

LACTOBACILLUS VIRIDESCENS NOV. SPEC., A HETEROFERMENTATIVE SPECIES THAT PRODUCES A GREEN DISCOLORATION OF CURED MEAT PIGMENTS<sup>1</sup>

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Niven *et al.* (1949) described, but did not name, a new species of heterofermentative lactobacillus that frequently is the cause of greening of cured meat pigments. These organisms were found to be physiologically and serologically homogeneous, and have been found widely distributed in and on cured meat products (Niven, 1951).

Members of this species have been found to possess some rather unusual nutritional requirements (Evans and Niven, 1951; Deibel *et al.*, 1955), and to show a wide range of heat tolerance (Niven *et al.*, 1954).

Because of the widespread occurrence of these unique but homogeneous organisms, it is desirable to name this new species, *Lactobacillus viridescens* nov. spec.

METHODS

This species is most readily isolated and maintained using APT medium (Evans and Niven, 1951). Care must be exercised to assure that the medium contains adequate thiamin (Deibel *et al.*, 1955).

The physiological, serological, and nutritional methods employed were those described in the publications mentioned in the introduction. Changes have been made in the basal medium employed for the physiological tests as our knowledge of the nutritional requirements of these organisms has progressed. In spite of these changes, the results have been consistent with those originally published.

DESCRIPTION OF ORGANISM

**Morphology.** The cells are gram positive, small rods (0.8 by 2.0  $\mu$ ) occurring singly or in pairs. The ends of the individual rods appear to be somewhat tapered. They are nonmotile and non-sporeforming.

**Physiology.** Growth is vigorous on or in APT medium, but is scant or absent in most other

media. They are facultatively anaerobic, catalase negative, and accumulate hydrogen peroxide when cultured under aerobic conditions. Vigorously growing cultures produce considerable CO<sub>2</sub> from glucose and accumulate optically inactive lactic acid as one of the fermentation products. The final pH in glucose broth is approximately 4.5.

Nitrate is not reduced, gelatin is not liquefied, and there is no change produced in litmus milk. Sodium hippurate, esculin, arginine, and starch are not hydrolyzed. Growth occurs at 5 C but not at 45 C, and in media containing 6.5 per cent sodium chloride.

Glucose, mannose, fructose, and maltose are fermented by all strains. Some strains ferment sucrose and synthesize large quantities of a polysaccharide from this sugar, resulting in the production of large mucoid colonies on sucrose gelatin agar. However, this characteristic is lost rapidly in stock cultures. Occasional strains ferment trehalose. No acid is produced from xylose, arabinose, galactose, lactose, raffinose, inulin, glycerol, or sorbitol. Rare strains ferment mannitol weakly.

**Nutrition.** Growth in complex media is greatly stimulated by the addition of manganese, citrate, and polyoxyethylene sorbitan monooleate ("tween 80"). In a casein hydrolyzate medium members of this species require thiamin, nicotinic acid, riboflavin, pantothenic acid, biotin, and asparagine, and may be stimulated by folic acid and pyridoxine. The quantitative requirement for thiamin appears to be unusually high.

**Serology.** Heat killed whole cells inoculated intravenously into rabbits resulted in the production of antibodies that give a precipitin reaction with Lancefield extracts of all members of this species, thus indicating their serological homogeneity. Extracts of other *Lactobacillus* species failed to give a positive test with the sera prepared.

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## DISCUSSION

This new species is not only homogeneous in its characteristics, but it differs in many respects from presently recognized species of heterofermentative lactobacilli. The major differences include a much lower minimum growth temperature, the fermentation of a very restricted group of carbohydrates, and generally more complex nutritional requirements. In particular, their failure to ferment arabinose, xylose, galactose, lactose, and raffinose should be noted.

The value of nutritional requirements in taxonomic studies of lactobacilli has been emphasized by Rogosa *et al.* (1953), and the value of serological studies of such organisms is in accord with the recent work of Sharpe (1955).

A typical strain of this new species (strain S38A) has been sent to the American Type Culture Collection.

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## SUMMARY

*Lactobacillus viridescens* nov. spec. has been described. It is homogeneous with respect to morphology, physiology, nutrition, and serology, and may be readily separated from other heterofermentative species of this genus. Members of

this species have been isolated from a variety of cured meat products produced in many different geographical areas. They are capable of causing a greenish discoloration of cured meat pigments, resulting in serious economic losses.

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