

Nutritional Requirements for Growth in Vitro of the Mycoplasma-like Organism Associated with Citrus Stubborn Disease¹

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ABSTRACT

The most suitable culture conditions for the mycoplasma-like organism, isolated frequently from stubborn-diseased citrus plants, were investigated. The requirements for PPLO broth, serum, fructose, glucose, sucrose, tryptone, and yeast extract for promoting growth were studied. No growth occurred when PPLO broth and horse serum were excluded. Growth was optimal with 2% PPLO broth, less with 4%, and very slight with 6%. Colonies were normal on media containing 0.0, 0.5, and 1% tryptone, but were small at the 1.5% level. No growth occurred on media with 1.5% fructose. Only a few small colonies grew on media containing 1.5% glucose. Levels of 0.0–0.5% fructose or glucose gave optimal growth. Similar results were obtained with sucrose concentrations of 0.06–0.12 *M* as well as with a concentration of 0.25 *M* with no other added sugars. However, 1 *M* sucrose prevented growth. Growth was progressively stimulated by adding fresh yeast extract up to 8–10%.

INTRODUCTION

The mycoplasma-like organisms are characterized of their peculiar morphology and colonial appearances. These characters might be affected by the conditions under which growth takes place. In the course of experimentation on the nutrition and metabolism of the mycoplasma-like organisms, it became necessary to obtain information to facilitate the handling of these organisms. The main objective of the present investigations was to improve culturing and growth *in vitro* of the mycoplasma-like organism *Spiroplasma citri* (2,3) of citrus stubborn disease.

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MATERIALS AND METHODS

Organism. The mycoplasmalike organism was obtained from aborted seeds of field-grown Hinckley sweet orange trees graft-inoculated with California 189 stubborn, as described by Fudl-Allah *et al.* (6). Primary and subcultures were grown in liquid and agar media at 30°C (5). Aliquots of 0.05 ml from the first subculture, 2 days old, were used for inoculating plates and flasks incubated at 30°C for 7 days.

Medium. For routine culture, the medium described by Fudl-Allah *et al.* (6) was used. It contains 0.1% glucose, 0.1% fructose, 0.5 tryptone, 2% PPLO broth, 2% sucrose, 10% of 25% fresh yeast extract, 20% horse serum, distilled water, and for agar media, 1% Bacto-agar. Phenol red was added as a pH indicator to a concentration of 0.0005% (w/v).

Size and colony morphology, grown on agar media, were observed for each treatment following incubation at 30°C for 7 days (5). Relative amounts of growth, in liquid cultures, were determined by measuring absorbancy at 500 m μ in a Beckman DB spectrophotometer.

RESULTS AND DISCUSSION

The typical colony of the mycoplasmalike organism associated with stubborn disease of citrus, grown on agar medium, was hemispherical, sometime slightly flattened, often with a central papilla or depression. The central spot, light brown in color, is produced by growth into the medium. The colony was translucent with a circular outline, and the surface was either faintly marked with irregular lines or appeared finely pitted.

No growth occurred in media lacking in either PPLO broth or horse serum. Growth was optimal with 2% PPLO broth, less with 4%, and very slight with 6%. There was no growth with 8% PPLO broth (Fig. 1A,B). Colonies, grown on agar media, with 2% PPLO broth were normal in their morphology (Fig. 1A), but were small in size and with the central spots poorly defined at the 4% level (Fig. 1B). Enrichment of media with an animal protein was needed for multiplication of the mycoplasmalike organism associated with stubborn disease of citrus (4). Cholesterol was compared with horse serum for its ability to promote growth. No growth occurred unless cholesterol was

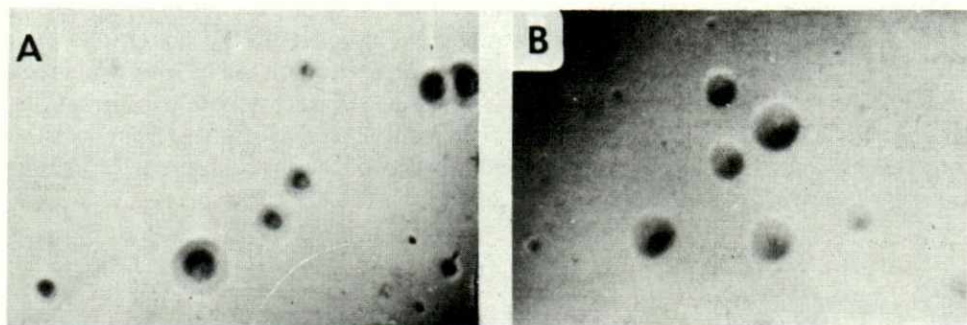


Fig. 1. Effect of PPLO broth concentration on the growth of the mycoplasmlike organism of citrus stubborn disease, on agar media with 2% (A), and 4% (B) PPLO broth.

added in amounts of 5–10 $\mu\text{g/ml}$; larger amounts (20 $\mu\text{g/ml}$) were inhibitory. The organism was maintained for long periods on horse serum containing media where it grew best, while it grew poorly with cholesterol.

Colonies were small in size on agar media with 1.5% tryptone (Fig. 2D), but were normal at levels of 0.0, 0.5, and 1% (Fig. 2A,B,C) with optimal size at 0.5% (Fig. 2B). This indicates that proteins and amino acids might have a stimulatory effect on the organism's growth (12,13,14).

No growth occurred on media containing 1.5% fructose while only a few small colonies grew on media with 1.5% glucose (Fig. 3D). Small dense colonies without central spots developed with 1% glucose (Fig. 3C) or fructose (Fig. 4C), while optimal growth occurred with concentrations 0.0–0.5% glucose (Fig. 3A,B) or fructose (Fig. 4A,B). Smith (14) reported that all fermentative mycoplasmas require hexoses as sources for carbon and energy. *Mycoplasma mycoides*, *M. gallisepticum*, and *M. pneumoniae* required glucose (7,8,13). *M. arthritidis* required pentoses for nucleic acid synthesis (9). *M. mycoides* (13) did not require pentoses.

Sucrose concentrations of 0.06–0.12 *M* (Fig. 5D,E) or 0.25 *M* in the absence of other added sugars (Fig. 5F), were optimal for growth. However, 1 *M* sucrose prevented growth of the mycoplasma-like organism of citrus stubborn disease. The relative resistance of mycoplasma organisms to osmotic shock has been described by several authors (1,10,15). A concentration of 0.12–0.25 *M* sucrose protected *Mycoplasma laidlawii*, *M. mycoides* var. *capri*, and *M. hominis* against lysis (11).

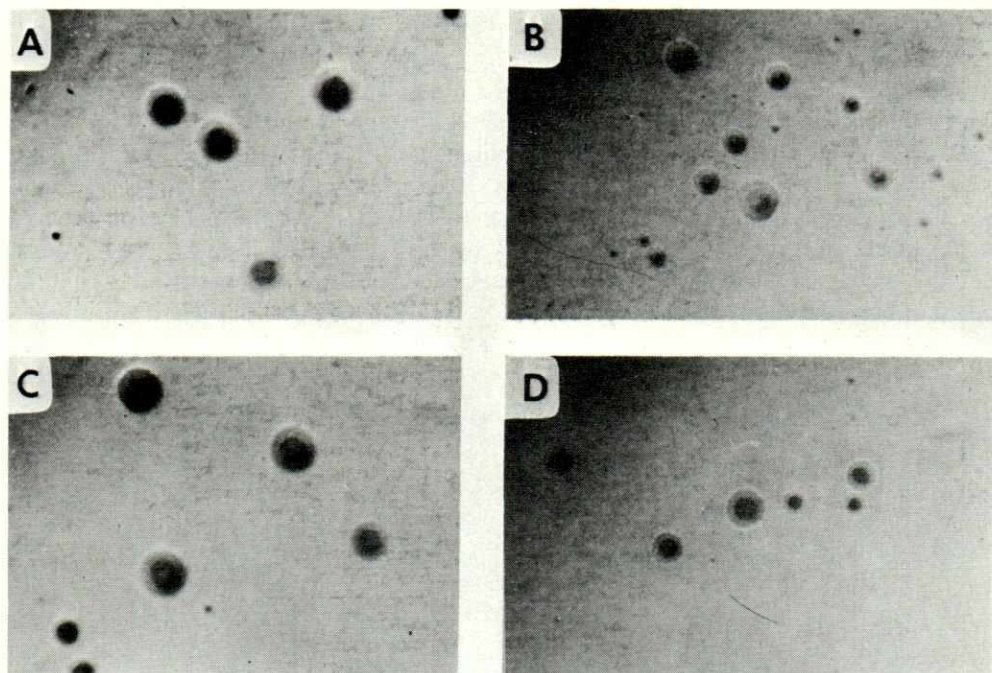


Fig. 2. Effect of tryptone concentration on the growth of the mycoplasma-like organism of citrus stubborn disease, on agar media with 0.0% (A), 0.5% (B), 1.0% (C), and 1.5% (D) tryptone.

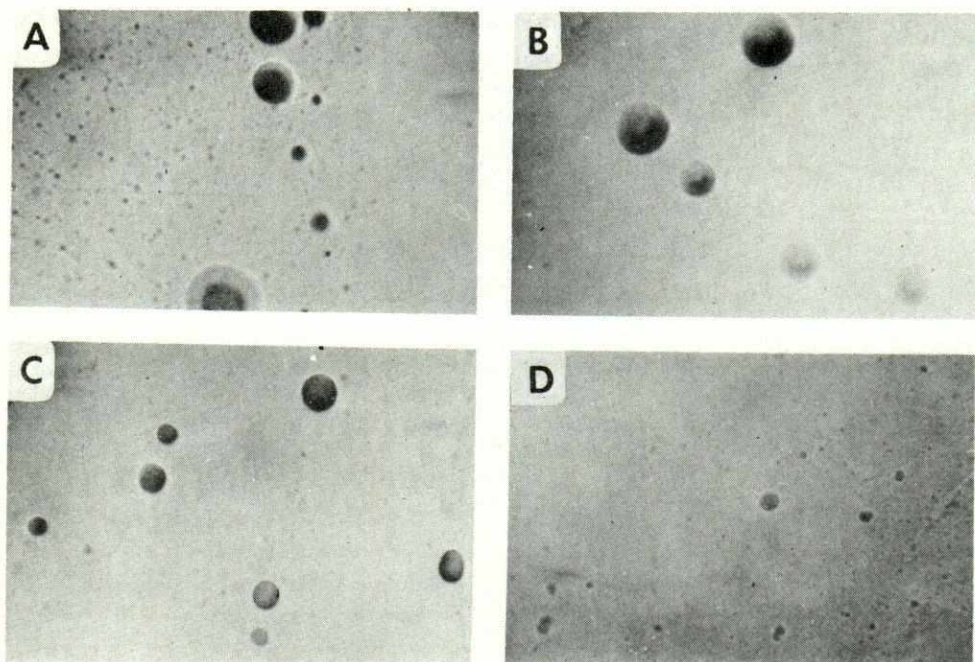


Fig. 3. Effect of glucose concentration on the growth of the mycoplasmalike organism of citrus stubborn disease, on agar media with 0.0% (A), 0.5% (B), 1.0% (C), and 1.5% (D) glucose.

The addition of 10% fresh yeast extract was found to improve the media for cultivating the mycoplasmalike organism associated with stubborn disease of citrus.

At present, it appears that a medium containing 5–20% horse serum, 2% PPLO broth, 0.5% tryptone, 0.1% fructose, 0.1% glucose, 10% fresh yeast extract, 3% sucrose, and distilled water was quite adequate for the growth of the mycoplasmalike organism of citrus stubborn disease. It should probably be the medium of choice due to its simplicity. Other growth conditions such as pH, temperature, and moisture should be taken in consideration (5).

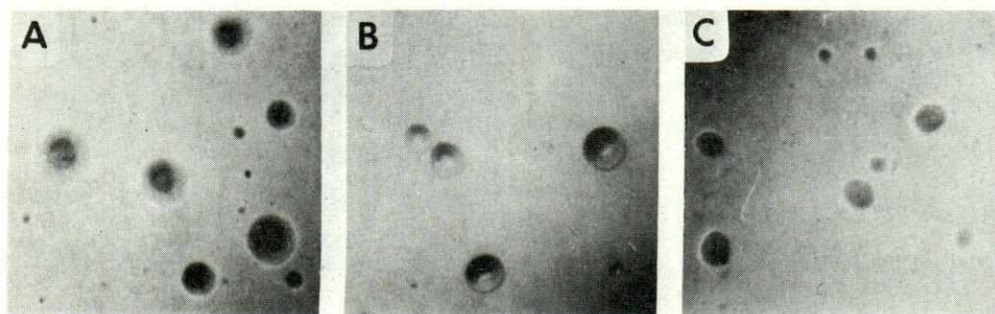


Fig. 4. Effect of fructose concentration on the growth of the mycoplasmalike organism of citrus stubborn disease, on agar media with 0.0% (A), 0.5% (B), 1.0% (C) fructose.

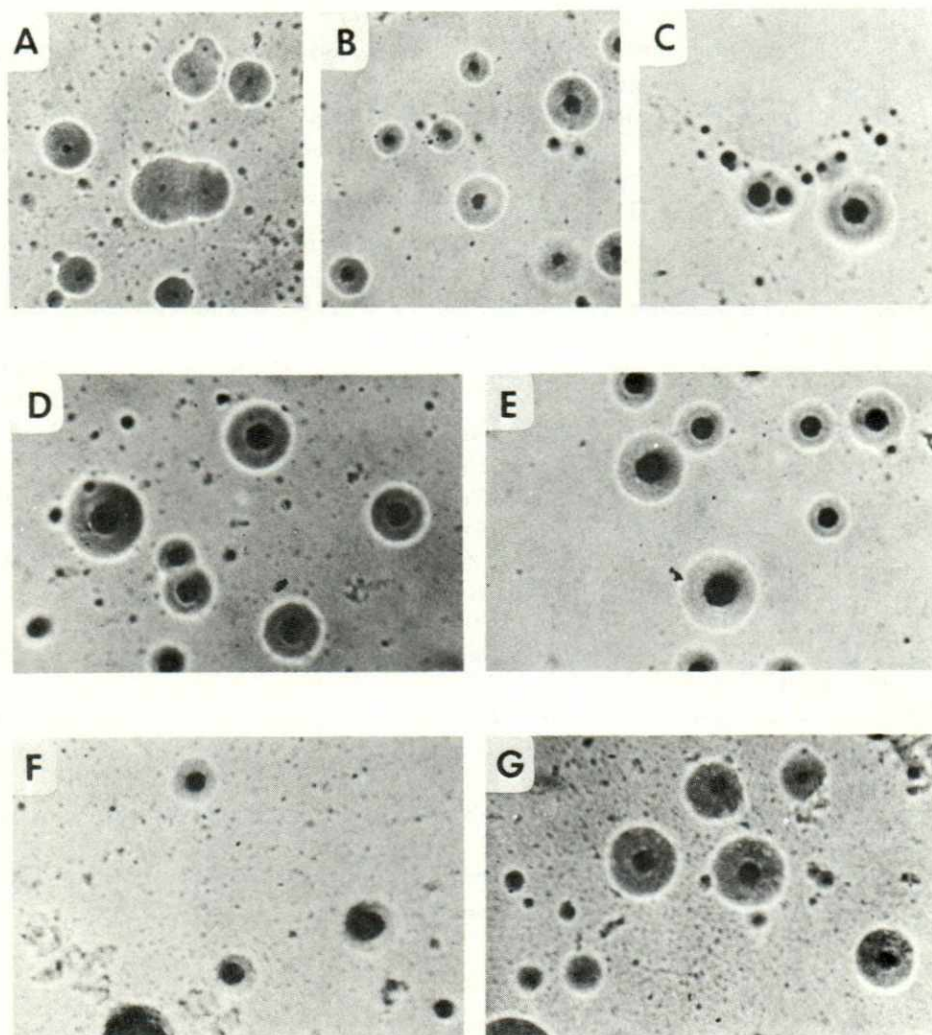


Fig. 5. Effect of sucrose concentration on the growth of the mycoplasma-like organism of citrus stubborn disease, on agar media with 0.0 *M* (A), 0.015 *M* (B), 0.03 *M* (C), 0.06 *M* (D), 0.12 *M* (E), 0.25 *M* (F), and 0.50 *M* (G) sucrose.

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الاحتياجات الغذائية الضرورية التي يجب توافرها
في البيئات الصناعية واللازمة لنمو الميكروب الشبيه
بالميكوبلازما المسبب لمرض قلة نمو وثمار أشجار
الحمضيات «ستبرن»

عبد الشافي عبد الله فضل الله

المستخلص

بحث الاحتياجات الغذائية المناسبة لتنمية الميكروب الشبيه بالميكوبلازما « سبيروبلازما سيتراي » والذي أمكن عزله في معظم الاحيان من أشجار الحمضيات المصابة بمرض قلة نمو واثمار الحمضيات « ستبرن » ، وتبين انه لتنمية هذا الميكروب في بيئة صناعية خارج العائل النباتي يلزم توافر المواد الغذائية التالية :

مستخلص غذائي ، مصّل حصان ، سكر الفاكهة ، سكر الجلوكوز ، سكر القصب ، مصدر بروتيني « تربتون » ، ومستخلص الخميرة .

ولم يتمكن هذا الميكروب من النمو عند ما استبعد المستخلص الغذائي أو المصل الحيواني من البيئة الغذائية ، وكان النمو مثاليا في وجود تركيز ٢٪ بينما تناقص النمو عند تركيز ٤٪ وكان ضعيفا عندما ارتفع التركيز الى ٦٪ من هذا المستخلص الغذائي .

وباستعمال بيئات تحتوي على تركيزات صفر ، ٠.٥ ، ١٪ من المصدر البروتيني « تربتون » تمت مستعمرات الميكروب بصورة طبيعية ، بينما كانت صغيرة في الحجم عندما ارتفع التركيز إلى ١.٥٪ من نفس المصدر البروتيني .

ولم يستطع الميكروب النمو في بيئات احتوت على تركيز ١.٥٪ من سكر الفاكهة ، بينما كانت المستعمرات قليلة العدد على بيئات احتوت على تركيز ١.٥٪ من سكر الجلوكوز ، ولكن كان النمو مثاليا في بيئات احتوت على تركيزات صفر ، ٠.٥٪ من سكر الفاكهة أو سكر الجلوكوز .

ولقد أمكن التحصل على نتائج مشابهة باستعمال بيئات احتوت على تركيزات من سكر القصب من ٠.٠٦ - ٠.١٢ مولر ، كذلك عند تركيز ٠.٢٥ مولر في غياب السكريات الأخرى ، بينما تسبب وجود سكر القصب بتركيز ١ مولر في البيئة في منع نمو الميكروب .

كما تبين ان اضافة مستخلص الخميرة الطازج و بتركيز ٨ - ١٠٪ إلى البيئة الغذائية يؤدي إلى تشجيع النمو باطراد .

ولقد استخلص ان البيئة المثالية لنمو الميكروب تحت الدراسة تتركب من ٥ - ٢٠ ٪
مصل حصان ، ٢ ٪ مستخلص غذائي ، ٥٠ ر ٪ مصدر بروتيني « تربتون » ، ١٠ ر ٪ سكر
الفاكهة ، ١٠ ر ٪ سكر الجلوكوز ، ٣ ٪ سكر القصب ، ١٠ ٪ مستخلص الخميرة الطازج
وماء مقطر . مع مراعاة درجة الحرارة المناسبة ودرجة حموضة البيئة المستعملة .