A Selective Medium for Nocardia[1]

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On the basis of colonial growth characteristics and the morphological appearance during certain phases of its life cycle, *Nocardia* can not be distinguished from certain bacteria. Specifically, the pleomorphism of the *Corynebacteria* resembles the hyphal stages of the *Nocardia* to a confusing extent. *Nocardia* can also be mistaken for the less aerial mycellated *Streptomyces*. Waksman (1959) states, ". . . no antibiotic or other antimicrobial substance has yet been found that would inhibit selectively the separate genera of the *Actinomycetes*." A search of the literature revealed no publication of an effort to separate the *Nocardia* from the *Corynebacteriacae* by means of a selective medium. A selective medium for the isolation of *Nocardia* from the soil would not only be helpful in laboratory work but also useful as a taxonomic aid in the classification of *Nocardia, Corynebacteria,* and *Streptomyces.*

MATERIALS AND METHODS

Selective factors considered in preliminary experiments were phenol, potassium tellurite, fructose, and pH values. pH values of 7, 8, 9, 10, and 11 were obtained in each medium. Allowances were made for deviations from the true pH values that resulted after sterilization.

Plates were inoculated with *Nocardia corallina, Arthrobacter simplex, Microbacterium lactecium, Corynebacterium hoagii, Corynebacterium bovis, Corynebacterium psuedodiphtheriticum, Corynebacterium xerosis,* and organisms from soil dilutions in order to determine the inhibitory effect

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of the various media on the organisms and to find the most useable concentrations of the experimental factors.

These preliminary experiments indicated that there was no inhibitory advantage in nutrient agar containing 1% fructose over nutrient agar containing no fructose ,although, more abundant growth of *Nocardia* was obtained on the nutrient fructose agar. Only nutrient agar containing 0.16% phenol, nutrient agar containing 0.02% potassium tellurite, and nutrient agar containing 0.1% phenol-0.01% tellurite combination were used for the final quanitative determinations. These media were tested at pH values of 7, 8, 9, 10, and 11. Since only *Nocardia, Corynebacteria,* and organisms from soil dilution grew at each pH on media containing the concentrations mentioned, these concentrations were considered to be the most useable for quanitative determinations. Triplicate plates were inoculated with 1 ml of soil dilution and incubated at 29° C, the optimum temperature for *N. corallina*. Plate counts were made the sixth day after inoculation. Identification and abritrary grouping of the organisms isolated from these plates were based on colonial growth appearance, microscopic examination of morphological characteristics, and Gram stained slides.

Results and Discussion

Growth of all test organisms occurred on nutrient agar at each pH. A search of the literature revealed only one report of bacterial growth under such alkaline conditions. A *Bacillus* growing at pH 11 was reported by Chislett (1961).

Growth of *Nocardia* and *Corynebacteria* on the control, on phenol, and on the tellurite media was more abundant at pH 8 than at pH 7 or 9. This indicates that pH 8 is the optimum pH for these organisms. *N. corallina, C. xerosis,* and *C. bovis* showed definite similarities in their adaptation to the extremes in media and pH, as well as optimum temperature. The three organisms grew well at 29° C; adapted to each pH value, with growth being most abundant at pH 8; tolerated the extremes in concentrations of phenol; and were similar in their adaptation to tellurite and to the phenol-tellurite combinations. The only factor that disturbed the similarities was that *N. corallina* growth was more abundant on the nutrient agar containing 1% fructose.

The results of the final quanitative determinations are indicated in Table I. Growth of *Nocardia* and *Corynebacteria* was best on nutrient agar containing 0.16% phenol. *Streptomyces* growth was best on nutrient agar containing 0.02% potassium tellurite. Concentrations of 0.1% phenol-0.01% tellurite were too selective to be considered of value. The addition of phenol was effective in inhibiting the growth of filamentous fungi. Preliminary experimental plates, even after much handling in the laboratory, seldom supported contaminating growths. Tellurite media were more susceptible to contamination. This is not clearly indicated in Table I since recognizable mold growths were disregarded in the identification of organisms that were present on the plates.

Conclusions

Evaluation of the results indicates: (1) highly alkaline conditions did decrease the abundance of growth but did not inhibit the growth of the seven test organisms; (2) pH 8 is the optimum for the cultivation of *Nocardia* and *Corynebacteria;* (3) nutrient agar containing 0.16% phenol with an alkaline pH influences the isolation of *Nocardia* and *Corynebacteria* from the *Streptomyces* and other soil bacteria; (4) nutrient agar containing 0.02% potassium tellurite influences the islolation of *Streptomyces*

(5) the similarities that exist in the adaptation of *N. corallina*, *C. xerosis*, and *C. bovis* suggests a closer relationship than that indicated by the present taxonomic classification.

TABLE I INHIBITORY EFFECT OF THE MEDIA AND IDENTIFICATION OF ORGANISMS ISOLATED.

pH	CONTROL		0.16% PHENOL		0.02% TELLURITE		0.1% pH-0.01% TE	
	% * present	type	% * present	type	% * present	type	% * present	type
7	100	20 *N.*** 27 *C.* 13 *S.* 40 ?	35	30 *N.* 45 *C.* 10 *S.* 15 ?	13	24 *N.* 13 *C.* 38 *S.* 25 ?	9	25 *N.* 37 *C.* 13 *S.* 25 ?
8	100	16 *N.* 33 *C.* 16 *S.* 35 ?	44	33 *N.* 45 *C.* 6 *S.* 16 ?	23	22 *N.* 22 *C.* 33 *S.* 23 ?	6	20 *N.* 25 *C.* 15 *S.* 40 ?
9	100	33 *N.* 16 *C.* 33 *S.* 18 ?	32	35 *N.* 30 *C.* 20 *S.* 15 ?	17	14 *N.* 14 *C.* 43 *S.* 29 ?	5	25 *N.* 25 *C.* 20 *S.* 30 ?
10	54	65 *N.* 18 *C.* 0 *S.* 17 ?	17	43 *N.* 42 *C.* 0 *S.* 15 ?	6	20 *N.* 20 *C.* 50 *S.* 10 ?	1.8	____
11	26	50 *N.* 20 *C.* 10 *S.* 20 ?	4	____	4	____	1.2	____

***N.* — *Nocardia*. *C.* — *Corynebacteria*. *S.* — *Streptomyces*. ? — Unknown.

* Based on number of colonies at pH 7 on nutrient agar.

LITERATURE CITED

Chislett, M. E., and D. J. Kushner. 1961. A strain of *Bacillus circulous* capable of growing under highly alkaline conditions. J. Gen. Microbiol. 24: 187-190.

Waksman, S. A. 1959. The *Actinomycetes*. Waverly Press, Inc. Baltimore, Md., Vol. 1, 327.