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Notes on Methodology

Preparation of uniform dispersions of cholesterol and other water-insoluble carbon sources in agar media*

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» Screening and maintaining microorganisms that use water-insoluble substrates such as sterols and hydrocarbons as sole sources of carbon have been hampered for want of a satisfactory solid medium. Most of these materials disperse poorly and thus present little surface area when added to agar. However, we have found that a Waring blendor finely disperses these materials in agar media. This procedure yields a preparation that presents more of the surface area of the insoluble substrates to organisms streaked on the agar surfaces. Several organisms that use these materials as sole sources of carbon have been shown to grow well on agar media prepared in this manner.

The carbon sources used in this study were cholesterol, anthracene, and Skellysolve B (a mixture of hydrocarbons containing approximately 50% normal hexane). Before use, the cholesterol and the anthracene were autoclaved dry for 20 minutes at 121°; the Skellysolve B was sterilized by passage through a sterile, sintered-glass filter.

Each of the organic substrates (0.2 g in each case) was placed into separate sterile blendor vessels containing 200 ml of a cooled, molten, salts–agar medium of the following composition: K_2HPO_4, 0.25 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; NaCl, 0.005 g; $Fe_2(SO_4)_3$, 0.0001 g; NH_4NO_3, 1.0 g; tap water, 1000 ml; pH, 6.8.

The mixtures were blended for 2 minutes, poured in sterile Petri plates (about 15.0 ml per plate), and allowed to harden. In each case, a medium with a homogenous, white opaqueness resulted. Control plates with no added source of carbon were also prepared and inoculated in each of the following experiments. Figure 1 shows the appearance of a blended, cholesterol—mineral salts agar plate as compared with one containing an equal amount of unblended cholesterol.

The plates containing the cholesterol medium were streaked with several actinomycetes isolated from soils in Houston, Texas. When growing in a cholesterol—mineral salts liquid medium, these actinomycetes used the sterol as a sole source of carbon. After incubation at 30° for 4 days, good growth with two patterns of sterol utilization was noted on the agar plates containing blended cholesterol. Some cultures caused a clearing of the cholesterol opaqueness surrounding the colonies while others did not (see Fig. 2). In Table 1, those isolants that caused a clearing in the cholesterol surrounding their colonies are referred to as the I-4 series of streptomycetes, and those that did not are called the I-3 series. All isolants, when classified according to the procedure of Gordon and Smith (1), appeared to be members of the genus *Streptomyces*. Other workers

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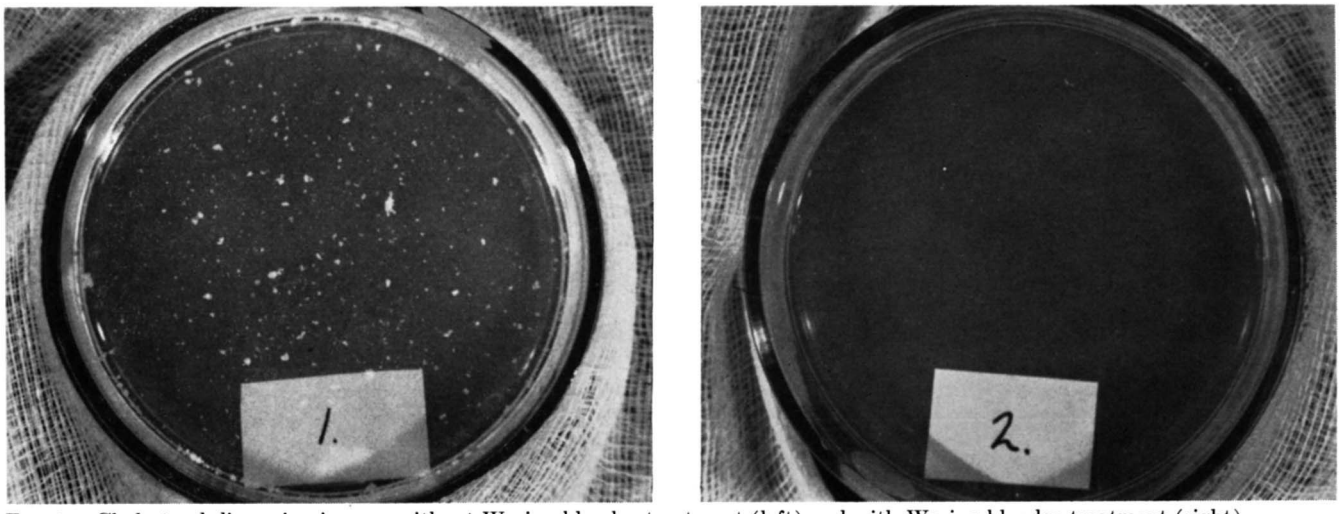

Fig. 1. Cholesterol dispersion in agar without Waring blendor treatment (left) and with Waring blendor treatment (right).

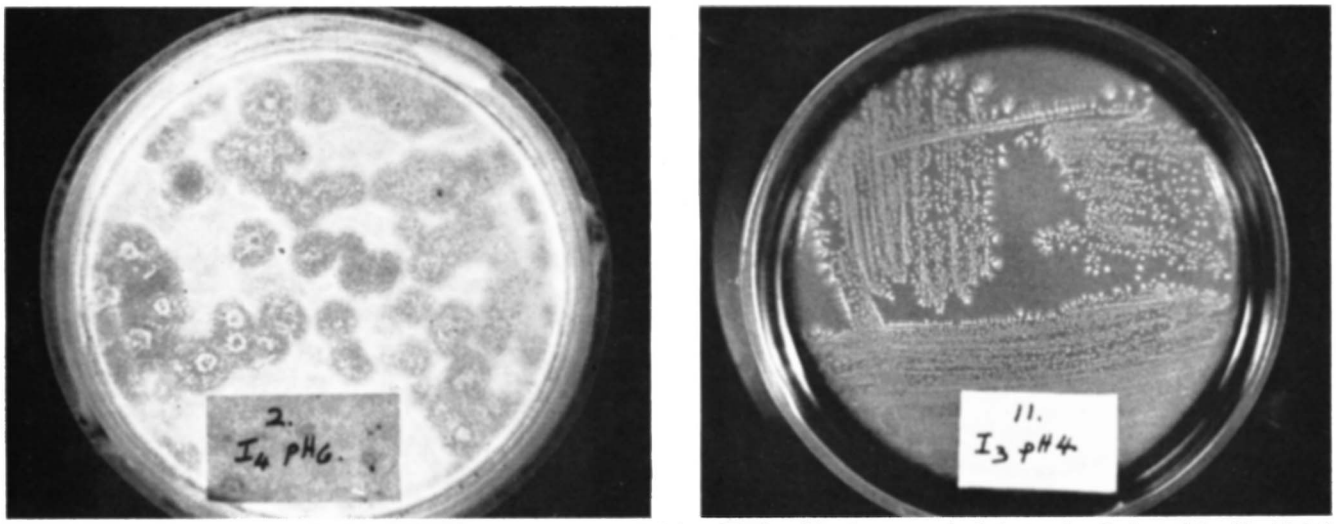

FIG. 2. Growth of cholesterol-using soil isolants on agar containing Waring blendor-treated cholesterol. Isolant I-3 pH_4 (right) does not clear agar while isolant I-4 pH_6 (left) does. (Numbers are laboratory code identifications of isolants.)

have noted naturally-occurring *Nocardia spp.*, *Mycobacterium spp.*, and certain "true bacteria" that degraded cholesterol in media usually containing supplementary sources of organic carbon (2, 3, 4, 5).

In addition, Table 1 shows that seven of 12 stock cultures of *Mycobacterium spp.* and three cultures of a hydrocarbon-using *Nocardia spp.* grew well on this cholesterol—mineral salts agar. Hence, cholesterol utilization by these species may be more common than generally considered. In addition, some of these organisms that used cholesterol as a sole carbon source were also tested for their ability to grow on agar containing blended anthracene or Skellysolve B as sole sources of carbon. Table 1 shows that several of the same organisms that grew on cholesterol as a sole source of carbon also grew on the anthracene and Skellysolve B substrates.

TABLE 1. GROWTH OF CERTAIN MICROORGANISMS ON A WARING BLENDOR-TREATED AGAR MEDIUM CONTAINING EITHER CHOLESTEROL, ANTHRACENE, OR SKELLYSOLVE B AS SOLE CARBON SOURCES

Organism	Growth Observed With Indicated Materials as Sole Carbon Source in Medium*			
	None	Cholesterol	Anthracene	Skellysolve B
Soil streptomycete I-3 pH_4	−	+	+	+
Soil streptomycete I-4 pH_8	−	+	+	+
Mycobacterium rhodocrous ATCC 271	−	+	−	+
Mycobacterium butyricum ATCC 357	−	±	−	−
Mycobacterium phlei ATCC 354	−	+	−	±
Mycobacterium paraffinicum ATCC 12670	−	+	−	+
Nocardia sp. A. 107–332	−	+	−	±
Nocardia sp. AMO	−	+	−	±
Nocardia sp. A. Bough	±	+	+	+
Mycobacterium avium ATCC 11755	−	+	N.T.	N.T.
Mycobacterium tuberculosis (4 isolants)†	−	−	N.T.	N.T.
Soil Streptomyces spp. I-3 pH_4 series (3 cultures)	−	+	N.T.	N.T.
Soil Streptomyces spp. I-4 pH_8 series (5 cultures)	−	+	N.T.	N.T.

* Scale: −, no growth; ±, scant—fair growth; +, good growth (see Fig. 2); N.T., not tested.

† Human pathogens. These cultures incubated for 3 weeks at 37°.

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