NEW DIFFERENTIAL PLATING METHODS FOR B. BIFIDUS (TISSIER) AND B. ACIDOPHILUS (MORO)

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In connection with the study of fecal bacteria the following media and methods have been found useful for the isolation of aciduric forms, especially for quantitative determinations of *B. bifidus* and *B. acidophilus*.

B. BIFIDUS

With laboratory media in common use the isolation of *B. bifidus* from fecal material containing large numbers of *B. coli* is not easy. The preparation of anaerobic plates is necessary, and with glucose agar, which is ordinarily employed, the few colonies of *B. bifidus* developing are generally overshadowed by those of *B. coli*. There is also no well marked difference between the types of colony formation, both surface and deep, of these bacteria in ordinary glucose agar. I have found during the course of a study of the Gram-positive aciduric bacilli of the intestinal tract that solid media prepared with an infusion of beef liver, instead of muscle tissue as a base, is particularly favorable. For the isolation of *B. bifidus* a glucose blood liver agar is employed. In brief, the preparation of this medium is as follows: Cut 500 grams of beef liver in small pieces and add to 1000 cc. of distilled water, boil for two hours in a double boiler, filter through flannel and cotton, and to the filtrate add 10 grams of peptone and 20 grams of agar. Heat in flask in the Arnold for one hour, adjust to the reaction desired and clear with eggs if necessary; to the clear filtrate add 10 grams of glucose and 1 gram of di-potassium phosphate. For *B. bifidus* the medium is

titrated to + 1 to phenolphthalein, and to each 10 cc. about 1 cc. of sterile defibrinated rabbit blood is added, just before the plate is poured. For its optimum development *B. bifidus* requires a certain degree of anaerobiosis, but as will be shown below, it is not an obligate anaerobe, and will grow fairly well aerobically on this medium.

To obtain the reduction of oxygen favorable for the luxuriant development of *B. bifidus*, it has been found advantageous to utilize Nowak's (1908) suggestion for the partial exhaustion of oxygen through the action of a member of the subtiloid group of bacteria. As far as I am aware this method has not been applied hitherto to plate cultures. Perhaps the nearest approach is Horton's (1914) divided test tube device. A plate, however, offers obvious advantages over a tube in that a larger surface of medium is available for seeding and the growth may be readily inspected. The apparatus used by the writer is similar to the anaerobic plate described by Zinsser (1906), except that the lower larger dish contains agar seeded with *B. cereus* (*B. subtilis* would probably prove satisfactory) instead of the pyrogallic acid sodium hydrate mixture.

A Petri dish 10 cm. in diameter and at least 2 cm. high is selected and the glucose blood liver agar is poured into it, taking care that the sides of the dish are kept free from the medium. The dish should be placed in the incubator until the sides are dry and there is no obvious moisture on the surface of the medium, when it may be seeded. This drying is important, as moisture on the sides of the dish may permit contamination by the culture used to absorb the oxygen. Into the cover of a Petri dish about 12 cm. in diameter are next poured about 15 cc. of nutrient 3 per cent agar seeded with the *B. cereus* culture. Before this agar has quite solidified the half-dish containing the seeded liver blood agar medium is inverted and placed in it. The agar in the lower plate on solidifying forms a seal. The apparatus is placed in the incubator in a moist chamber to prevent the exposed agar medium from drying out.

The *B. bifidus* colonies are visible in twenty-four hours, but are not especially distinctive in appearance. After forty-eight

hours incubation, however, they may be readily recognized as raised, more or less *globular*, opaque colonies 1 to 3 mm. in diameter, *buff to reddish brown in color*. In fact the principal novelty in this mode of cultivation lies in the distinctive appearance of the *B. bifidus* colonies (fig. 2), while the method used for obtaining conditions of partial anaerobiosis is of secondary importance. Most strains of *B. acidophilus* form on this medium flat, dingy colonies with a serrated edge, although a few pro-

FIG. I. ANAEROBIC PLATE FOR B. BIFIDUS

duce more or less convex whitish or yellowish growths. The *B. coli* colonies are easily distinguished. Hence, if the quantitative count of the viable *B. acidophilus* or *B. coli* has been determined for the specimen, by ascertaining the ratio of the colonies of *B. bifidus* appearing on the plate to those of *B. acidophilus* or *B. coli*, the count for *B. bifidus* may be estimated.

Glucose blood liver agar is so favorable for *B. bifidus* that it will grow on this medium even under aerobic conditions. The colonies in primary plate cultures, however, are generally very small, and appear after forty-eight hours incubation as minute colorless cones. Some strains adapt themselves quickly to

aerobic conditions and give rise to a rather thick staphylococcus-like growth, while others continue to produce a very thin growth on slants of this medium. Both types may be kept alive indefinitely growing on slants under aerobic conditions, if transplants are made about every seven days.

With most strains of *B. bifidus* aerobic growth on liver glucose agar tends to encourage polymorphism, the profuse and variegated branching giving rise at times to coral-like aggregations.

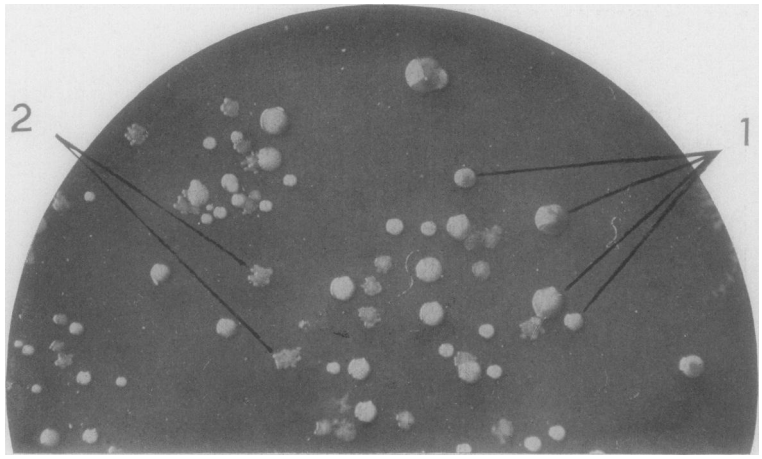

FIG. II. PHOTOGRAPH, SLIGHTLY ENLARGED, OF COLONIES ON A GLUCOSE BLOOD LIVER AGAR PLATE (ANAEROBIC) SEEDED FROM A DOG'S STOOL. 1. B. BIFIDUS COLONIES. 2. B. ACIDOPHILUS COLONIES.

Also, bifid forms are more likely to develop on liver medium than on media prepared with muscle tissue infusion as a base, when cultivated under conditions of partial anaerobiosis.

B. ACIDOPHILUS

Acid glucose agar or oleate glucose agar has generally been recommended heretofore for the cultivation of *B. acidophilus*. I have not found either of these media satisfactory for a quantitative determination of bacilli of this type in fecal specimens. In searching for a substitute it was found that glucose liver agar constitutes a decidedly favorable medium for bacteria of this

type, and furthermore that the typical colony formation facilitates quantitative determinations. The medium is prepared as described above for *B. bifidus*, except that no blood is added to it. *B. acidophilus* will develop on this medium with a range of reaction from neutral to phenolphthalein to +5 acid. A reaction of +3 acid appears to be most favorable, but for fecal work +4 acid is used in order to inhibit the development of streptococci and most strains of *B. coli*.

With a little experience the colonies formed by members of the *B. acidophilus* group may be recognized readily. The type most frequently encountered forms a small fluffy deep colony resembling a fleck of cotton. Certain other strains form globular deep colonies with a serrated border. Acid-tolerating colon bacilli give rise to lenticular deep colonies or sharp pointed triangular ones. Anaerobic plates are not necessary, as with this medium the development of the *B. acidophilus* colony is quite as satisfactory under aerobic as under anaerobic conditions.

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