Influence of Various Chemicals in the Isolation of Nocardia From Soil[1]

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A selective medium for isolation of *Nocardia* should stimulate the growth of *Nocardia* while inhibiting the other genera of the *Actinomycetes*. It should also inhibit closely related organisms, such as the *Corynebacteria* and *Mycobacteria*, as well as other microorganisms such as molds and bacilli. The presence of such fast growing and spreading microflora invalidated the value of a medium as a differentiating tool.

In this research, various concentrations and combinations of chemicals have been incorporated into solid media in an effort to formulate a selective medium that could be used to isolate *Nocardia* from soil.

Corke and Chase (1956) suggested the use of cycloheximide in the isolation of *Streptmyces*. Actidione, a cycloheximide; phenol, and alkaline pH were considered in this research for their fungicidal properties.

Gilbert and Humphreys (1956) found that potassium tellurite would inhibit motile *Bacillus*. Lichstein and Soule (1944) used sodium azide in 0.03% concentration as a bactericide for various *Bacillus* organisms. The effects of these chemicals were considered in the control of bacilli on the selective media.

Other factors considered were various substances that would stimulate the growth of *Nocardia*, or substances that could be utilized by *Nocardia*, but could not be metabolized by other soil organisms. Since *Actinomycetes* can tolerate higher saline conditions than bacteria (Krassilnikov, in Waksman, 1959), the effects of sodium chloride were determined. Crooke, Carpenter, and Klens (1950) used sodium propionate in the cultivation of *Actinomycetes*.

McClung (1954) found that all the *Nocardia* species that he worked with could utilize d-mannose. Gordon and Smith (1955) showed that

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Streptomyces and *Nocardia* are not similar in their utilization of mannose. Turfitt (1947) discovered that all the *Nocardia* species that he tested could use the steroid, cholesterol acetate. Asparagine, fructose, and glycerin have often been incorporated into media for the cultivation of *Actinomycetes* (Waksman, 1961).

In this preliminary report of results, the influence of these chemicals in various combinations and concentrations on the growth of *Nocardia corallina, N. cellulans, N. rubra, N. gardneri, N. gibsonii, N. convoluta, N.* species (ATCC 12288), and *N. vaccinii*, has been studied. The inhibitory effect of the chemicals on soil organisms was determined.

Methods and Materials

Nutrient agar and asparagine agar were used as a base for the addition of various chemicals. The chemicals in solution were added aseptically after sterlization. This simplified the formulation of media with different combinations and concentrations of chemicals, and also eliminated discoloration of the medium, pH changes, and possible chemical reactions that may have occurred during autoclaving; pH 8 was considered the optimum for the cultivation of *Nocardia*.

Seventy test media were inoculated by the streak method with the eight species of *Nocardia* and with soil dilutions. Effects of the media on the growth of *Nocardia* were determined. The effectiveness of the media in the isolation of *Nocardia* from soil dilution was evaluated.

Identification of organisms isolated from soil dilution plates was based on colony characteristics and microscopic examination of stained specimens.

Results and Discussion

Of the chemicals tested; Actidione, fructose, d-mannose, glycerin, asparagine, cholesterol acetate, sodium propionate, potassium tellurite, phenol, and sodium azide, the majority had a deterimental effect on *Nocardia* or were of no value as a differentiating agent in a selective medium for the isolation of *Nocardia* from soil.

The pH 8 was as effective as the cycloheximide, Actidione, in the control of fungus contaminants.

Potassium tellurite has value in the control of bacilli but this advantage, when considering its properties for the isolation of *Nocardia*, is lost. Potassium tellurite influences the growth of *Streptomyces* and supports the molds.

Sodium azide has possibilities for use in the control of rapid spreading bacilli. On all nutrient base media containing sodium azide, growth of organisms was not effected to such an extent as to prohibit its use in a selective medium.

The most productive and unusual growth patterns occurred on the nutrient agar-cholesterol acetate medium containing azide. All of the *Nocardia* species tested grew better on this medium than on the nutrient agar-cholesterol control. The addition of 0.02% sodium azide increased the colony count, based on the nutrient control, from 15 to 32%. Addition of 0.03% sodium azide to the nutrient agar-cholesterol medium increased the colony count to 60%.

From a random selection of organisms, isolated from nutrient agar-cholesterol media and arbitrarily identified, the percentage of *Nocardia* isolates increased as the concentration of sodium azide increased. *Streptomyces* growth decreased with the increased concentration of sodium

azide. Only one mold was present on the nutrient agar-cholesterol acetate media. This was on the control. Bacilli were inhibited to less than 2% on all the nutrient agar media containing cholesterol acetate and sodium azide. The pH 8 and sodium azide were assumed to be responsible for this inhibition of contamination.

Perlman (1953) found that a *Streptomyces* grown in a steroid enriched medium was inhibited by azide. Since sodium azide is considered as a bacterial inhibitor, the results obtained on the nutrient agar-cholesterol-azide medium are being studied in connection with steroid metabolism of *Nocardia*, as well as determining its value in the selective isolation of *Nocardia* from soil.

SUMMARY

In an effort to formulate a selective medium for the isolation of *Nocardia*, the chemicals; fructose, d-mannose, glycerin, asparagine, cholesterol acetate, sodium propionate, sodium chloride, potassium tellurite, phenol, Actidione, and sodium azide were incorporated into basal medium. The effects of these chemicals on the growth of *N. corallina*, *N. cellulans*, *N. rubra*, *N. gardneri*, *N. gibsonii*, *N. convoluta*, *N.* species (ATCC 12288), *N. vaccinii*, and of soil organisms were determined.

Basal nutrient medium containing cholesterol acetate and sodium azide appeared to be the most selective for *Nocardia*, and worthy of further investigation. Sodium azide with the cholesterol acetate stimulated the growth of organisms. Cholesterol acetate, alone, was not as effective. Sodium azide controlled obvious bacilli growths. Mold contaminants were inhibited by pH 8.

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