MICROBIOLOGICAL STUDIES OF PHYTOPATHOGENIC BACTERIA

On 2-Ketogluconic Acid Fermentation by the Bacteria Belonging to the *Erwinia amylovora* Group (Preliminary Report)

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It is a well known fact that the genus ***Erwinia*** comprises gramnegative, non-sporulating, peritrichous phytopathogenic bacteria which are assigned to the family ***Enterobacteriaceae*** according to Bergey's Mannual of Determinative Bacteriology, 7th edition (1957). They are divided according to their characteristic symptons on host materials into two groups ; the first group includes strains identical with ***Erwinia amylovora*** that causes dry necroses, galls or wilts in plants but not a soft rot (***Erwinia sensu stricto***) and the second group includes strains identical with ***Erwinia carotovora*** that normally causes soft rots in plants (belong in the genus ***Pectobacterium*** Waldee).

A great number of the genera of the family ***Enterobacteriaceae*** have been profoundly investigated from the biochemical point of view, while the genus ***Erwinia***, especially the first group centering around ***Erwinia amylovora***, has received very little attention in this respect.

In the course of a comparative investigation on the fermentation pattern of carbohydrate between the bacteria of the genus ***Erwinia*** and the other genera of the ***Enterobacteriaceae***, the authors found an interesting phenomenon in which strains of ***Erwinia amylovora*** group produced a large amount of 2-ketogluconic acid from glucose in growing culture under aerobic conditions.

EXPERIMENTAL

Experiments were carried out on a shaker at 28°C with a medium containing 10% glucose, 0.3% polypeptone, 0.05% yeast extract, 0.1% K_2HPO_4, 0.2% NaCl, 0.04% $MgSO_4 \cdot 7H_2O$, 5 mg% $FeSO_4 \cdot 7H_2O$ and 8% $CaCO_3$ (sterilized separately). The medium was dispensed in 120 m*l* quantities in 500 m*l* shaking flasks and then sterilized. Two m*l* of a 3 days' culture of ***Erwinia amylovora*** ATCC 7400, which was grown aerobically in a potato-medium were used as the inoculum.

In Figure 1, an example of fermentation was shown. Similar results were obtained with different strains of ***Erwinia amylovora*** group; ***E. amylovora*** ATCC 7398 and 7401, ***E. tracheiphila*** ATCC 11417, and four strains of ***E.***

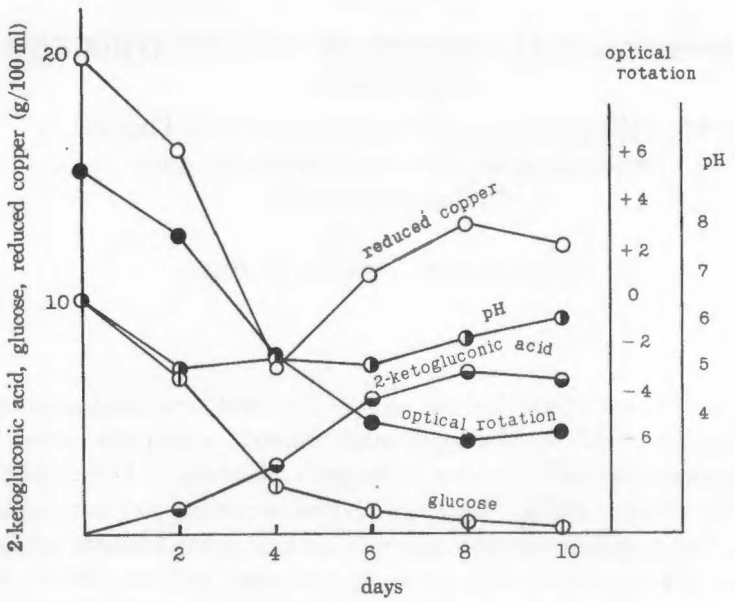

Fig. 1. Chemical changes in 2-ketogluconic acid fermentation.

milletiae which were isolated from the galls on the Milletia plants in the garden of the Ohara Institute. When the maximum negative rotation was observed, the culture was centrifuged. To the supernatant an equal volume of ethanol was added slowly with stirring and the mixture kept to stand overnight at 0°C. A precipitate formed was collected by decantation and recrystallized from the minimal amount of water to obtain the pure preparation. This preparation had reducing power on Fehling's solution and gave no colored products on reaction with naphthoresorcinol, phloroglucinol or orcinol. And also it appeared as deep olive green spot on the paper with orthophenylenediamine spray. The melting point of its potassium salt was 152°C, not depressed by the admixture with authentic potassium salt of 2-ketogluconic acid. And its methyl ester melted at 171—172°C. Results of analysis of its methyl ester were as follows:

Found: C, 40.46; H, 6.06
Calcd. for $C_7H_{12}O_7$; C, 40.38; H, 5.82

These results were in good accordance with values of methyl ester of 2-ketogluconic acid found in the literature (Ohle and Wolter, 1930).

The outstanding feature of the 2-ketogluconic acid fermentation by strains of ***Erwinia amylovora*** group was that 2-ketogluconic acid was the only main product (the yield was about 60—80% on glucose consumed) and such by-product as 5-ketogluconic acid was not detected.

The results of our further studies on the genus ***Erwinia*** have revealed that all the strains studied are divided according to their fermentation pattern of carbohydrate into two groups which correspond on the whole to two species of the genus

Erwinia.

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LITERATURE

Ohle, H. and Wolter, R. 1930. Zur Kenntnis der 2-Keto-gluconsäure (II. Mitteil.). Ber. 63 : 843—852.