Selective Isolation of Aerobic Actinomycetes

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ABSTRACT

EL-NAKEEB, MOUSTAFA A. (Rutgers, The State University, New Brunswick, N.J.) AND HUBERT A. LECHEVALIER. Selective isolation of aerobic actinomycetes. Appl. Microbiol. **11**:75–77. 1963.—The composition of an arginine-glycerol-salt medium (AGS), suitable for the selective isolation of aerobic actinomycetes, was given. When soil samples were treated with calcium carbonate and plated on the AGS medium, higher total and relative plate counts of actinomycetes were obtained than when other media and methods were used.

Actinomycetes are filamentous, branching bacteria with a fungal type of morphology. They are part of the microbial flora of most natural substrates. Numerous methods have been advocated to facilitate the isolation of actinomycetes and to separate them from their relatives. It is not difficult to isolate actinomycetes from an intimate mixture with fungi, since the physiological properties of these two groups of microorganisms are different. For example, strictly antifungal antibiotics, which do not affect the growth of actinomycetes, can be used successfully. It is more difficult to separate actinomycetes from true bacteria. Nevertheless, some selective media have been suggested and also various means for increasing the actinomycetic flora of the soil samples before plating out.

The purpose of our present study was to compare an arginine-glycerol-salt (AGS) medium with other currently used media and to compare various methods advocated for the selective isolation of actinomycetes.

MATERIALS AND METHODS

Media. AGS had the following composition (in g/liter of distilled water): arginine monohydrochloride, 1.0; glycerol (sp gr not less than 1.249 at 25 C), 12.50; K_2HPO_4, 1.0; NaCl, 1.0; $MgSO_4 \cdot 7H_2O$, 0.5; $Fe_2(SO_4)_3 \cdot 6H_2O$, 0.010; $CuSO_4 \cdot 5H_2O$, 0.001; $ZnSO_4 \cdot 7H_2O$, 0.001; $MnSO_4 \cdot H_2O$, 0.001; and agar, 15.0 (pH 6.9 to 7.1).

The following media were also used: chitin medium (Lingappa and Lockwood, 1961); modified Benedict's medium (Porter, Wilhelm, and Tresner, 1960); soybean meal-glucose medium (Tsao, Leben, and Keitt, 1960); Gauze's agar medium (Řeháček, 1959); and Czapek's agar medium, egg albumen medium, glucose-asparagine medium, and glycerol-asparaginate agar II (Waksman, 1961).

Soils. Three different soil samples (corn field, cow barn yard, and forest) were collected, air-dried, sifted, and stored for use.

Preparation of soil suspensions. Dry soil (1 g), or its equivalent, was stirred for 5 min with 100 ml of sterile distilled water in a Waring Blendor (the cup of the blender was previously sterilized). Serial dilutions of the supernatant were prepared after the suspension had been allowed to stand for 30 min.

Inoculation and incubation. Over the surface of solidified agar plates, 0.2-ml samples of the proper dilution, as indicated in the results, were spread with a sterile glass rod. The plates were then incubated at 28 C for 10 days at which time differential counts were made.

Calcium carbonate soil treatment. The air-dried soil (1 g) was mixed in a mortar with 1 g of calcium carbonate (Tsao et al., 1960). The mixture was incubated for 10 days at 28 C in a closed inverted sterile petri dish in which a high relative humidity was maintained by water-saturated discs of filter paper.

Sodium propionate method. Sodium propionate was added in a 0.4% (w/v) concentration to the AGS medium before sterilization (Crook, Carpenter, and Klens, 1950).

Phenol treatment. Dry soil (10 g) was stirred in 100 ml of sterile distilled water for 5 min as described before. The suspension was mixed with an equal volume of 1.4% (w/v) phenol solution in sterile distilled water (Lawrence, 1956). After 10 min the supernatant was serially diluted with sterile distilled water to give 1:1,000 final dilution.

Centrifugation method. The soil suspension was centrifuged for 20 min at a centrifugal force of about 1,600 × *g* at the bottom of the tube (Řeháček, 1959). The supernatant was then diluted as usual and plated out.

RESULTS AND DISCUSSION

El-Nakeeb (1961) compared the value of numerous nutrients as ingredients for a medium that would be highly selective for aerobic actinomycetes. The relative value of various sources of carbon and nitrogen, and also of inorganic compounds, was compared. Combinations of compounds and concentrations of nutrients were investigated. Experiments were designed to permit statistical evaluation of the data. The medium that evolved from this study was the AGS medium previously discussed. Its composition is similar to the modification of Lindenbein's medium suggested by Benedict and his co-workers (Porter et al., 1960).

Chitin, which is widely distributed in both animal and plant kingdoms, has been found to be decomposed and utilized as the sole carbon and nitrogen source by different microorganisms. Several chitin-decomposing bacteria, actinomycetes, and fungi have been isolated from different soils (Veldkamp, 1955). Recently, a chitin medium has been used by Lingappa and Lockwood (1961) for the isolation and cultivation of actinomycetes. The AGS medium was compared in a series of experiments with the chitin medium. Three soil samples were used. The AGS medium gave superior results (Table 1), as shown mainly by its high selectivity for actinomycetes.

It should be emphasized that a selective medium for the isolation of actinomycetes does not have to be one on which their growth is luxuriant. It is enough that they grow, be it meagerly, while bacteria and fungi do not. It is known that actinomycetes can survive and grow to some extent on very small amounts of nutrients which may be found as impurities in some of the nonnutrient substances such as agar. This is why such a medium as water and agar may serve as a selective medium for isolation of actinomycetes. One should not be too surprised then to note in Table 1 that chitin is not an essential constituent of the "chitin medium." Almost the same counts of actinomycetes were obtained both in the presence and absence of chitin. [After the completion of this paper, Lingappa and Lockwood (1962) have also shown the value of water agar.] However, the growth of individual colonies of actinomycetes was better in the presence of chitin than in its absence. In the case of the AGS medium, removal of the apparent carbon and nitrogen sources (glycerol and arginine) gave about 50% reduction in the counts of actinomycetes. This is probably due to the care with which the AGS medium was developed. Almost every possible alteration in its composition was tested and analyzed statistically. It might be, therefore, that the particular combinations and concentrations of salts in this medium are toxic for some actinomycetes in the absence of carbon and nitrogen sources.

The AGS medium and the corn field soil were used to compare the value of various methods previously reported to be helpful in the isolation of actinomycetes (Table 2). In our hands, the calcium carbonate treatment (Tsao et al., 1960) was the most effective, since it gave not only the highest total counts of actinomycetes, but also the lowest relative numbers of bacteria and fungi.

TABLE 1. *Comparison of the AGS medium with the chitin medium and effect of the sources of carbon and nitrogen in these media**

Media	Soils	Actinomycetes, per cent of total microbial population	Actinomycetes per g of soil (thousands)
AGS medium	CB	94	560
	CF	87	245
	F	80	172
AGS medium without arginine and glycerol	CB	94	269
	CF	83	143
	F	74	78
Chitin medium	CB	85	511
	CF	57	152
	F	54	150
Chitin medium without chitin	CB	88	503
	CF	57	108
	F	67	127

* Soils not treated with calcium carbonate. Counts from eight plates inoculated with 1:1,000 dilution. Symbols: CB = cow barn yard soil; CF = corn field soil; F = forest soil.

TABLE 2. *Comparison of various methods recommended for the isolation of actinomycetes**

Method	Actinomycetes, per cent of total microbial population	Actinomycetes per g of soil (thousands)
Calcium carbonate treatment†	81	9,750
Sodium propionate method‡	58	90
Phenol treatment‡	35	21
Centrifugation method‡	45	13
No treatment‡	33	89

* Corn field soil plated out on AGS medium. Counts from four plates.

† Plates inoculated with 0.2 ml of 1:10,000 dilution.

‡ Plates inoculated with 0.2 ml of 1:1,000 dilution.

TABLE 3. *Comparison between eight different media using three calcium carbonate-treated soils**

Media	Soils	Actinomycetes, per cent of total microbial population	Actinomycetes per g of soil (millions)†
AGS	CB	96	29.4
	CF	96	10.9
	F	94	11.2
Modified Benedict's medium	CB	95	18.0
	CF	95	6.8
	F	93	6.9
Egg albumen	CB	93	*19.9*
	CF	90	9.2
	F	94	10.7
Glucose-asparagine	CB	84	14.5
	CF	84	5.2
	F	87	7.1
Glycerol-asparaginate agar II	CB	92	16.6
	CF	81	5.6
	F	88	7.9
Czapek's agar	CB	91	*21.1*
	CF	80	8.5
	F	90	8.8
Soybean meal-glucose	CB	86	*23.8*
	CF	70	7.2
	F	82	8.5
Gauze's agar	CB	94	*19.8*
	CF	86	7.7
	F	67	3.3

* Counts from four plates inoculated with 1:20,000 dilution. CB = cow barn yard soil; CF = corn field soil; F = forest soil.

† Little variation between replicate plates was noted. Using a *t*-test analysis, the counts in italics were found to differ from the AGS count at the 0.1% level of significance.

The three soil samples, therefore, received the calcium carbonate treatment and were then plated out on the AGS medium and on seven other media described as suitable for isolation and cultivation of actinomycetes. The results in Table 3 suggest that the AGS medium is more advantageously selective for actinomycetes than any of the seven media used.

One should remember that our studies were quantitative in nature. It is therefore a possible limitation of the calcium carbonate treatment method, that it might favor the repeated isolation of the same types of actinomycetes. The AGS medium, however, supports the isolation of different types of actinomycetes. This can be judged simply by the different morphological characters of the colonies and the diffusible and nondiffusible pigments produced on plating the soil sample on the AGS medium. The other media tested did not give such easily recognized differentiation between the colonies of actinomycetes which developed on them. In addition, the AGS medium permitted adequate growth of many different types of actinomycetes from our culture collection.

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