Tanzania Journal of Natural and Applied Sciences (TaJONAS)
Nov.-Dec. 2011: Volume 2, Issue 2, 424- 432

ISSN 1821-7249

Growth of *Streptomyces* isolates from four soils in Morogoro, Tanzania, under culture-media pH conditions other than their original environmental pH

Ernest Semu* and Doris Akishule

Department of Soil Science, Sokoine University of Agriculture,

P.O. Box 3008, Morogoro, Tanzania

***ABSTRACT**: A study was conducted to assess the ability of Streptomyces isolates to grow under pH conditions other than their original environmental pH. The study involved isolation of Streptomyces from soils in four different locations in Magadu area, Morogoro, Tanzania. The Streptomyces were isolated using the starch-casein agar medium and subsequently grown under different simulated pH conditions, both buffered and unbuffered, in the oat-meal medium. The results of the isolation revealed that the soils had a diversity of Streptomyces isolates, growth of each of which was favoured by particular pH conditions. For the soils with an original pH of 5.0 the growth of two cultures increased and one seemed to show a peak growth when the pH was decreased to 4.5. Others did not show much change in growth. The soils which had a pH of 6.3 had three cultures whose growth was improved when pH was increased beyond pH 6.3. Others were almost unchanged. Almost all cultures from soils of pH 7.0 had their growth decreased when pH of the growth medium was increased pH 7.0. The Streptomyces isolates from soils with an original pH of 8.1 showed little change in growth as pH was altered. The colors of the mature colonies that formed when the Streptomyces were grown at various pH levels were cream, white, blue, gray, brown and red, with the cream color being the dominant one. When results of growth of Streptomyces on an unbuffered medium were compared to that on buffered medium, there was generally poor growth of Streptomyces with the latter medium. It was concluded that these Streptomyces which would grow at the buffered pH levels were those that were adapted to those pH conditions. However the unbuffered medium could allow the development of micro-sites with different pH and wider range of pH values conducive to growth of a wider variety of strains.*

Key words: *Streptomyces*, unbuffered medium, buffered medium, simulated soil pH

INTRODUCTION

Bacteria of the genus *Streptomyces* are Gram-positive microorganisms that grow as branched filaments. *Streptomyces* occur in great numbers in the top few inches of soil and decrease with depth. In addition to their ecological roles of decomposing organic matter in the soil [1,2,3] and of improving soil structure through binding of soil particles by their hyphal threads, *Streptomyces* are a very important group of bacteria that have long been known to produce antimicrobial substances [4]. These compounds have revolutionized the treatment of bacterial and fungal diseases, and have had substantial impact in the medical and veterinary fields. Potential is also envisaged in the agricultural field, as Dicklow *et al.* [5], for example, found that a strain of *Streptomyces* resulted in a significant reduction of tomato root galling due to the nematode *Meloidogyne incognita*, thus contributing to significant increases in tomato yields as compared to untreated controls. Other plant diseases also found to be susceptible to *Streptomyces* include fungal root rot and seed rot [6], and others caused by *Sclerotium rolfisii* and *Fusarium oxysporum* [7].

* To whom correspondence may be addressed: E-mail: semu@suanet.ac.tz
Tel.: +255 754 565808,

While *Streptomyces* can be found in different habitats [4], most *Streptomyces* are primarily soil organisms [8,9,10]. The *Streptomyces* are the most abundant actinomycetes in soil, and they may form over 60% of colonies when cultured on plates [11].

There are a number of factors which influence the growth and activity of *Streptomyces* in their natural environment. Soil pH is one such factor. Populations of *Streptomyces* are high in soils of neutral pH levels. These bacteria are less tolerant to low pH (high acidity), and do not proliferate much in soils or water below pH 5.0 [10]. While actinomycetes usually prefer neutral or slightly alkaline soils, Williams *et al.* [12] isolated from acid soils some *Streptomyces* spp. that could grow in culture at even lower pH values, in the range of 3.5 - 5.5. It is probable that imposition of experimental conditions of pH which imitate some more favorable natural conditions may help in isolating previously unknown strains [13]. Recovery of such types will depend on good simulation of natural conditions in which the *Streptomyces* thrived.

With respect to production of antibiotics by the genus *Streptomyces*, over 50% of the 3000 antibiotics known are produced by the genus *Streptomyces* [14]. It is estimated that 75% of *Streptomyces* isolates can produce an antibiotic of one type or another [15]. This potential seems to be endless. Erwealor and Njoku-obi [16] in Nigeria also isolated a strain of *Streptomyces* which had the ability of producing antibiotics which inhibited a number of microorganisms including *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Yersinia enterocolitica, Candida albicans, Aspergillus fumigatus,* etc.

To take advantage of this potential, the organisms should be able to survive and grow well, both in their natural environment and under artificial culture. In both cases, the conditions of the growth medium, especially pH conditions, can profoundly affect the growth of these bacteria. It is especially important to be able to provide the most optimal conditions in artificial culture to maximize production of the desired substances. This paper reports on the influence of pH adjustments *in vitro* on tolerance and growth of *Streptomyces* isolated from soil in the Morogoro area of Tanzania.

MATERIALS AND METHODS

Location of soil sampling

The study was conducted using soils obtained from the Magadu area of the Sokoine University of Agriculture farm. The area is in Morogoro region, at the base of the Uluguru Mountains. The altitude of the location is about 500 meters above sea level. The temperature regime of the area is generally hot (25 – 30°C), and the average annual rainfall is 700 - 900 mm. The dominant vegetation consisted of an assortment of grasses which were used for grazing cattle.

Surface (0-10 cm) soil samples were collected from eight different points in attempts to obtain samples of different pH conditions. After sampling the samples were each divided into two portions. One portion was air-dried, ground and sieved through a 2mm sieve for pH determination. The other portion was not dried but was stored in a refrigerator without processing and used for isolation of *Streptomyces*. Only four samples were selected and used on the basis of their pH values.

The soil pH was determined in water (at the soil:water ratio of 1: 2.5). Duplicate ten-gram samples of soil were placed in 100 ml beakers and 25 ml of distilled water were added. The suspensions were stirred for thirty minutes, left to stand for a few minutes and then pH measured using the glass electrode. The soils selected for the study were those of pH 5.0, 6.3, 7.0, and 8.1.

Isolation of *Streptomyces* from soil

Media were prepared that simulated the soils' original pH values, i.e. pH 5.0, 6.3, 7.0 and 8.1. The media used were basically based on the starch-casein agar medium [17], with the following ingredients:

dilutions required for plating. One ml portions from three dilutions, i.e. 10^3, 10^4 and 10^5, were plated in the different media in four replicates and incubated at 25°C to 28°C for 14 days.

sub-cultured at simulated medium-pH values of 4.0, 4.5, 5.0, 5.5 or 6.0 to obtain a profile of their pH tolerance. Similarly, a range of simulated-pH values was tested for other colonies earlier cultured in media at the original soil pH

Starch	10 g
Casein (vitamin-free)	0.3 g
KNO_3	2 g
NaCl	2 g
K_2HPO_4	2 g
$MgCO_3$	0.05 g
Fe $SO_4.7H_20$	0.01 g
Agar	18 g
Distilled water	1000 ml.

The pH of the basic medium was adjusted, to give the different pH values of the culture media, using two approaches. In the first approach, where the pH of the media was not buffered, HCl was added either to lower the pH of the medium or NH_4OH to raise the pH of the medium to simulate the desired pH values. In the second scenario, where the media-pH was buffered, different volumes of 0.1 M K_2HPO_4 and 0.1 M KH_2P0_4 solutions were combined to make buffer solutions with the desired pH levels in which the ingredients (above) were dissolved, instead of using distilled water. The simulated pH values of the media were as shown in Table 1.

The media were sterilized in an autoclave at 15 pounds per square inch and 121°C for 15 minutes. The sterilized media, cooled to 70°C, were fortified prior to plating by aseptically adding penicillin or actidione (cycloheximide) solution to suppress growth of bacteria and fungi, respectively [18]. Petri-dishes and pipettes were sterilized in an oven at 170°C for 2 hours prior to use.

Ten grams of moist soil, oven-dry basis, were weighed and transferred to bottles containing 90 ml of sterile distilled water, and shaken thoroughly by hand to detach microbial cells

Table 1. Table showing the original soil pH (OpH) and simulated medium-pH (MpH) values used to culture *Streptomyces*

Original soil pH (OpH)	Simulated medium-pH (MpH)
5.0	4.0
	4.5
	5.0
	5.5
	6.0
6.3	5.0
	5.5
	6.0
	6.5
	7.0
7.0	6.0
	6.5
	7.0
	7.5
	8.0
8.1	6.5
	7.0
	7.5
	8.0
	8.5

Assessment of growth of *Streptomyces* under different simulated media-pH conditions

Seven colonies from the original soil pH conditions, representing all the color types, were selected and aseptically transferred to petri-dishes containing oat meal agar medium at the simulated pH values tested. Portions of each well-grown colony were transferred to and

conditions (Table 1). Subsequent growth of the colonies was assessed in terms of the diameters of the colonies, and three classes were assigned to estimate the growth: i. colony diameters of 6 - 8 mm (very good growth, and represented as +++), ii. colony diameters of 3-5 mm (good growth, ++), or iii. colony diameters of < 2 mm (poor growth, +). These procedures were adopted for both types of pH adjustment of the growth media: buffered and unbuffered.

Determination of color of and size *Streptomyces* colonies

After a growth period of seven days, each colony was assigned a color [11]. The colony size was measured in terms of the diameter of the colonies, in mm.

RESULTS AND DISCUSSION

Growth of *Streptomyces* in media adjusted to different unbuffered pH values

The growth of *Streptomyces* under the simulated different pH values is shown in Table 2. For the soils whose original pH (OpH) was 5.0, growth of three cultures increased with increasing medium pH (MpH) up to pH 6.0. One culture seemed to peak when medium pH was decreased to 4.5. Other cultures did not show much change in growth as pH was altered. In the case of soils with original pH of 6.3, growth of three cultures increased as MpH was raised beyond 6.0. Growth of the other cultures was almost unchanged.

For the soils with OpH of 7.0, there was an observable decrease in growth for almost all cultures beyond MpH 7.0. In the case of the soils which had OpH of 8.1, there was not much change of growth as MpH was altered

Table 2. Relative growth of *Streptomyces* at different simulated pH levels

Original pH of soil (OpH)	Medium pH (MpH)	Growth after 1 week						
		Colony1	2	3	4	5	6	7
5.0	4.0	+	trace	+	+	+	+	+
	4.5	+	+	++	+	+	++	+
	5.0	+	+	+	+	+	+	+
	5.5	++	++	++	+	+	+	+
	6.0	++	++	++	+	+	+	+
6.3	5.0	+	+	trace	+	+	trace	trace
	5.5	+	+	+	++	+	+	trace
	6.0	++	+	+	++	++	++	trace
	6.5	+++	++	++	+	+	+	+
	7.0	++	+	+	+	+	+	+
7.0	6.0	+	+	++	+	++	++	+++
	6.5	++	++	++	++	++	++	+++
	7.0	+	+++	++	++	+++	++	+++
	7.5	+	+	+	+	trace	+	+
	8.0	++	+	+	+	+++	+	+
8.1	6.5	++	trace	+	++	+	+	+
	7.0	++	++	+	+	+	+	+
	7.5	+	trace	+	+	+	+	+
	8.0	+	++	+	+	+	+	++
	8.5	++	++	+	+	+	+	++

+++- (6 - 8) mm-very good growth;++ - (3-5) mm-Good growth;+ - <2 mm-Poor growth

In soils with OpH of 5.0, the increased growth of *Streptemyces* in the three cultures as the MpH increased to 6.0 could be explained in terms of improvement of the growth conditions as pH was increased. This means that the original soil pH of 5.0 suppressed growth of those *Streptomyces* in the natural acidic conditions. Williams *et al.* [12] observed that few actinomycetes could grow in soils more acid than pH 5.0. Therefore, the MpH ranges 4.0, 4.5 and 5.0 were too low for the growth of those colonies *Streptomyces*, as was the OpH of 5.0, and this led to their poor growth. The fact that some cultures did not show much change in growth as the pH was varied, and other colonies which grew poorly at MpH 5.0 showed better growth at the more acid conditions of MpH 4.5, implies that those isolates were adapted to a wider range of pH conditions, including low pH [12].

The better growth at higher media MpH levels of 5.5 and 6.0 for the soils with OpH of 5.0 is compatible with the observation [12] that these actinomycetes preferred neutral or slightly alkaline soils.

For the soils which had OpH of 6.3, the good growth of colonies as the MpH was adjusted to pH 6.0 or pH 6.5 was probably because the adjusted pH levels and the pH of the original soils were almost similar in that there was less departure of the simulated pH values from the original soil pH conditions. Thus, the growth environment was not radically changed.

The fact that *Streptomyces* from soils with an OpH of 6.3 showed poor growth at MpH of 7.0 is in contrast with other observations [10] that higher populations, probably as a result of better growth and proliferation of the bacteria, were encountered at around neutrality. The rise in pH from the original pH of 6.3 could have interfered with the normal metabolic processes and hence suppressed growth of the cultures. Campbell [19] showed that pH not only affected the organisms directly but also the equilibrium between carbon dioxide, bicarbonate and carbonate ions, and hence the availability of carbon which is necessary to enhance growth.

In the case of *Streptomyces* from soils with OpH 7.0, the decrease in growth of most cultures in MpH beyond 7.0 is because most actionmycetes prefer neutral or slightly alkaline soils [10].

For the soils with OpH 8.1 the unchanged growth of some cultures as pH was altered, and the poor growth of most others, was due to the prevailing alkaline conditions beyond MpH of 8.1. However, the two cultures that grew fairly well at the various simulated pH levels of at MpH 6.5, 7.0 and 8.5 shows that these particular isolate of *Streptomyces* may be adapted to perform well at a wide range of pH values, i.e. from slightly acidic all the way to alkaline conditions.

Growth of *Streptomyces* in media adjusted and buffered at different pH values

The growth of *Streptomyces* from soil OpH of 7.0 and 8.1, under buffered conditions of MpH, is shown in Table 3.

Table 3. Relative growth of *Streptomyces* isolates at different simulated pH values

Original pH of soil (OpH)	Media pH (MpH)	Colony 1	2	3	4
7.0	6.0	+++	+	+	+
	6.5	+++	+	++	++
	7.0	+++	+	++	++ +
	7.5	++	trace	+	tra ce
	8.0	+	trace	+	++ +
8.1	6.5	+	+	+	+
	7.0	+	+	+	+
	7.5	trace	+	trac e	+
	8.0	+	+	+	+
	8.5	++	+	+	+

+++ - (6-8) mm - Very Good growth
++ - (3-5) mm - Good growth
+ - <2mm - Poor growth

More cultures from soil of OpH 7.0 showed decreased growth beyond MpH 7.0. *Streptomyces* isolates from the soil of original

pH of 8.1 grew poorly across all the MpH values. The poor growth could be due to sustained alkaline conditions, which suppressed growth of most of the colonies.

Comparison of growth of *Streptomyces* in unbuffered and buffered media

When the relative growth of *Streptomyces* from the unbuffered media and the buffered media were compared for soils with OpH 7.0, some differences were apparent. For the unbuffered medium, 19 colonies (54%) grew well out of a total of 35 colonies assessed. For the buffered medium, nine (45%) colonies grew well out of 20 colonies assessed. From this observation it can be concluded that the unbuffered medium led to growth of more *Streptomyces* strains as compared to the buffered medium. In the case of soils with original pH 8.1, nine colonies (25%) grew well out of 35 colonies examined in the unbuffered medium. For the buffered medium 100% of the colonies grew poorly, again leading to the conclusion that the unbuffered medium resulted in growth more strains of *Streptomyces* as compared to the buffered medium.

This observation can be explained by the contention that in the unbuffered medium micro-sites with a more favorable environment developed around the growing colonies as a result of metabolic activities of the growing cells. This can occur because being unbuffered, metabolic activities could easily change the pH in the zone immediately surrounding the colonies to result in a more favorable environment in that micro-site as compared to the rest of the environment of the medium farther away from a colony. However, in the medium that was buffered, the buffer would resist any pH changes due to metabolic activities in the micro-sites. Thus, the unbuffered medium would offer favorable micro-site environments conducive for growth of a wide array of *Streptomyces* strains under all the MpH ranges tested because the medium is not buffered. In contrast, the buffered medium, precisely because it is buffered, will not permit development of micro-sites having a different pH other than that at which it is buffered. Therefore, the buffered medium will offer conditions that would be specific only for a narrower range of the *Streptomyces* which are able to grow in that particular buffered pH level. This, then, explains the better growth of more strains in the unbuffered media.

Generally, the recovery of a relatively broad diversity of *Streptomyces* isolates under the unbuffered media may be a reflection of a rich diversity of strains actually potentially present, whose expression of growth is masked by the prevailing original pH. However, altering (without buffering) the pH of the growth medium could enhance or stimulate their growth, thereby recovering the suppressed types. Buffering limits recovery only to those types strictly adapted to the buffered pH, thus narrowing the diversity of the recovered types.

Diversity of *Streptomyces* isolates from the soils

An indication of the diversity of the *Streptomyces* flora of these soils can be provided by examining the colors of the isolates from the different soils and culture media used.

Diversity of colors of Streptomyces isolates in unbuffered media

Table 4 shows the range of colors of colonies of some selected *Streptomyces* isolated from the experimental soils, with unbuffered medium. Five classes of colour were observed, often with color intergrades seen within a class. The classes were cream, brown, blue, gray and white. Similar color classes were observed by other workers [11,20]. The cream type of color class dominated in the present studies.

Table 4. Colors of colonies of *Streptomyces* isolated from soils using the unbuffered media

Original Soil pH (OpH)	Medium pH (MpH)	Colony 1	Colony 2	Colony 3	Colony 4	Colony 5	Colony 6	Colony 7
5.0	4.0	cream	cream	whitish cream	whitish cream	whitish cream	whitish cream	white
	4.5	"	"	"	"	"	"	"
	5.0	"	"	"	"	"	"	"
	5.5	"	"	"	"	"	"	"
	6.0	"	"	n.c.	"	"	"	"
6.3	5.0	cream	cream	light brown	white	cream	whitish cream	n.c.
	5.5	"	"	"	creamy white	"	"	n.c.
	6.0	"	"	"	brownish cream	brownish cream	"	n.c.
	6.5	"	"	"	"	creamy white	"	n.c.
	7.0	"	"	n.c.	creamy white	"	"	n.c.
7.0	6.0	brownish gray	bluish gray	brownish cream	brownish cream	gray	bluish gray	bluish gray
	6.5	"	"	"	"	"	"	"
	7.0	"	gray	"	"	"	"	gray
	7.5	"	gray	whitish cream	"	"	"	"
	8.0	"	gray	"	"	"	"	"
8.1	6.5	bluish gray	cream	bluish gray	gray	bluish gray	bluish gray	cream
	7.0	"	"	"	"	"	"	"
	7.5	gray	"	gray	"	cream	gray	"
	8.0	gray	"	gray	brownish cream	creamy gray	creamy gray	"
	8.5	gray	"	gray	"	gray	gray	"

n.c. = not clear

From Table 4, the variation in the colors of the colonies of isolates indicates that color is a reflection of the diversity or variability of the isolated *Streptomyces* strains. It was observed that as the OpH of the soils increased from 5.0 to 8.1 there was an increase in diversity of *Streptomyces* isolates in terms of the wide array of colors of the colonies. In soils with original pH of 5.0 only two colors were observed while in OpH 6.3, 7.0 and 8.1 more than four colors were observed at each soil pH (Table 4).

3.4.2 Diversity of colors of Streptomyces isolates in the buffered media

Table 5 shows the range of colors of colonies of some selected *Streptomyces* isolated from the

experimental soils, with the buffered media. Here, there was a much narrow spectrum of colors observed under buffered media, again with the cream type of color dominating.

Table 5. Colors of colonies of *Streptomyces* isolated from soils using the buffered media

Original soil pH	Medium pH	Colony 1	2	3	4
7.0	6.0	red	whitish cream	white	brownish gray
	6.5	"	"	"	"
	7.0	"	"	"	"
	7.5	"	"	"	"
	8.0	"	"	"	"
8.1	6.5	cream	cream	cream white	cream white
	7.0	"	"	"	"
	7.5	"	"	"	"
	8.0	"	"	"	"
	8.5	"	"	"	"

For the buffered media that simulated the soils with OpH of 7.0 and 8.1, the narrow range of colors observed may be an indication that the buffered environments of the media supported entirely different types of *Streptomyces,* with a narrower diversity of *Streptomyces*.

CONCLUSIONS

This study has shown that there is a wide diversity of *Streptomyces* strains isolated from the Magadu, Morogoro, soils in relation to soil pH. This was indicated by the different colors of colonies that were derived from the different soils, each soil having a different pH level. At any given pH of the culture medium as adjusted relative to the original soil pH level, the relative growth of some isolates (colonies) was favored while at the same time the growth of some other isolates was suppressed. Thus, the pH greatly affected the relative growth, and recovery, of colonies/strains of these organisms. The seemingly different strains that grew at different pH levels (as indicated by their colors) give an indication that different pH levels supported growth of entirely different strains. The study has shown the ranges of pH of culture media and of soils, within which *Streptomyces* continue to grow well or otherwise. One approach in isolating from soils *Streptomyces* that can yield isolates with potential beneficial applications is manipulating, within some limits, the pH of the isolation/culture medium. This may also have implications in assessing production of metabolites by the organisms so isolated as pH of the growth medium is varied in attempts to maximize production or to discover new types of metabolites produced under different pH environments.

ACKNOWLEDGEMENTS

We wish to gratefully acknowledge the financial assistance provided for this research by the Department of Soil Science, Sokoine University of Agriculture

REFERENCES

1. S.A. Waksman and A. Lomanitz, Contribution to the chemistry of decomposition of proteins and amino acids by various groups of microorganisms, *Journal of Agricultural Research* 30 (1925) 203.
2. D.M. Reynolds, Extracellular chitinase from a *Streptomyces* spp., *Journal of General Microbiology* 11 (1954) 150.
3. S.T. Williams, The role of actinomycetes in biodeterioration, *International Biodeterioration Bulletin* 2 (1966) 125.

4. M. Alexander, *Introduction to Soil Microbiology,* 2nd Ed. Wiley Eastern Ltd, New Delhi, (1983) 467 pp.
5. M.B. Dicklow, N. Acosta, and B.M. Zuckerman, A Novel *Streptomyces* species for controlling plant parasitic nematodes, *Journal of Chemical Ecology* 19 (1993) 159-173.
6. W.M. Yuan and D.L. Crawford, Characterization of *Streptomyces lydicus* WYEL 108 as a potential biocontrol agent against fungal rot and seed rot, *Applied and Environmental Microbiology* 61 (1995) 319-3128.
7. R.S. Mehrotra, and G.R. Claudius, Biological control of root rot and wilt diseases of *Lens culinaris* Medic., *Plant and Soil* 36 (1972) 657 - 664.
8. T.D. Brock, M.T. Madigan, J.M. Martiko and J. Parker, *Biology of Micro-organisms.* 7th Ed. Prentice-Hall International, Inc., Englewood Cliffs, (1994) 909 pp.
9. T.D. Brock and K.M. Brock, *Basic Microbiology with Application,* Prentice - Hall, Englewood Cliffs, (1973) 406 pp.
10. R. Mitchel, *Introduction to Environmental Microbiology,* Prentice-Hall Inc., Englewood Cliffs, (1974) 355 pp.
11. G. Sykes and F.A. Skinner, *Actinomycetales: Characteristics and practical importance,* Academic Press, London, (1973) 339 pp.
12. S.T. Williams, F.L. Davies, C.I. Mayfield and M.R. Khan, Studies on the Ecology of Actinonmycetes in soil II. The pH requirements of *Streptomyces* in acid soils, *Soil Biology and Biochemistry* 3 (1971) 187-195.
13. D.B. Steele and M.D. Stowers, Techniques for selection of industrially important micro-organisms, *Annual Review of Microbiology* 45 (1991) 89-106.
14. B.D. Davis, D. Renato, N.E. Herman and S.G. Harold, *Microbiology including Immunology and Molecular Genetics,* 3rd Ed. Harper and Row, Philadelphia, (1980) 1355 pp.
15. M. Alexander, *Introduction to Soil Microbiology,* 2nd Ed. John Wiley and Sons, New York, (1977) 467 pp.
16. I.A. Erwealor, and A.N.U. Njoku-obi, Antimicrobial activity of a *Streptomyces* species isolated from Nukka soil, *World Journal of Microbiology and Biotechnology* 6 (1990) 337 - 339.
17. E. Kuster, and S.T. Williams, Selection of media for isolation of *Streptomyces, Nature (London)* 202 (1964) 928 – 929.
18. S.T. Williams and F.L. Davies, Use of antibiotics for selective isolation and enumeration of actinomycetes in soils, *Journal of General Microbiology* 38 (1965) 251 - 262.
19. R.E. Campbell, *Microbial Ecology.* Blackwell Scientific Publications, London, (1977) 148 pp.
20. T.G. Pridham, Color and *Streptomyces*: report of an international workshop on determination of color of Streptomyces, *Applied Microbiology* 13 (1964) 43 - 61.