

VIABILITY STUDY OF SOME LOCALLY ISOLATED STREPTOMYCETES

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Summary

Maintaining and preserving actinomycetes cultures are essential elements for systematic and biodiversity investigations. The influence of long-term preservation on the survival of some locally isolated streptomyces cultures was studied. The storage duration varies from 5 to 15 years. 37.5 % of the viable streptomyces strains had been kept on Sabouraud-dextrose agar while the rest (62.5 %) was kept on Malt extract agar. Streptomyces violaceusruber produced its characteristic pigment even after 14 years under these conditions. Four of the recovered strains were tested for their antimicrobial action. The activity was retained to 82-95 % from the initial one. The method of preservation was found to be suitable for long-term storage of strains producing antibiotics.

Key words: *actinomycetes, paraffin oil, preservation, viability.*

Introduction

Actinomycetes population has been identified as one of the major groups of the soil population [11]. They are Gram-positive organisms assumed to be the transition group between fungi and bacteria. Actinomycetes have characteristic biological aspects such as mycelial forms of growth that accumulate in sporulation and the ability to form a wide variety of secondary metabolites including most of antibiotics. Actinomycetes make three quarters of all known antibiotics of which the *Streptomyces* species are especially prolific. The strains important for industry, were usually obtained as a result of long-term selection. A reversion to the wild type accompanied with decreasing of bio-synthetic activity had been very frequently observed. Actinomycetes grow easily under laboratory conditions, but continuous subculturing has negative effects on their production of secondary metabolites including antibiotics. For example, *S. ambofaciens* loses about 20 % of its spiramycine production ability after 4 sub-cultures [8]. The storage of highly productive variants at suitable conditions is one way to

prevent the production of biologically active substances from sharp decrease. Several methods of preservation are required to ensure the viability of morphological, physiological and genetic integrity of the culture over time. The cost and convenience of each method, however, also must be considered. Among the ways for preservation are dispersal of spores in sterile distilled water [6, 7], maintenance of cultures under mineral oil [4], in sterile soil [3], deep freezing [14], lyophilization [5] and cryopreservation [2].

Methods for handling actinomycetes cultures are varying. Restoration conditions, medium, temperature, air, tube, plate or shaking flask cultivation affect the growth of the preserved culture and the interpretation of the preservation methods. The conditions used to restore the culture may differ from those used for the cultivation of a culture for a specific process. The importance of restoration conditions is often overlooked. However, for recovering cultures it may be useful to take into account the culture conditions at the time of conserva-

tion and to try to reproduce them as nearly as possible. It is needed to prepare some media used for conservation such as Sabouraud-dextrose agar and Malt extract agar. Each strain showed the same morphological characteristics on both media.

In the present work, 100 locally isolated cultures of streptomycetes stored under paraffin oil 15 years were investigated for their viability profile. Some selected cultures were further investigated for their biological activities.

Materials and Methods

Microorganisms. Previously, the streptomycetes were the target of various studies in the Microbial Chemistry Department, National Research Centre, Cairo, Egypt. The initial cultures were maintained by periodical cultivation; 60 strains were kept on Sabouraud-dextrose agar while the rest (40) on Malt extract agar both overlaid with paraffin oil.

Culture media. The viability of the streptomycetes strains was evaluated on plates with three different media namely Brain heart-infusion agar (BHIA), Malt extract agar (MEA) and Sabouraud-dextrose agar (SDA) adopting the method given by Lennette et al. [12].

Morphological and physiological characteristics. Morphological characteristics of streptomycetes colonies were observed micro-

scopically. Physiological criteria were determined by routine methods for genus determination [10].

Genus identification. Identification was confirmed by direct observation of both morphological and microscopic characteristics as well as biochemical tests [10, 15, 17].

Biological assays. The antibiotic activities of 14-day old cultures were determined according to the diffusion method on agar plate and the results were given in mm diameter sterile zone [9]. The tested microorganisms were *Staphylococcus aureus*, *Micrococcus luteus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Saccharomyces cerevisiae*, *Candida albicans* and *Aspergillus niger*.

Results

The number of viable strains and culture life-spans are reported in Table 1, from which it is clear that 16 strains were recovered without apparent morphological or physiological changes. Fifteen of the recovered strains were 13-15

years old and had never been subcultured. Only one strain could be recovered from the strains that were subcultured at least once. *S. violaceusruber* produced its characteristic pigment even after 10 years in storage.

Table 1 Number of viable cultures and life spans.

Genera and species <i>Streptomyces</i>	Number of viable isolates/ tested strains	Life span years	Culture medium
Subculture			
<i>S. albus</i>	0/5	5	SDA
<i>S. griseus</i>	0/7	5, 13	SDA
<i>S. violaceusruber</i>	0/12	5	SDA
<i>S. galbus</i>	1/8	10	SDA
<i>S. albogriseolus</i>	0/10	10	SDA
<i>Streptomyces</i> sp.	0/3	10	SDA
Subtotal	1/45		
Never subcultured			
<i>S. albus</i>	1/7	15	SDA
<i>S. griseus</i>	0/5	15	MEA
<i>S. violaceusruber</i>	2/10	14	MEA
<i>S. galbus</i>	3/6	14, 15	SDA
<i>S. albogriseolus</i>	2/9	13	MEA
<i>S. lavendulae</i>	1/2	15	SDA
<i>S. lydicus</i>	1/3	15	MEA
<i>Streptomyces</i> sp.	5/13	13, 15	MEA
Subtotal	15/55		

All strains were acid-fast negative and gram-stain positive. The degradation of the substrates casein, tyrosine and xanthine were also observed. Colonies are slow growing, aerobic, chalky, heaped, folded, varied in colour and have an earthy odour. Microscopically, the filaments are extensively branched producing long chains conidia. *S. violaceusruber* has a characteristic purple colony with reverse leaking of the same colour to the surrounding medium. The other species do not produce any visible pigment. These results confirm the genus assignment to the family *Streptomycetaceae*.

Since there is no existing record of biological activity of all strains except *S. lavendulae*

lae, *S. galbus*, *S. albogriseolus* and *Streptomyces* sp., it was found to be of interest to know if these strains lost or never had this ability. The results showed relatively low decrease in the antibiotic activity of *S. lavendulae* from initial culture (100 %) to 89 and 92 % against *B. subtilis* and *S. aureus*, respectively after 15 years preservation (Table 2). The antimicrobial activity of *S. galbus* decreased from initial culture to about 91 and 82 % against *M. luteus* after storage duration from 10 and 14-15 years. In addition, the results showed slight decrease of the antibiotic activity of *S. albogriseolus* and *Streptomyces* sp. to 95 and 90 % against *B. subtilis* after preservation of 13 and 13-15 years.

Table 2 Antimicrobial activity of *Streptomyces* species after preservation.

<i>Streptomyces</i> species	Preservation (years)	Test-microorganism	Antibiotic activity (mm sterile zone)	% compared to initial culture
<i>S. lavendulae</i>	0	<i>B. subtilis</i>	29	100
	10		24	89
<i>S. lavendulae</i>	0	<i>S. aureus</i>	26	100
	10		24	92
<i>S. galbus</i>	0	<i>M. luteus</i>	34	100
	10		31	91
	14-15		28	82
<i>S. albogriseolus</i>	0	<i>B. subtilis</i>	48	100
	13		46	95
<i>Streptomyces</i> sp.	0	<i>B. subtilis</i>	52	100
	13-15		47	90

Discussion

The main purpose of this work is to test the viability of old preserved cultures. Four of them (*S. lavendulae*, *S. galbus*, *S. albogriseolus* and *Streptomyces* sp.) showed previously antibacterial activity. The obtained results indicated that some old strains preserved by traditional methods are still viable after 15 years. Similar results were obtained by Taddei et al. [16] who examined the viability of 89 actinomycetes strains kept under paraffin oil overlay for a period between 10-30 years from which 13 strains were recovered (viable).

The newer techniques such as lyophilization and cryopreservation are more effective methods for the conservation of microorganisms. However, they are expensive for small collections with little funding. These methods

are recommended by the American Type Culture Collection [1]. Yocheva et al. [18] mentioned that the method of lyophilization was suitable for long-term preservation of antibiotic activity of streptomycetes cultures. The activity was retained to 80-90 % from initial one after 8-28 years. In addition, cryoconservation is excellent for preservation of most microorganisms where they retained their activity to 96-99 % after 10 years [13].

On the other hand, the present investigation assessed the suitability of the traditional paraffin oil lay methods for maintaining streptomycetes strains when they are in good conditions and free of contamination. The author recommended the oil overly method as it is a low cost one and appropriate for long preservation of actinomycetes.

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ПРОУЧВАНЕ НА ЖИЗНЕНОСТТА НА НЯКОЛКО ИЗОЛИРАНИ В ЕГИПЕТ СТРЕПТОМИЦЕТИ

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Резюме

Поддържането и запазването на актиномицетните култури са съществени елементи при изследванията в областта на систематиката и биоразнообразието. Проучено е влиянието на дълготрайното съхранение върху преживяемостта на няколко изолирани в Египет стрептомицети. Периодът на съхранение варира от 5 до 15 години. 37,5 % от жизнените стрептомицетни щамове са поддържани на Сабуро-декстрозен агар, докато останалите (62,5 %) са съхранявани на агар с малцов екстракт. *Streptomyces violaceus ruber* продуцира характерния си пигмент дори след 14 години при тези условия. Четири от възстановените щамове са тествирани за антимикуробно действие. Активността се запазва до 82-95 % спрямо изходната. Прави се заключение, че използваният метод е подходящ за дълготрайно съхранение на щамове, продуциращи антибиотици.