Acetobacter acidum-mucosum Tosic & Walker, n.sp., an Organism Forming a Starch-like Polysaccharide

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SUMMARY: A new species of *Acetobacter, A. acidum-mucosum,* isolated from a sample of brewery yeast is proposed. In a malt extract medium at pH 4·5 or lower it forms large capsules and a voluminous viscid sediment, whereas at pH values above 4·9 the capsules are smaller and the deposit is powdery. Its ability to form a starch-like polysaccharide is a characteristic which has not been noted in the case of any other species of *Acetobacter.*

During studies of *Acetobacter* species obtained from natural sources, Walker & Tosic (1942) isolated an organism which possessed all the generic characters of *Acetobacter*, but which showed marked specific differences from eighteen authentic cultures of known species of this genus. This paper describes the characters of the new organism.

METHODS

The standardized procedure for characterization of *Acetobacter* species, described in an earlier communication (Tosic & Walker, 1946) was employed throughout. The giant colonies shown in Pl. 1, fig. 3 were prepared by Dr Dora Kulka, by the method described by Kulka, Preston & Walker (1949).

DESCRIPTION OF THE ORGANISM

Morphological characters

Shape, size and arrangement of cells. In standard malt extract at 30°, 24–48 hr. after inoculation the majority of the cells were coccoid forms while others appeared as short rods. Their dimensions varied from $0·6 \times 0·8\mu$. to $0·8 \times 0·8\mu$. In hanging-drop preparations mainly single cells were seen, but some pairs, short chains and irregular groupings were also noted. Cells taken from a malt extract agar culture after incubation at 30° for 48 hr. were mostly $0·8 \times 1·1\mu$. in size (see Pl. 1, fig. 1). *Motility* was not observed.

Thread and swollen cell formations. Cultures grown for 24 hr. in malt extract medium at 30° were raised in temperature to 40°. Sixteen hours later a large number of cells appeared as long threads and after a further 36 hr. swollen forms were also seen.

Staining. Carbol fuchsin and Loeffler's methylene blue gave satisfactory results. The Gram stain was negative. Neither *endospores* nor *flagella* were detected.

Capsules were detected by Baker's technique (Baker, 1920). The size of capsules in malt extract medium varied, depending on the pH to which the medium was adjusted. Thus, at pH 4·5 or below, cells approximately $0·7 \times 1·4\mu$.

were seen within capsules of average dimensions $3{\cdot}2 \times 4{\cdot}8\,\mu$., but some capsules were very large, even up to $8\,\mu$. in length. At pH 4·9 or above, the majority of the capsules measured $1{\cdot}6 \times 2{\cdot}0\,\mu$. and enclosed cells of dimensions $0{\cdot}6 \times 0{\cdot}8\,\mu$.

Cultural characters

Single colonies on malt extract agar plates at 25°. At 7 days, surface colonies were round, 1·5–2·5 mm. in diameter, convex with entire margin, glistening, smooth and wet in appearance, opaque and of greyish yellow colour, pasty, and the cells could readily be suspended in water. At 14 days the appearance was similar, but the average size was about 3 mm. in diameter.

Giant colony. Convex, semi-transparent, very shiny (see Pl. 1, fig. 3). For comparison there are shown on the same plate giant colonies of (I) *A. suboxydans*, a very delicate, opaque film, not coherent; (II) *A. acetigenum*, a dome-shaped, completely opaque and very coherent colony; (III) *A. acetosum*, raised growth (but not dome-shaped), opaque and of butyrous consistency.

Streak on malt extract agar at 30°. Growth visible at 24 hr. At 3 days, growth moderately strong, echinulate (lower part) and nodose, glistening, translucent with a greyish tinge. At 7 days, similar growth but becoming slightly raised and opaque. *Stab in malt extract agar at* 30°. Slight beaded growth along line of inoculation.

Streak on malt extract gelatin at 20°. Growth visible after 48 hr.; at 3 days, slight nodose growth; after 7 days, moderately strong, echinulate, greyish and glistening. Growth very weak in a stab culture.

Streak on nutrient broth agar at 30°. Growth visible after 28 hr. At 3 days, growth slight, beaded and greyish in colour. In the stab culture only slight beaded growth developed.

Streak on glucose yeast-water agar at 30°. Growth moderately strong and in appearance similar to that on nutrient broth-agar.

Growth in standard malt extract liquid medium, 30°, pH 5. Growth visible at 18 hr. At 3 days a delicate bluish ascending film had formed on the moderately turbid liquid. At 7 days turbidity was somewhat more pronounced and a scanty powdery sediment had formed. The liquid became less acid, the pH value rising from 5·0 to 6·0.

Growth in pasteurized beer. This type of growth was entirely different from that in malt extract. At 3 days a gelatinous, friable and greyish coloured surface cover had formed on the turbid medium. Turbidity increased and at 7 days a voluminous and viscid sediment had been deposited. The liquid was not ropy. In cultures in beer a strong odour of acetic acid and a fall of pH value were noted soon after growth became evident.

Nutrient broth. Growth was slower and weaker than in malt extract or in pasteurized beer. At 7 days a bluish ascending film was noted, the liquid was turbid and a scanty powdery sediment was present.

Yeast-water (10 %, w/v). Growth slight, enhanced by addition of any one of the following: ethanol, propanol, ethyleneglycol, glycerol, L-arabinose, D-xylose, D-glucose, D-fructose, maltose. Addition of lactose or of sucrose did

not stimulate growth. In all these cases an even turbidity, a delicate bluish ascending film and a powdery sediment were produced.

Effect of pH *value on the type of growth.* This was investigated on observing the difference in type of growth in such related media as malt extract and pasteurized beer. As it was shown that neither the yeast extract and hop antiseptic present in pasteurized beer, nor the lower specific gravity of the beer as compared with malt extract medium affected the type of growth, the effect of pH value was studied. It was found that malt extract adjusted to pH 4·5 or below by addition of acetic, lactic or sulphuric acid, produced the type of growth characteristic of the beer cultures. Alternatively, the same result was attained by addition of sufficient ethanol to the medium to provide, on oxidation, that quantity of acetic acid necessary to lower the pH value to below 4·5. The authors have begun a study of the viscid product formed at pH values below 4·5.

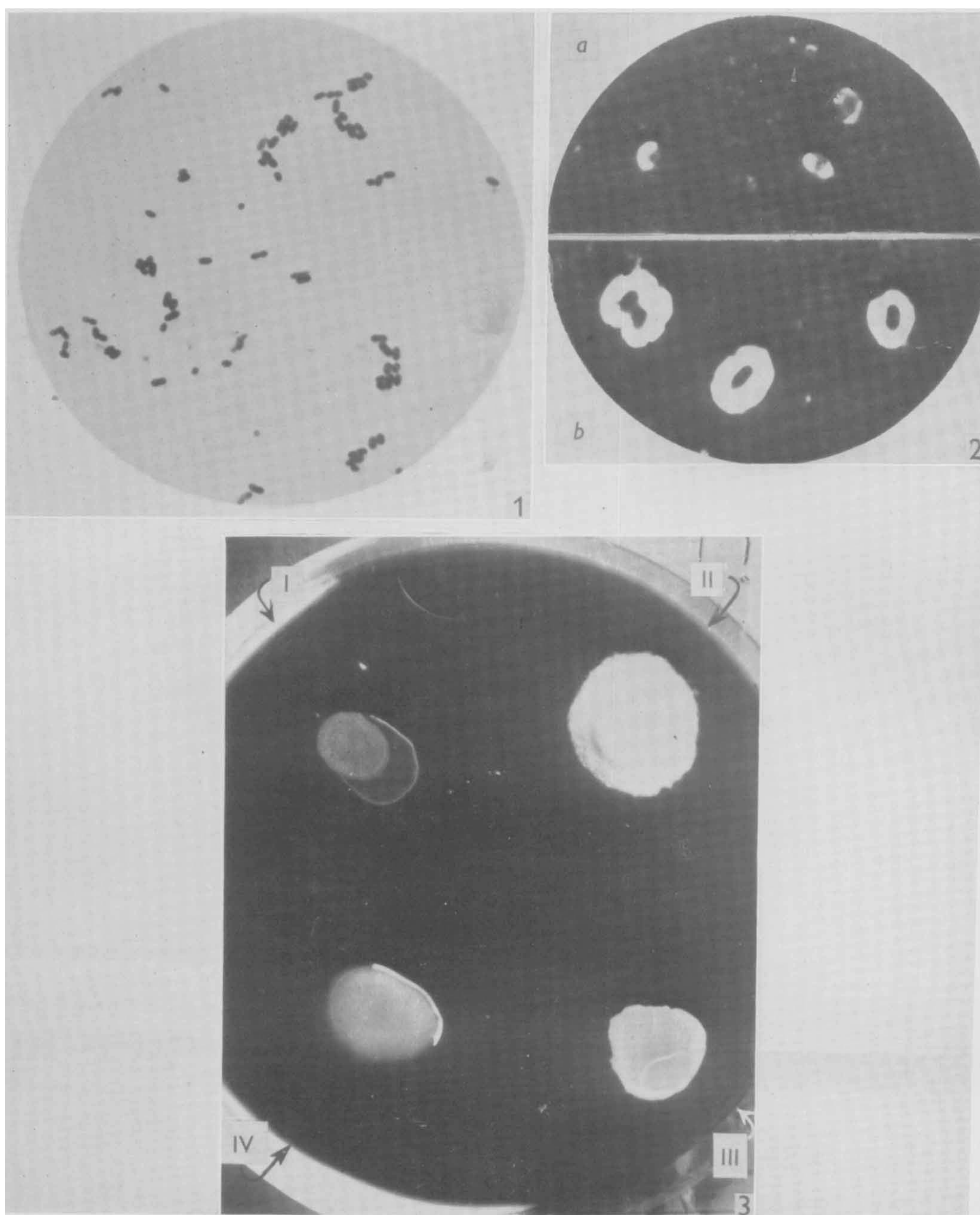
Physiological characters

Relation to temperature. In the malt extract medium the optimum range for growth was 25–30°. Slow growth occurred at 14° and in course of time the development became as strong as that at 30°. Growth at 37° was weaker than at 30° and the organism died when the inoculated medium was incubated at 42°. *Relation to oxygen.* The organism is aerobic, but tolerates a deficiency of oxygen to a greater degree than can *A. suboxydans*; but it cannot grow at an oxygen deficiency which was tolerated in a comparative study by *A. turbidans. Relation to hydrogen ion concentration.* In malt extract, growth is strong between pH 6·0 and 3·7. Growth was delayed at pH 7·0 and 3·10; no development at pH 3·0. *Resistance to ethanol.* Addition of 8 ml. of ethanol to 100 ml. of the malt extract delayed growth for 48 hr., and 10 ml. of ethanol increased the lag-phase of growth to 21 days.

Biochemical characters

(*a*) *Gas formation at* 30°. The organism did not produce gas in Durham tubes in any of the liquid media mentioned in this communication. (*b*) *Acid formation in different media at* 30°. Acid was produced in yeast-water cultures containing 2 % (w/v) of ethanol, propanol, ethyleneglycol, D-xylose or D-glucose. No acid was formed when glycerol, L-arabinose, D-fructose, D-galactose, sucrose, lactose, maltose, mannitol, salicin and dextrin, respectively, in yeast-water, were employed as the medium. Acetic acid was formed on oxidation of ethanol. (*c*) *Utilization of acetic acid.* In yeast-water cultures containing 2 % (w/v) of acetic acid, the organism utilized 38 % of the acid in 14 days at 30°. (*d*) *Catalase reaction*, positive. (*e*) *Voges-Proskauer reaction*, negative. (*f*) *Gelatin liquefaction*, none. (*g*) *Nutritional nitrogen requirement.* The organism cannot utilize ammonium salts as the sole source of nitrogen.

Formation of a substance giving positive tests for starch. It was noticed that the abundant surface growth produced by the organism on malt extract agar and also the cell material of giant colonies on the same medium, gave an intense blue-black coloration when treated with Lugol's iodine-potassium iodide solu-



Figs. 1–3

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EXPLANATION OF PLATE

Fig. 1. Cells of *Acetobacter acidum-mucosum* n.sp. grown on malt extract agar at 30° for 48 hr. (×1200.)

Fig. 2. Cells of *A. acidum-mucosum* grown in malt extract medium at 30° for 10 days, showing capsules formed (*a*) at pH 7·0, and (*b*) at pH 4·0. (×2100.)

Fig. 3. Giant colonies of *Acetobacter* spp., grown on malt extract agar at 17–21° for 14 days. Photographed with top lighting (×1). I, *A. suboxydans*; II, *A. acetigenum*; III, *A. acetosum*; IV, *A. acidum-mucosum.*

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