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Lactobacillus frigidus n.sp. Isolated from Brewery Yeast

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SUMMARY: Details are given of a new species of the genus *Lactobacillus* isolated from a sample of brewery yeast. The cells appear as short thick rods, mainly in pairs and small clumps. This organism, which ferments arabinose, xylose, glucose, fructose, mannose and sucrose, has the low optimum temperature of 22-23° and fails to grow above 30°. It has been named *Lactobacillus frigidus*.

During studies of lactic acid bacteria which occur as contaminants of brewery yeasts fifty-four cultures of the former were isolated (Bhandari, 1951); four of these consisted of rod-shaped organisms very similar to each other but unlike previously described lactobacilli. These cultures were provisionally designated HH1, HH2, HH3 and HH5, the last being chosen as the type. The characters and behaviour of HH5 are now described.

METHODS

The attributes of the organism were studied in liquid and in solid media made from beer (hopped and also unhopped) and from unhopped wort. Glucose nutrient broth was also used. The fermentation characteristics were observed in a medium which consisted of casein double-digest prepared according to Davis (1939), with the addition of yeast autolysate (1 ml./100 ml. digest).

DESCRIPTION OF THE ORGANISM

Morphological characters

Shape, size and arrangement of cells. In unhopped beer at 25° cells were seen as short rods, some being almost coccoid forms; their size was usually $0.7 \times 1.1 \mu$. Single cells, pairs, small clumps and chains of four to six cells were observed. The organism was not motile.

Staining. The cells were Gram-positive. Capsules, flagella and endospores were sought in cultures on the several suitable media but were not detected.

Cultural characters

Single colonies on solid media in an atmosphere of CO₂. After 7 days on beer gelatin plates at room temperature colonies were from 1 to 1.5 mm. in diameter, entire and colourless. Growth on beer agar slopes was weak.

Stab. Moderately strong beaded growth in beer gelatin, scanty near the surface.

Growth in liquid media at 25°. In 2-3 days the organism developed well in unhopped beer giving rise to the silky turbidity which characterizes the growth of several species of *Lactobacillus* in this medium; the liquid subse-

quently cleared when a small deposit formed. No growth occurred in hopped beer containing 0.003 % of humulone, which is the principal bacteriostatic constituent of the hop and exercises inhibitory effects on the growth of *Lactobacillus* species. HH5 developed well during incubation for 3 days in unhopped wort, and growth was also strong in glucose Lemco peptone broth.

Physiological characters

Relation to temperature. In unhopped beer the optimum temperature for growth is 22–23°; minimum at 15°, maximum at 30°. *Relation to pH value.* In unhopped beer the optimum pH range is from 5.0 to 5.6; no growth occurs at pH 3.6 or at pH 7.3. *Relation to oxygen:* facultative anaerobe. *Resistance to heat:* survives for 15 min. at 60–65° in unhopped beer. *Resistance to ethanol:* growth is delayed in beer containing 8 % (v/v) of ethanol and fails in presence of 10 %.

Biochemical characters

Catalase reaction: negative. *Nitrate* not reduced to nitrite. *Indole* not formed. *Acetylmethylcarbinol* not formed. *Gelatin* not liquefied. *Litmus milk:* no change observable.

Utilization of carbohydrates. In a casein double-digest supplemented with yeast autolysate growth occurred and acid was formed from: arabinose, xylose, glucose, fructose, mannose, sucrose or maltose; gas formation did not occur. The organism showed slight growth when mannitol, salicin or inulin was used as carbon source but acid was not detected in these cultures.

The acid produced from glucose. The behaviour of HH5 in the glucose medium was heterofermentative, yielding lactic and acetic acids in the molecular ratio 100 : 16.

CLASSIFICATION

The ability of the organism to ferment certain carbohydrates and the fact that it is a facultative anaerobe which is catalase-negative, does not reduce nitrate to nitrite and is non-motile, non-spore forming and Gram-positive, places it in the tribe Lactobacilleae Winslow *et al.* of the family Lactobacteriaceae Orla-Jensen. The additional features, a rod-shaped cell which produces lactic acid from glucose, allow the organism to be classed as a species of *Lactobacillus*.

The description of this organism has been compared with such information concerning *Lactobacillus* species as is available in the literature (Pederson, 1938; Orla-Jensen, 1942; *Bergey's Manual*, 1948; Shimwell, 1949). On this evidence the only species which is somewhat similar to HH5 is *L. pastorianus* van Laer. However, the following differences are to be observed between the two organisms: cells of *L. pastorianus* appear as rods 5–10 μ . in length and even up to 35 μ . as filamentous forms, whereas HH5 is about 1.1 μ . in length. Maximum temperature for growth of *L. pastorianus* is 38° with optimum range 29–33°. HH5 has the low optimum 22–23° and fails to grow at 30°. Similarly, in its pH range HH5 is markedly restricted in growth below pH 4.2 and fails to grow above pH 6.9, whereas *L. pastorianus* can be culti-

vated over the pH range 3.4–8.0. Finally, *L. pastorianus* ferments galactose, lactose, raffinose and salicin, none of which is attacked by the new organism. It is concluded, therefore, that HH5 represents a species of *Lactobacillus* hitherto undescribed. Since one of the main features of HH5 is its comparatively low optimum temperature and the low maximum temperature at which growth can occur, it has been named *L. frigidus*.

***Lactobacillus frigidus* n.sp.**

Cells short thick rods, $0.7 \times 1.1 \mu$. Non-motile. No endospores, capsules or flagella. Gram-positive. Colonies on beer-gelatin 1.0–1.5 mm. diameter, entire, convex, colourless.

Facultative anaerobe. Optimum temperature, 22–23°. Optimum hydrogen ion concentration, pH 5.0–5.6. Rapid growth in unhopped beer, unhopped malt wort, glucose nutrient broth and in a casein double-digest with added yeast autolysate. No growth in milk.

Ferments arabinose, xylose, glucose, fructose, mannose, sucrose and maltose, in all cases with production of acid but not of gas. Slight growth on mannitol, salicin and inulin but acid not detected. Heterofermentative on glucose.

Isolated from a sample of brewery 'top-yeast' (*Saccharomyces cerevisiae*), Manchester, England, February 1950. Subcultures from the type culture have been deposited at the National Collection of Industrial Bacteria (The Chemical Laboratory, D.S.I.R., Teddington, Middlesex) as Culture no. NCIB 8518, at the National Institute for Research in Dairying, Shinfield, Reading, and at the Laboratories of the Brewing Industry Research Foundation, Nutfield, Surrey. The authors have been informed that subcultures from the NCIB have been sent to the American Collection of Type Cultures.

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