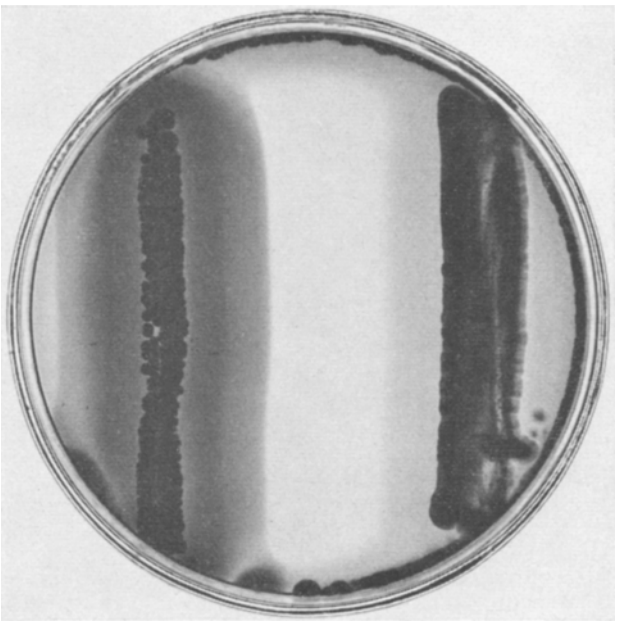
Egg-Yolk Agar as a Diagnostic Medium for Streptomycetes

Egg-yolk agar is widely used in diagnostic work with bacteria of various genera. The formation of an opaque precipitate due to decomposition of the lecithin molecule by lecithinase (α-toxin of *Clostridium perfringens* or enzymes related to this lecithinase C) is characteristic for some species of the genera *Clostridium* and *Bacillus*; it is a useful aid in the recognition of *C. perfringens* and some other clostridia as well as of *B. cereus* and *B. laterosporeus* and their differentiation from other species of these genera[1]. There are several reports on the occurrence of a 'positive lecithovitellin test' in other bacteria such as *Serratia*[2], *Pseudomonas*[3], *Listeria monocytogenes*[4], *Corynebacterium*[5], as well as in fungi[6,7]. Although it has been known for some time that lecithin is degraded by streptomycetes and used as a carbon source for growth[8,9] and antibiotic production[10], the lecithinase reaction does not appear to have been used as a taxonomic criterion in this group of organisms. Formation of this enzyme seems to have been tested only in a study on oral actinomycetes[11]. Moreover, *Streptomyces coelicolor* was included in a study on lecithinase activity among fungi and spore formers[6,7]; an 'egg-yolk reaction' was observed with this organism only after 18 days of incubation.

In our search for new physiological tests which might be useful for the characterization of members of the genus *Streptomyces*, we have tested about 300 strains for their activity on egg-yolk agar. The material which includes a large number of strains carrying a species name is essentially the same as that used in our study on urease activity[12,13]. The following 2 basal media were used (g/l): (a) peptone 10, yeast extract 5, NaCl 10; (b) glucose 5, peptone 10, meat extract 3, NaCl 10. Egg-yolk emulsion (oxoid) 10% was added to the basal media after sterilization and cooling to 50 °C. Agar plates were streak-inoculated with aerial mycelium from cultures 10–20 days old, and the plates were incubated at 28 °C; the changes produced by the growing streptomycetes were recorded after 4, 7 and 12 days.

The following types of activity were observed: (a) Clearing of the slightly turbid medium, probably due to a proteolytic activity. (b) Lipolysis of free fat indicated by the formation of a 'pearly layer' zone of different width (1–15 mm) along the growth streak, due to the precipitation of insoluble fatty acids; this zone can easily be recognized when the plates are viewed under reflected light. In addition, a zone with a slight precipitate of fine granular appearance developed beneath the 'pearly layer'. (c) Formation of a very dense, yellowish precipitate similar to that produced by typical lecithinase producers such as *C. perfringens* and *B. cereus* (Figure). Besides these 3 changes which could easily be differentiated there occurred with some cultures the formation of a narrow zone of a slight, whitish precipitate not as striking as the zone described under (c) but more distinct than the granular precipitate beneath the pearly layer. This change may or may not be due to a rather weak lecithinase activity. Further studies will be necessary to learn the nature of this activity.

Whereas formation of a clear zone as well as of a pearly layer (lipase activity) occurred with numerous streptomycetes, the very opaque precipitate – probably due to a lecithinase activity – was observed only with a limited number of cultures. The most active streptomycetes producing an opaque zone of several mm width within 2 or 4 days were: *S. albireticuli*, *S. cinnamomeus*, *S. flavopersicus*, *S. netropsis*, FAL G 169 (*S. griseocarneus*?). It appears to be remarkable that these species belong to the morphological group of verticillate streptomycetes[14] which have been placed into a separate genus, *Streptoverticillum*[15,16]. No more strains of this group were available at the time of this study; thus no conclusion can be drawn whether or not lecithinase activity may be particularly common in these organisms. Other streptomycetes showing a positive lecithovitellin test within 4–7 days were: *S. hygroscopicus*, *S. lavendulae*, *S. michiganensis*, *S. platensis*, *S. virginiae*. There were several other strepto-



Changes produced on egg-yolk agar by 2 streptomycetes after 7 days. Left, *S. netropsis*, positive lecithovitellin reaction; strong precipitate extending 12 mm from the growth streak. Right, *S. spec.*, pearly layer extending 9 mm from the growth streak. Although the difference of the nature of the precipitate obtained in the LV-reaction (left) and with lipase activity (right) may not become as obvious in the photograph, it is easily recognized when inspecting the plates in reflected light.

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mycetes producing an opaque precipitate only after 10–14 days. Furthermore, the lecithovitellin reaction is to a certain degree influenced by the composition of the medium. However, the species mentioned above as strongly positive gave consistent results and 'negative' strains were negative under all conditions.

So far the results indicate that egg-yolk agar is a useful diagnostic medium for the characterization of streptomycetes[17].

Zusammenfassung. Eigelb-Agar erwies sich als ein geeignetes Medium für die Charakterisierung von Streptomyceten. Während zahlreiche der 300 geprüften Kulturen auf diesem Medium proteolytische und lipolytische Aktivität zeigten, beschränkte sich eine positive Lecithovitellin-Reaktion auf nur wenige Arten. Soweit bisher festgestellt wurde, waren Angehörige der morphologischen Gruppe «Verticillatus» (Gattung *Streptoverticillium* Baldacci) besonders starke Lecithinase-Produzenten.

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Calcium Alginate: A New Approach in the Artificial Culturing of Insects, Applied to *Spodoptera littoralis* (Boisduval)

Most of the artificial media used as gels for the nutrition of insect larvae, and all those containing an agar carrier, require heating at some stage of their preparation. Avoidance of heating is beneficial in 4 ways: (a) by conserving the organoleptic properties, if not the actual chemical entity of some nutrients; (b) when insect pathogens are to be tested on or produced by the host through incorporation in the larval food (in this case substances which inhibit or destroy these pathogens must be excluded and the larval food renewed often enough); (c) in toxicological studies when toxicants are to be homogeneously distributed through the insect's food and (d) in mass production, by elimination of a time-consuming step.

A novel approach is used here in the preparation of gelled insect media. Advantage is being taken of the reaction between alginic acid and calcium ions to produce, under suitable conditions, an insoluble and irreversible gel at room temperature[1]. Using this reaction, we were able to develop media for the Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisd.), and lately the codling moth, *Carpocapsa pomonella* (L.). The medium used for the continuous rearing of *S. littoralis* will serve as an illustration. While artificial media for rearing this insect have been developed by us[2], LEVINSON and NAVON[3], and by BOT[4], they all contained agar and required heating.

The larval medium consists of 3 fractions. Fraction A contains 200 cm³ distilled water, 0.7 g triethanolamine, 10.0 g vitamin-free casein, 45.0 g finely ground full-fat soy, 35.0 g Torula yeast, 1.3 g methyl-*p*-hydroxybenzoate (Nipagin), 0.7 g sorbic acid and 2.7 g dibasic calcium phosphate. Fraction B consists of 300 cm³ distilled water and 11.0 g sodium alginate (Protanal L, produced by Protan, Drammen, Norway). Fraction C comprises 50 cm³ distilled water, 5.4 g glucono-δ-lactone and 3.32 g ascorbic acid.

The ingredients of A are blended in the order given, with 5 min allocated for dissolving the casein before the remainder is added. After B has been blended separately to a smooth paste, it is transferred to the mixer bowl together with A and both fractions are mixed thoroughly. Finally, fraction C is mixed rapidly with A and B and the mixture allowed to set.

In another diet for *S. littoralis* containing agar[2], ascorbic acid was included to obviate a possible deficiency; it was also one of the nutrients in the agar medium of BOT[4]. It has been shown by LEVINSON and NAVON[3] with a semi-synthetic diet that ascorbic acid was indeed essential for *S. littoralis* and that the optimum amount was 0.5% w:w, which is the level used in the present medium. However, glucono-δ-lactone being an ascorbic acid analogue, we made separate tests with this semi-synthetic diet to check whether ascorbic acid could be replaced by its analogue: all the larvae died in various instars. Glucono-δ-lactone thus cannot be substituted for ascorbic acid in the larval nutrition of *S. littoralis*.

Triethanolamine is used in the present medium in lieu of potassium hydroxide in order to dissolve casein and the soy protein as well as to emulsify the lipids.

Thus far, larvae have been bred collectively through 4 serial generations, and 3 individually, with the food changed daily. The average adult yield in individual breedings was 64.0%. At 25 $\pm$ 1 °C, larval development lasted an average of 21 $\pm$ 0.2 days, and the mean pupal weight was 283 $\pm$ 12 mg. The sex ratio did not depart significantly from unity in any of these generations; fecundity and fertility also were normal.

Since the approach taken is new and adaptable to the development of similar media for other insects, a somewhat detailed discussion of the principles involved seems warranted.

Alginic acid is obtained from various seaweed species and is a polymer of mannuronic and guluronic acids. Since the proportion of these acids varies according to the seaweed species, alginates of different origin have different properties[1], which may explain why there has been no report of actual alginate gels being used in insect media. While alginic acid is insoluble, its sodium salt is very soluble in water and can enter into a controlled chemical reaction with a calcium salt to produce an insoluble calcium alginate gel at room temperature. This

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