

# HILGARDIA

*A Journal of Agricultural Science Published by  
the California Agricultural Experiment Station*

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VOLUME 19

APRIL, 1949

NUMBER 4

**LACTOBACILLUS TRICHODES NOV. SPEC.,  
A BACTERIUM CAUSING SPOILAGE  
IN APPETIZER AND DESSERT WINES**

**JOHN C. M. FORNACHON, HOWARD C. DOUGLAS,  
and REESE H. VAUGHN**

**THE TAXONOMY OF LACTOBACILLUS  
HILGARDII AND RELATED  
HETEROFERMENTATIVE LACTOBACILLI**

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and JOHN C. M. FORNACHON**

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A new species of *Lactobacillus* which has been observed in wines of high alcohol content for many years is described and named. The name *L. trichodes* is proposed because of the marked tendency to grow as long intertwined chains and filaments resembling a mass of hair.

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*Lactobacillus hilgardii* is redescribed and compared with other heterofermentative lactobacilli. The criteria of optimum and maximum temperature relations, fermentation of pentoses and other carbohydrates, rate of growth, type of growth, growth requirements, cell morphology, and the effect of pH on fermentation indicate that this and other "inactive" lactobacilli—*L. fructivorans*, *L. trichodes*, and *Betabacterium caucasicum*—are well-defined species. The name *Lactobacillus desidiosus* is proposed to replace *Betabacterium caucasicum*, in order to conform to accepted nomenclature.

### ERRATUM:

In *Hilgardia* Volume 19, Number 4, the first five lines under "Distinguishing Characteristics," page 135, should read:

The characteristics of *Lactobacillus hilgardii* which differentiate it from the other heterofermentative species investigated are shown in table 1. It ferments *d*-xylose, galactose, sucrose, maltose, and *l*-malic acid; of these, *L. fructivorans* ferments only *l*-malic acid (weakly), *Betabacterium caucasicum* only galactose (weakly), and *L. trichodes* only sucrose and maltose (both weakly).

## **LACTOBACILLUS TRICHODES NOV. SPEC., A BACTERIUM CAUSING SPOILAGE IN APPETIZER AND DESSERT WINES<sup>1</sup>**

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EVIDENCE for the existence of an unusual species of *Lactobacillus* capable of growing in wines of high alcohol content has accumulated during the past two decades. The organism is exacting in its growth requirements. Hence the pure-culture studies carried out independently in Australia and California have not been made until recently.

Douglas and McClung (1937)<sup>5</sup> isolated from California wines a bacterium capable of causing spoilage in the presence of more than 20 per cent of alcohol by volume and commonly occurring as masses of long entangled filaments. These authors described some of the characteristics of the organism, but owing to difficulties experienced in culturing it they did not describe it fully nor determine its systematic position.

Fornachon (1943) isolated a similar organism from Australian wines, described it, and placed it in the heterofermentative group of the genus *Lactobacillus*. He did not give the organism a specific name, but referred to it as *Lactobacillus* Type I.

Subsequent investigations have indicated the identity of the organisms studied by the Californian and Australian workers. As the morphology, physiology, and cultural characters of the organism distinguish it from other species of *Lactobacillus* which have been described, we propose that it be recognized as a species. For this new species we propose the name *Lactobacillus trichodes* because of its marked tendency to grow as long intertwined chains and filaments resembling a mass of hair.<sup>6</sup> The typical morphology of this species is shown in figure 1.

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<sup>5</sup> See "Literature Cited" for citations, referred to in the text by author and date.

<sup>6</sup> We are indebted to Dr. R. E. Buchanan for assistance with the nomenclature.

DESCRIPTION OF *LACTOBACILLUS TRICHODES* NOV. SPEC.

## Morphology (at 25° C)

Rods 0.4 to 0.6 by 2 to 4 microns, occurring singly, in pairs and in chains. The organism has a very marked tendency to grow into very long, threadlike chains and filaments, which frequently form a tangled mass. Nonmotile. Without endospores. Gram-positive in young cultures, but in old cultures some cells are Gram-negative.

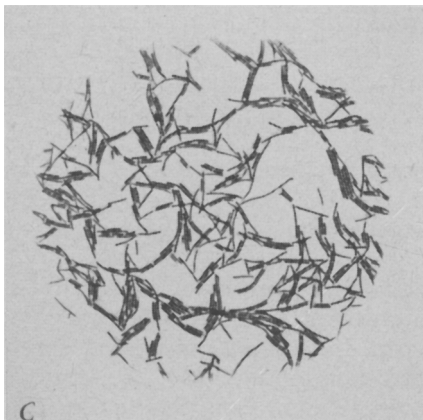
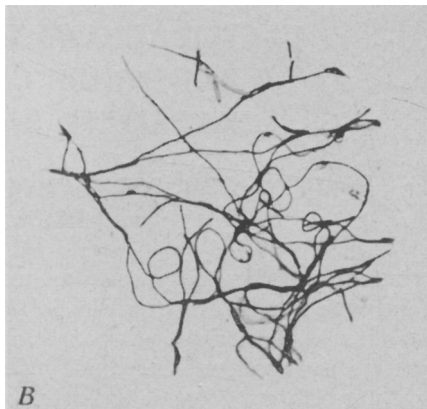
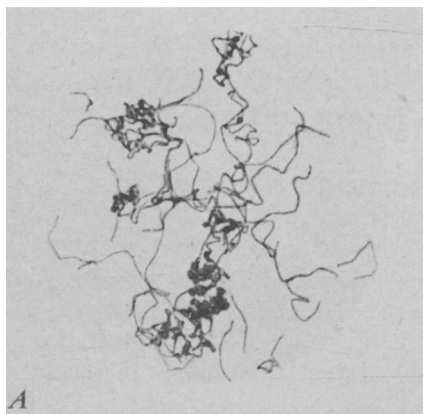


Fig. 1. Morphology of *Lactobacillus trichodes*: A, Deposit from wine infected with *L. trichodes*. Note the beaded appearance of old tangled filaments. B, Pure culture of *L. trichodes* showing long tangled filaments. Grown for 16 days at 23° C in autolyzed yeast glucose broth containing 15 per cent of alcohol. C, Pure culture of *L. trichodes* showing rods and short filaments after repeated subculture in alcohol-free, autolyzed yeast glucose broth. This culture was incubated 6 days at 23° C. (All  $\times 1,000$ .)

## Cultural Characteristics

*Autolyzed yeast glucose gelatin stab*: Growth, if any, scant. No liquefaction.

*Autolyzed yeast glucose agar slant*: Growth, if any, scant.

*Autolyzed yeast glucose agar colonies*: Colonies develop slowly; are small, subsurface, creamy white, and irregular in shape.

*Nutrient agar*: No growth.

*Potato slant*: No growth.

*Autolyzed yeast glucose broth*: Medium becomes turbid after 3 to 5 days and exhibits a very pronounced silky, wavy appearance when shaken gently.



After 2 to 3 weeks the organism settles and forms a compact sediment. Sometimes the organism grows as a flocculent deposit consisting of long, tangled chains and filaments while the liquid above remains almost clear. No growth in nutrient broth, glucose peptone broth, bacto-yeast extract broth, or grape juice.

*Litmus milk*: No growth.

### Biochemical Characteristics

*Catalase*: This enzyme is not produced.

*Substances fermented*: Acid formed in glucose and fructose. Sometimes a little acid formed in sucrose and maltose. Arabinose, xylose, galactose, mannose, lactose, raffinose, glycerol, mannitol, malic acid, citric acid, and tartaric acid are not attacked.

*Products of fermentation*: Lactic and acetic acids, carbon dioxide, and alcohol are the chief products from glucose; and lactic and acetic acids, carbon dioxide, and mannitol are formed from fructose.

### Requirements for Growth

*Oxygen relations*: Micro-aerophilic.

*Optimum temperature range*: Optimum range for growth is 25° to 30° C in alcohol-free media and 20° to 25° C in wine or other media of high alcohol content.

*pH range*: Optimum initial pH range for growth is between 4.5 and 5.5. The organism usually fails to grow when the initial pH of the medium is above 5.8 or below 3.5.

*Alcohol tolerance*: Grows vigorously in wine containing 20 per cent of alcohol by volume. A few strains grow in 21 per cent alcohol.

*Suitable media*: Vigorous growth has been obtained only in wine and in media containing yeast autolysate.

### Ecology

*Sources from which isolated*: Dessert and appetizer wines and lees.

*Geographical distribution*: Widely distributed where dessert and appetizer wines are made and handled in California and other parts of the United States and in Australia. Viable cultures have also been obtained from samples of Spanish sherry and Italian vermouth.

*Common names*: In California, the bacterium is commonly referred to as the "hair bacillus" and, less frequently, as the "cottony bacillus," "cottony mold," or "Fresno mold."

### Distinguishing Characteristics

*Lactobacillus trichodes* may be distinguished from all other lactobacilli because it has been found only in appetizer and dessert grape wines containing 20 per cent of alcohol by volume; regularly grows as a mass of tangled chains of cells and long filaments forming a flocculent sediment in the depths of these wines leaving the supernatant clear; requires yeast autolysate for its growth and must be trained to grow well in the absence of alcohol, although alcohol is apparently not required for the nutrition of the organism.

**LITERATURE CITED**

DOUGLAS, H. C., and L. S. McCLUNG.

1937. Characteristics of an organism causing spoilage in fortified sweet wines. *Food Research* 2(6):471-75.

FORNACHON, J. C. M.

1943. Bacterial spoilage of fortified wines. 126 p. Australian Wine Board, Adelaide.

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