# MICROMONOSPORIN, AN ANTIBIOTIC SUBSTANCE FROM A LITTLE-KNOWN GROUP OF MICROORGANISMS[1]

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The genus *Micromonospora* belongs to one of the least-known genera of the *Actinomycetales* (Waksman and Henrici, 1943). It comprises both mesophilic and thermophilic forms and is found in soils, in manure and composts, in lake bottoms, and in other natural substrates. The micromonosporae are characterized by the formation of single spores on side branches. Several species have now been recognized.

In a survey of the distribution of antagonistic properties among the actinomycetes (Waksman, Horning, Welsch, and Woodruff, 1942), a strain of *Micromonospora* was found to produce an antibiotic substance that was active against various gram-positive bacteria. This organism possessed rather interesting physiological properties. It grew in stationary liquid media or on solid media only to a very limited extent, producing an orange to brown slimy growth, with a smooth and shiny surface, which later turned brown or almost black. However, in an aerated submerged condition, this organism grew very abundantly in the form of orange-colored compact clumps of "colonies" which nearly filled the culture flask; the medium itself remained clear.

The organism grew well on glucose and starch media, the latter being rapidly transformed to reducing sugar, which accumulated in the medium. Organic sources of nitrogen were preferred to the inorganic forms. The organism was, therefore, grown in a starch tryptone medium in a submerged state. Under these conditions, the organism hydrolyzed all the starch (900 mg dry raw starch per 100 ml of medium) and produced, in 5 days, 250 mg cell material on a dry basis; about 225 to 250 mg of reducing sugar was accumulated. The pH of the medium usually became alkaline in glucose media and acid in starch media.

Five- to seven-day-old culture filtrates gave an activity of 1,000 to 3,000 dilution units against *Bacillus subtilis*, but only 100 units against *Staphylococcus aureus* and *Sarcina lutea*; gram-negative bacteria were not affected. The results of a typical experiment are brought out in table 1. When the culture filtrate was removed and fresh medium added, a considerable amount of activity was again produced. The nature of the replacement medium did not seem to have any appreciable effect, since even distilled water gave nearly as high activity as the complete medium.

The antibacterial substance produced in the medium did not dialyze through

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a collodion membrane; it was adsorbed by norit, but could be removed therefrom either by aqueous buffer solutions or by common organic solvents.

The active substance was not stable when the pH was adjusted to less than 3.0 or to greater than 9.0; about two-thirds of the activity was lost in 1 hour when the filtrate was heated at 100 C. Pepsin and trypsin were without effect on its activity.

TABLE 1

*Influence of carbon source on the growth of Micromonospora sp. and on the production of micromonosporin*

| CARBON SOURCE | NITROGEN SOURCE | INCUBATION DAYS | GLUCOSE MG PER 100 ML OF MEDIUM* | GROWTH MG | UNITS OF ACTIVITY | | |
|---|---|---|---|---|---|---|---|
| | | | | | *B. subtilis* | *B. mycoides* | pH |
| Glucose | Tryptone | 4 | 820 | 55 | >300 | 100 | |
| Starch | Tryptone | 4 | 190 | 178 | >300 | 25 | |
| Starch | $NaNO_3$ | 4 | 0 | Trace | 30 | 0 | |
| Glucose | Tryptone | 6 | 745 | 85 | >1,000 | 100 | |
| Starch | Tryptone | 6 | 197 | 277 | 100 | 30 | |
| Starch | $NaNO_3$ | 6 | 22 | 57 | <10 | 10 | |
| Glucose | Tryptone | 9 | 715 | 98 | 500 | 30 | 7.7 |
| Starch | Tryptone | 9 | 135 | 351 | 150 | 10 | 5.6 |
| Glucose | Tryptone | 13 | 685 | 134 | 300 | 30 | 8.0 |
| Starch | Tryptone | 13 | 62 | 356 | >300 | 30 | 6.1 |

* Original medium contained 920 mg reducing sugar.

TABLE 2

*The isolation and activity of micromonosporin*

| PREPARATION | VOLUME OR WEIGHT | ACTIVITY IN DILUTION UNITS PER MG OR PER ML | | | |
|---|---|---|---|---|---|
| | | *S. aureus* | *B. mycoides* | *B. subtilis* | *S. lutea* |
| Culture filtrate | 1,500 ml | 25 | 20 | 300 | 300 |
| Filtrate from $(NH_4)_2SO_4$, ppt. | — | <100 | <100 | <100 | <100 |
| Final dialyzed solution | 75 ml | 200 | 100 | 3,000 | 1,000 |
| Residue after lyophilizing | 78 mg | 30,000 | 80,000 | 800,000 | 300,000 |
| Acetone extract of mycelium | 100 ml | 3,000 | 1,000 | 30,000 | 10,000 |
| Residue from acetone extract | 168 mg | 200,000 | 200,000 | 20,000,000 | 6,000,000 |

The antibiotic could be precipitated from the filtered culture medium by saturation with ammonium sulfate. The chocolate-brown precipitate was removed on filter cel, washed with a saturated solution of ammonium sulfate, and dissolved in 75 ml of a 5 per cent solution of sodium chloride. This solution was dialyzed for 1 day against running tap water and for 2 days more against distilled water, and finally lyophilized. The dry residue now dissolved only with difficulty in 5 per cent sodium chloride. The product gave a very

strong positive test for carbohydrate by the Molisch reaction, the phloroglucinol, orcinol, and naphthoresorcinol reactions being negative; it contained 6.7 per cent nitrogen, and on hydrolysis with 0.5 ml of boiling 6 N hydrochloric acid for 16 hours, it gave 5.5 per cent amino nitrogen.

The mycelium removed by filtration from this culture was treated with acetone. The acetone extract was of a bright orange color. On adding an alcoholic ferric chloride solution, there was no color change. The orange color of the acetone extract was changed to red on adding dilute potassium hydroxide. Sodium hydrosulfite bleached the color to yellowish, and the extract oxidized hydriodic acid to iodine. Würster reagent was not affected. The antibiotic activity of the different fractions is given in table 2.

A more detailed chemical analysis of the preparation obtained from the culture filtrates showed it to be a highly pigmented and very unstable protein, associated with a carbohydrate, which has the solubility of an albumin. The preparation obtained from the mycelium by extraction with organic solvents appears to be a pigment with some of the characteristics of a quinone. The bacteriostatic spectra of the two substances are much alike. Neither is effective against gram-negative bacteria, such as *Escherichia coli*. Both show their greatest action against *B. subtilis*, with progressively less action against *S. lutea*, and least activity against *S. aureus* and *Bacillus mycoides*. As a working hypothesis, one may suggest that the organism secretes a pigment in combination with a protein, whereas the mycelium contains the free pigment.

## REFERENCES

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