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Oxygen and the Micro-aerophilic Bacteria: Growth and Peroxidase Activity of *Lactobacillus casei*

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The total growth and the specific growth rate of *Lactobacillus casei* ATCC 7469 are maximal and constant between 1 and 46 % of the atmospheric oxygen. The specific activity of the inducible NADH peroxidase rises to a maximum during the first hour of the exponential growth. The organism responds to increasing oxygen concentration with higher specific activity of this enzyme. The specific activity of NADH oxidase is independent of the growth phase and of the concentration of dissolved oxygen in the medium.

The bacteria of the genus *Lactobacillus* are usually regarded as micro-aerophilic or anaerobic.[1,2] The weak respiratory metabolism in these species is mediated by flavoproteins yielding hydrogen peroxide which can cause auto-intoxication. The survival of these bacteria in the presence of oxygen depends on the activity of NADH peroxidases which are also flavo-proteins.[3,4]

Brown and VanDemark [5] have studied the respiratory enzymes of a strain of *L. casei* indifferent to atmospheric oxygen. They demonstrated the presence of NADH oxidase and peroxidase in the cells. The total oxidase and peroxidase activities were NADPH dependent to the extent of less than 10 %. Aerobic growth increased the NADH oxidase and peroxidase activities twofold. The authors did not report the oxygen concentrations used in the experiments nor did they compare the enzyme activities with the growth of the organism.

Electrometric oxygen determinations have been used to study the effect of oxygen on the growth of only one microaerophilic organism, *L. pastorianus*.[6] The maximal total growth was obtained between 1 and 4 % oxygen in the gas phase. A further increase in oxygen concentration up to 20 % had only a slight inhibitory effect.

In the present work, the effect of oxygen on the total growth and the exponential (= maximum) specific growth rate of *L. casei* was studied. Oxidative metabolism and detoxication of hydrogen peroxide were investigated during growth at different oxygen concentrations by measuring NADH oxidase and NADH peroxidase activities.

EXPERIMENTAL

Organism. L. casei ATCC 7469 was used in the experiments.

Culture methods. The bacteria for the inoculum were grown in a stationary liquid nutrient medium [7] for 1 day at 37°C. An inoculum of 5 ml was used and previously adjusted to a concentration of 0.75 mg/ml of cells (dry weight). It was also important to saturate the inoculum with the gas mixture before inoculation. The organism was grown at 37°C in 250 ml batches of nutrient medium which was continuously flushed with a gas mixture through a tube terminating in a sintered glass disc. The oxygen concentration was varied from 0.1 to 100 %, corresponding to 0.005—0.950 μmoles oxygen/ml nutrient medium. The lowest oxygen concentration, 0.1 % (0.005 μmoles oxygen/ml), was obtained by using commercially available (flask) nitrogen alone. The gas mixtures were prepared by mixing this nitrogen and air or 100 % oxygen through rotameter type flowmeters. The exact concentrations of dissolved oxygen were measured using a G.M.E. Oxygraph with a vibrating platinum electrode. The bacterial density was determined on samples taken at 2 h intervals from the cultures. All the other culture conditions were kept constant.

In the preliminary experiments it was found that the mixing system for gases was adequate giving constant values for dissolved oxygen during the growth cycle, provided that the nutrient medium was flushed with each gas mixture at a rate of at least 1 ml per minute per ml of medium. To minimize evaporation caused by this high flow rate, the gas mixtures were saturated with water at 37°C.

Preparation of cell-free extracts. The cells were collected from the samples by centrifugation, washed twice with distilled water and suspended in cold (+4°C) 0.05 M phosphate buffer (pH 6.8) to give a concentration of 1 mg/ml of cells (dry weight). 10 ml of this suspension was saturated with flask nitrogen and shaken for 60 min with 5 g of Ballotini beads (No. 12) in a Mickle tissue disintegrator at the maximum speed and amplitude. The broken cells and Ballotini beads were removed by centrifugation and the cell-free supernatant was used in the analyses.

Measurement of enzyme activities. The NADH oxidase and NADH peroxidase activities in the cell-free extracts were measured as described by Strittmatter.[7]

Definitions of specific enzyme activities. One unit of NADH oxidase catalyses the consumption of 1 μmole/min of oxygen in 1 ml of cell-free extract under specified conditions.

One unit of NADH peroxidase catalyses the disappearance of 1 μmole/min of hydrogen peroxide in 1 ml of cell-free extract under specified conditions.

The specific activity is expressed in both cases as enzyme units/mg cells (dry weight) originally present in the suspension.

RESULTS

Growth behaviour of *L. casei*

The effect of oxygen on the growth of *L. casei* is shown in Fig. 1 where two parameters, total growth and exponential (= maximum) specific growth rate, are used to define the optimal growth conditions for the micro-organism. The specific growth rate and the total growth were maximal between 1 and 46 % oxygen in the gas phase. Practically no growth was observed when the gas was flask nitrogen (0.1 % oxygen).

The total growth was the same with 60 % and 0.5 % oxygen in the gas phase, and the cell mass produced with 100 % oxygen was equal to the cell mass produced with 0.2 % oxygen. Outside the broad range of optimum concentrations, at oxygen concentrations of 0.5, 60, and 100 %, the specific growth rate was about half of the maximum.

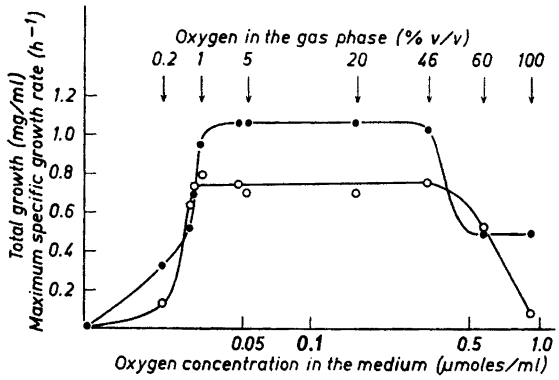

Fig. 1. Total growth and maximum specific growth rate of *Lactobacillus casei* ATCC 7469 as functions of the oxygen concentration in the medium. The total growth (open circles) is expressed as milligrams of cells (dry weight) per ml of nutrient medium. The maximum specific growth rate, μ, (closed circles) was calculated from data for the exponential growth phase

$$\mu = \frac{1}{x}\frac{dx}{dt}$$, where x is the dry weight of the cells at time t.

Enzyme activities during the growth cycle

The specific activity of NADH oxidase rose during the first hour of the lag phase to a rather low value which remained constant during subsequent growth phases of the bacterium. On the other hand, the specific activity of peroxidase increased rapidly during the late lag phase and the acceleration phase. The activity of the peroxidase rose to a maximum during the first hour of the usually 5-hour-long exponential phase and then decreased during subsequent growth. When the specific activity had decreased to the level in the inoculum, exponential growth ceased in all cultures regardless of the oxygen

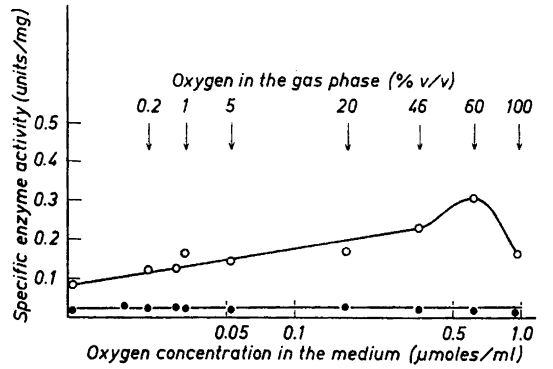

Fig. 2. The specific activities of NADH peroxidase (open circles) and NADH oxidase (closed circles) in *Lactobacillus casei* ATCC 7469 as function of oxygen concentration in the medium. For definitions of unit enzyme activities, see Methods.

concentration. When the oxygen concentration in the gas phase was 0.1 or 100 % the exponential growth continued for 1.5 h only, and the maximum specific activity of peroxidase was reached even in the acceleration phase.

The effect of oxygen on the enzyme activities

The metabolic response of *L. casei* to different oxygen concentrations in the medium is illustrated in Fig. 2. The specific activity of NADH oxidase did not vary with the oxygen concentration. The growth at 20 % oxygen in the gas phase increased the activity of NADH peroxidase twofold compared to the peroxidase activity of the cells grown at 0.2 % oxygen. A fourfold increase and a maximum specific peroxidase activity was found in the cells grown at 60 % oxygen in the gas phase. At 100 % oxygen, the peroxidase activity was lower.

DISCUSSION

The observed wide limits of oxygen tolerance of *L. casei* are contrary to earlier statements as to the micro-aerophilic nature of the organism, but agree with results obtained with *L. pastorianus* by Weinfurtner *et al.*[6] They observed, however, some growth even in the complete absence of oxygen, whereas we failed to obtain growth at 0.1 % oxygen in the gas phase (flask nitrogen). This discrepancy is probably due not only to the different species, but also to the fact that Weinfurtner and co-workers did not saturate their inocula with nitrogen before the growth experiments and thus carried oxygen over to the media.

Brown and VanDemark[5] reported that the NADH oxidase and NADH peroxidase activities increase in *L. casei* grown in aerobic conditions. We have confirmed and amplified these observations with NADH peroxidase. An increase in the oxygen concentration of the gas phase did not, however, lead to a higher NADH oxidase level in *L. casei* ATCC 7469. This may be due to the different strains. Unfortunately, Brown and VanDemark did not specify their strain of *L. casei*.

In our opinion, the present data show that NADH peroxidase is an inducible enzyme in *L. casei*. The synthesis of this enzyme ceases at the beginning of exponential growth, *i.e.* when the most rapid division ensues. The dividing cells metabolize using the enzyme already synthesized. This leads to a decrease in the specific activity until there is too little peroxidase left in the cells to remove the growth inhibiting hydrogen peroxide.

The aerobic metabolism of *L. casei* does not depend on the concentration of dissolved oxygen determined by the maximum specific activity of NADH oxidase. On the contrary, the bacterium responds to increasing oxygen concentration by synthesizing more peroxidase and thus maintains the optimum conditions for growth up to an oxygen concentration of 46 % in the gas phase. At higher oxygen concentrations, partial auto-intoxication is not prevented by a further increase of peroxidase. Nevertheless, on the basis of the present data *L. casei* cannot be classified as micro-aerophilic in the sense that normal atmospheric oxygen pressure is toxic to its growth.

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