five isolates, which showed a great tendency to inhibit some plant pathogens, had different morphologies of their spore chains. They generally differed in the colour of their aerial mycelia, and in that of their soluble pigments. These differences may imply that these *Streptomyces* isolates may be genetically distinct from each other. These observations may be reasonable to some extent, although colour is not a basic criterion in the systematics of *Streptomyces* (Lechevalier *et al.* 1971). Thus, while some isolates of the present studies may have had some similarities as already suggested, it is also probable that others may have been distinctly different.

Thirdly, this sensitivity of the test pathogens to the antibiotics produced by the *Streptomyces* may also imply that the test plant pathogens may have not been exposed to similar antibiotics previously. As a result, they were still susceptible to such compounds. This may be reasonable because antibacterial antibiotics have, generally, not been used much in plant disease control at the field level. This sensitivity implies that a scope may exist for using such antibiotics in a small scale, for example under limited acreage to control bacterial diseases so as to obtain disease-free seed/grain material. The observed greater resistance of *X. oryzae* pv. *oryzae* as well as of other pathogens to these *Streptomyces* is difficult to explain at present in terms of any possible previous exposure. These results may indicate a need to initiate a programme to screen the most promising *Streptomyces* isolates via-a-vis the more sensitive plant pathogens, as well as to characterize the promising isolates in greater detail.

As seen in Table 4, 4% of the isolates did not show any antibiosis towards the test organisms. But it is probable that they produced antibiotics which might be effective against other plant pathogens not tested presently. It is also possible, on the other hand, that those isolates were not antibiotic producers at all. However, Porter (1971) stated that probably all *Streptomyces* possessed some antimicrobial properties if proper conditions were taken into consideration during culturing of these organisms for purposes of evaluating antibiotic production.

Overall, about 96% of all isolates were observed to have the ability to produce growth inhibition of one or other of the plant pathogens tested. This is in agreement with the conclusion (Alexander, 1977) that over 75% of all soil *Streptomyces* were capable of producing antibiotics. This may be an indication of the potential of the environments sampled in the present study to harbour antibiotic-producing *Streptomyces*.

#### Evaluation of *Streptomyces* isolates for antimicrobial activity against animal pathogenic bacteria

Table 5 shows an evaluation of the ability of the *Streptomyces* isolates to inhibit growth of bacteria potentially pathogenic to livestock and/or humans. A pattern somewhat similar to that seen in the case of the plant pathogens was also observed for the animal pathogens. However, more of the animal pathogens tested seemed to show resistance to antibiosis activity of some of the *Streptomyces* isolates which had shown good antibiosis against some plant pathogens.

Therefore, most of the *Streptomyces* isolates which inhibited growth of plant pathogens showed weak antibiosis against animal pathogens. This greater extent of resistance of the animal pathogens may be due to previous exposure to antibiotics routinely used in disease control which may have been similar to those produced by the present *Streptomyces* isolates. This exposure may have led these animal pathogens to develop resistance to those antibiotics.

Only eight of the *Streptomyces* isolates inhibited (to various extents) most or all of the animal pathogens. Those eight isolates were effective against both the Gram-negative and the Gram-positive bacteria tested. The remaining isolates inhibited the growth of either the Gram-positive test pathogens or the Gram-negative ones.

From Table 5, 52% of all isolates were observed to display ability to produce antibiotics which, to various extents, inhibited one or other of the animal pathogens tested.

The fact that some of the *Streptomyces* isolates inhibited growth of both Gram-positive and Gram-negative bacteria tested may imply that the antibiotics they produced were of broad spectrum. Further evaluation of these isolates may ascertain the range or spectrum of bacteria whose growth their antibiotics may inhibit.

In conclusion, the isolates of *Streptomyces* obtained from these soils, some of them seemingly different, and the antimicrobial properties they seem to possess, may provide evidence that these soils harbour species or strains which may produce useful secondary metabolites.

Table 5. Extent of antibiosis activity of *Streptomyces* isolates against animal pathogens.

Number of animal pathogens inhibited	Extent of inhibition (mm)	Number of <i>Streptomyces</i> isolates showing inhibition	% of all isolates
None	—	30	47.61
1	2–25	12	19.04
2	5–18	6	9.52
3	6–25	7	11.11
4	3–37	4	6.34
5	3–36	4	6.34
Total	—	63	99.96

## Acknowledgements

We gratefully acknowledge the financial assistance provided by the SUA-NORAD Tan 091 project to the senior author. We also thank Mr J.S. Matabaro of the Tanzania Official Seed Certification Agency, Morogoro, for providing us with the test plant pathogens, and Prof. S.F.H. Jiwa and Mr M.M. Shoo of the Faculty of Veterinary Medicine, Sokoine University of Agriculture (SUA), for the test animal pathogens. This work is a part of the first author's MSc dissertation submitted to SUA, Morogoro, Tanzania.

## References

- Alexander, M. 1977 *Introduction to Soil Microbiology*, 2nd edn. New York: John Wiley and Sons. ISBN 0-85226-013-X.
- Arjunarao, V. 1971 Biological control of cotton wilt III. *In vivo* effect of antagonists on the pathogen *Fusarium vasiafectum* Atk. *Proceedings of the Indian Academy of Science* **B74**, 54–62.
- Brock, T.D., Madigan, M.T., Martiko, J.M. & Parker, J. 1994 *Biology of Micro-organisms*, 7th edn. Englewood Cliffs: Prentice-Hall International Inc. ISBN 0-13-176660-0.
- Dicklow, M.B., Acosta, N. & Zuckerman, B.M. 1993 A novel *Streptomyces* species for controlling plant-parasitic nematodes. *Journal of Chemical Ecology* **19**, 159–173.
- Erwealor, I.A. & Njoku-obi, A.N.U. 1990 Antimicrobial activity of a *Streptomyces* specie isolated from Nukka soil. *World Journal of Microbiology and Biotechnology* **6**, 337–339.
- Iwai, Y. & Omura, S. 1982 Culture conditions for screening of new antibiotics. *Journal of Antibiotics* **35**, 123–141.
- Johnson, F.L. 1954 Antibiotics in relation to *Pythium* root rot of sugarcane and corn. *Phytopathology* **44**, 69–73.
- Korn-Wendisch, F. & Kutzner, H.J. 1992 The family *Streptomycetaceae*. In: *The Prokaryotes*, Eds. Balows, A., Trüper, H.G., Dworkin, M., Harder, W., & Schliefer, K.H., pp. 921–994. New York: Springer-Verlag. ISBN 3-540-97258-7.
- Krasil'nikov, N.A. 1958 *Soil Micro-organisms and Higher Plants*. Moscow: Academy of Sciences of the USSR. PST Cat. No. 206.
- Küster, E. 1959 Outline for a comparative study of criteria used in the classification of Actinomycetes. *International Bulletin for Bacterial Nomenclature and Taxonomy* **11**, 91–98.
- Küster, E. & Williams, S.T. 1964 Selection of media for isolation of *Streptomyces*. *Nature (London)* **202**, 928–929.
- Lechevalier, H. 1992 Actinomycetes and their products: a look at the future. *World Journal of Microbiology and Biotechnology* **8** (Suppl. 1), 72–73.
- Lechevalier, H.A., Lechevalier, M.P. & Gerber, N.N. 1971 Chemical composition as a criterion in the classification of actinomycetes. *Advances in Applied Microbiology* **14**, 47–72.
- Mehrotra, R.S. & Claudius, G.R. 1972 Biological control of root rot and wilt diseases of *Lens culinaris*, Medic. *Plant and Soil* **36**, 657–664.
- Ndonde, M.J.M. 1998 Isolation, preliminary characterization, and antibiosis potential of *Streptomyces* from soils of different ecological zones in Tanzania. MSc dissertation. Morogoro, Tanzania: Sokoine University of Agriculture.
- Porter, J.N. 1971 Prevalence and distribution of antibiotic-producing actinomycetes. *Advances in Applied Microbiology* **14**, 73–92.
- Prescott, S.C. & Dunn, C.G. 1959 *Industrial Microbiology*, 3rd edn. New York: McGraw-Hill, pp. 762–835. Library of Congress Catal. Card No. 58-10002.
- Pridham, T.G., Hesseltine, C.W. & Benedict, R.G. 1958 A guide for classification of *Streptomyces* according to selected groups: placement of strains in morphological sections. *Applied Microbiology* **6**, 52–79.
- Pridham, T.G. 1964 Colour and *Streptomyces*: report of an international workshop on determination of colour of *Streptomyces*. *Applied Microbiology* **13**, 43–61.
- Rothrock, C.S. & Gottlieb, D. 1984 Role of antibiosis in antagonism of *Streptomyces hygroscopicus* var *geldanus* to *Rhizoctonia solani* in soil. *Canadian Journal of Microbiology* **30**, 1440–1447.
- Shirling, E.B. & Gottlieb, D. 1966 Methods for the characterization of *Streptomyces* species. *International Journal of Systematic Bacteriology* **16**, 313–340.
- Thomashow, L.S. & Weller, D.M. 1990 Role of antibiotics and siderophores in biocontrol of take-all diseases of wheat. *Plant and Soil* **129**, 93–99.
- Thomashow, L.S., Weller, D.M., Bonsall, R.F. & Piersoson, L.S. 1990 Production of antibiotic phenazine-1-carboxylic acid by fluorescent *Pseudomonas* species in the rhizosphere of wheat. *Applied and Environmental Microbiology* **56**, 908–912.
- Yuan, W.M. & Crawford, D.L. 1995 Characterization of *Streptomyces lydicus* WYEC 108 as a potential biocontrol agent against fungal root and seed rot. *Applied and Environmental Microbiology* **61**, 3119–3128.