Studies on the Antibiotic Substances from Actinomyces

(2nd Report)

On the Classification of Antibiotic Actinomyces by "Streak Plate Method"
(So-called "Primary Screening")

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In the previous paper¹⁾ it was stated, that "streak plate method," which was used at first by Ishida²⁾ in our laboratory and by Emerson *et al*³⁾ in other country, was found to be most simple, convenient and sensitive for the isolation and identification of the antibiotic strains of actinomyces. The present paper deals with the results of the experiments, in order to select, identify and classify 360 active strains out of 1800 actinomyces strains using this method, which was called by the authors "Primary Screening."

EXPERIMENTAL

For each strain of actionmyces four plates were employed, two of which were glucose agar plates (A) (meat extract, peptone, glucose, 1% each, Agar 3%) and other two were glucose glycerol agar plates (B) (the former with addition of 1% glycerol). In the centre of these plates the spore suspensions of each strain of actinomyces were inoculated in a form of a circle of 15 mm in diameter and incubated at 27°C for 4 days.

Twelve kinds of test microorganisms (*E. coli* (Umezawa), streptomycin resistant *E. coli* (Umezawa), *Staphylococcus aureus* (F.D.A. 209-P), *B. anthracis* (Umezawa), *Alkaligenes faecalis* on two A plates and *Ps. aeruginosa*, *K. pneumoniae*, *S. sonnei*, *My. avium*, *My. phlei* and *My. smegmatis* on two B plates) were streaked radially around the actinomyces colonies and incubated for 24-48 hours at 37°C.

The lengths of inhibition zones were measured by the nonius in mm and the mean value obtained from each of two plates was considered to represent the inhibitory power of each strain of actinomyces.

Results

By the method mentioned above a "antibacterial spectrum" was shown for each of all strains of actinomyces used in the experiment. Table I shows, for example, those of streptomycin producing strain (*Streptomyces olivaceus*, on which the report will be made afterwards), of streptothricin producing strains (No. 36, No. 24 and Seki), of special substance producing strain (No. 406) and of an actinomyces strain, especially strong inhibitory upon acid-fast bacteria only (No. 364).

TABLE I
Antibacterial Spectra of Several Strains of Actinomyces

Strain of actinomyces	Group	Gram negative bacteria						Gram positive bacteria			Acid fast bacteria		
		<i>Ps. aeruginosa</i>	<i>Alkalig. faecalis</i>	<i>E. coli</i>	SM resistant <i>E. coli</i>	<i>K. pneumoniae</i>	<i>Shigella sonnei</i>	<i>St. aureus</i> 209-P	<i>B. subtilis</i> NRRL 588B	<i>B. anthracis</i>	<i>My. smegmatis</i>	<i>My. avium</i>	<i>My. phlei</i>
No. 346	I	0	9.0	13.0	0	5.0	13.0	13.5	13.0	17.4	17.5	8.2	11.0
No. 36	I	0	7.7	9.0	9.0	6.7	11.0	11.0	4.5	9.2	10.7	10.9	13.2
No. 24	I	0	10.7	9.8	8.0	6.1	10.5	11.7	10.7	11.0	9.0	11.0	9.5
Seki	I	0	6.7	6.8	6.7	4.0	7.0	8.5	9.3	5.3	5.5	6.0	7.0
No. 406	I	0	11.2	7.7	4.8	0	5.0	7.0	7.5	6.5	6.0	6.8	8.0
No. 17	II	0	0	0	0	0	0	10.7	6.9	7.8	8.7	8.3	8.6
No. 382	III	0	0	0	0	0	0	4.5	3.2	4.8	0	0	0
No. 364	VI	0	0	0	0	0	0	0	0	0	4.0	6.9	6.7

360 strains of active streptomyces were selected by the method mentioned above and classified by their antibacterial spectra into following 4 groups:

- I. group: inhibitory upon Gram positive, Gram negative and acid fast organisms 201 (56%) strains
- II. group: Inhibitory upon Gram positive and acid fast organisms 130 (36%) strains
- III. group: Inhibitory upon Gram positive organisms only..... 14 (4%) strains
- IV. group: Inhibitory upon acid-fast organisms only 15 (4%) strains

The strains belonging to I group of actinomyces, including streptomycin, streptothricin, chloramphenicoln, aureomycin producingstrains, were the most in number. The strains inhibitory upon Gram negative bacteria

only were not found. The classification mentioned above was not so distinct in the following cases, viz., a strain belonging to II group may appear to belong to III group, if it is low potent, and so forth.

The strains of each group were divided into several subgroups according to their special antibacterial spectra.

I. group. 201 strains belonging to this group were divided as follows:

- 1) Sta.>coli154 strains (including 97 chromogenic and 57 non-chromogenic strains)
 - i) Ant>Sub76 strains (including 48 chromogenic and 28 non-chromogenic strains)
 - a. Coli>O, SM resistant coli=O ...6 strains (all non-chromogenic) (SM producing strains)
 - b. Coli≠SM resistant coli>O70 strains (including 48 chromogenic and 22 non-chromogenic strains)
 - ii) Ant≠Sub, Coli≠SM resistant coli>O30 strains (including 13 chromogenic and 17 non-chromogenic strains)
 - iii) Ant<Sub, Coli≠SM resistant coli>O48 strains (including 36 chromogenic and 12 non-chromogenic strains)
- 2) Sta=Coli26 strains (all chromogenic)
 - i) Ant>Sub.....13 strains
 - ii) Ant≠Sub..... 5 strains
 - iii) Ant<Sub..... 8 strains
- 3) Coli>Sta19 strains (including 16 chromogenic and 3 non-chromogenic strains)
 - i) Ant>Sub..... 6 strains (including 5 chromogenic and 1 non-chromogenic strains)
 - ii) Ant≠Sub..... 3 strains (including 2 chromogenic and 1 non-chromogenic strains)
 - iii) Ant<Sub.....10 strains (including 9 chromogenic and 1 non-chromogenic strains)
- 4) Inhibitory upon *Shigella sonnei* only, except Gram positive and acid fast organisms...2 strains (including 1 chromogenic and 1 non-chromogenic strain)

As shown above streptomycin producing 6 strains of actinomyces (Coli>O, SM resistant coli=O) were easily isolated, one of which was confirmed to belong to *Streptomyces olivaceus*, as will be reported afterwards. Some discrepancies were found between both antibacterial spectra, one pro-

duced by "streak plate method" and the other produced by "cup assay" using culture filtrates or purified antibiotic substances, as will be reported later in other paper. These discrepancies will be probably due to the production of two or more different substances by one and same organism, one of which may scarcely be proved in culture filtrates according to their small amounts of production.

II. Group. 130 strains belonging to this group were divided further into following 3 subgroups:—

- 1) Ant>Sub.....80 strains (including 36 chromogenic and 44 non-chromogenic strains)
- 2) Ant=Sub.....13 strains (including 9 chromogenic and 4 non-chromogenic strains)
- 3) Ant<Sub.....37 strains (including 21 chromogenic and 16 non-chromogenic strains)

III. group. Active upon Gram positive bacteria only.....
14 strains (including 8 chromogenic and 6 non-chromogenic strains)

- 1) Sta>Ant>Sub.....1 strain (chromogenic)
- 2) Sta>Sub>Ant.....2 strains (including 1 chromogenic and 1 non-chromogenic strain)
- 3) Active on Staphyloc. only...4 strains (including 3 chromogenic and 1 non-chromogenic strain)
- 4) Active on Anthracis only ...5 strains (including 4 chromogenic and 1 non-chromogenic strain)
- 5) Active on Subtilis only2 strains (all non-chromogenic)

IV. group. Active upon acid-fast bacteria only.....
 15 strains (including 9 chromogenic and 6 non-chromogenic strains)

The strains of actinomyces belonging to this group inhibited the acid-fast organisms stronger than other bacteria. This characteristic property was proved in their culture filtrates too, showing, for example, the dilution titer of 1:640 against *My. avium*, while no potencies against *Sta. aureus* and *E. coli*.

Kelner and Moton⁴⁾ reported previously on "antismegmatis factor" which appeared probably to be a similar substance with those produced by the strains belonging to this group.

SUMMARY

1. The advantages of "streak plate method" for the selection and identification of the antibiotic actionmyces over many other methods lie in its sensitivity, simplicity and characteristic properties, the last of

which enabled us to yield a production of the antibiotic substance or substances by one and same strain of actinomyces in much more number as well as in much more amount in comparison with other methods.

2. Three hundred sixty active strains of actinomyces were classified by their antibacterial spectra into following 4 groups:

- I. group: Inhibitory upon Gram positive, Gram negative and acid-fast organisms..... 201 (56%) strains
- II. group: Inhibitory upon Gram positive and acid-fast organisms130 (36%) strains
- III. group: Inhibitory upon Gram positive organisms only.....14 (4%) strains
- IV. group: Inhibitory upon acid-fast organisms only.....15 (4%) strains

The strains of each group were further divided into several subgroups.

3. "Streak plate method," being called by the authors "Primary Screening," is a first step of our systematic investigation of antibiotic substances produced by actinomyces.

References

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