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Physiological Role of Pyruvate Oxidase in the Aerobic Metabolism of *Lactobacillus plantarum*

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Under aerobic growth conditions *Lactobacillus plantarum* produced acetic acid in addition to lactic acid. It was found that lactic acid was predominantly produced at first, and then when the carbohydrate was nearly exhausted, lactic acid was metabolized further to acetic acid. The most likely enzyme involved in the aerobic metabolism of *L. plantarum* is pyruvate oxidase. Its activity is enhanced in the presence of oxygen and is reduced in the presence of glucose. The specific activity of pyruvate oxidase is highest at the beginning of the stationary-growth phase, where a strong increase in acetic acid production was also observed.

It has been reported that oxygen serves as a hydrogen acceptor in certain lactobacilli (2, 11). The question is whether the aerobic metabolism is associated with energy yield. Molar growth yields of *Lactobacillus* species, determined under anaerobic and aerobic growth conditions, were identical (Y = 18.8 g/mol of glucose) (8), indicating that no additional energy was produced under aerobic growth conditions. However, Dirar and Collins (3, 4) found higher growth yields for *Lactobacillus plantarum* under aerobic conditions than under anaerobic growth conditions when low concentrations of galactose were used. Under aerobic growth conditions 93% of the galactose was converted to acetate. However, the pathway by which *L. plantarum* converts pyruvate to acetate and the enzymes involved in the aerobic metabolism are still unclear.

L. plantarum L809 was cultivated in a semisynthetic medium (Table 1). The cultivation was performed in a 10-liter fermentor (New Brunswick Scientific Co., Inc.) at 30°C. Under aerobic growth conditions the pH was normally held constant at 5.8 with 4 N NaOH; the medium was gassed with air (0.8 liter/min/per liter of medium); the agitation speed was set at 400 rpm. Under anaerobic growth conditions the pH was also held constant at 5.8, but the medium was constantly gassed with a slow stream of nitrogen; the agitation speed was reduced to 50 rpm. Growth was followed by measuring the optical density at 578 nm with an Eppendorf spectrophotometer and by dry weight determination from 100 ml of culture. The molar growth yield is defined as grams (dry weight) of bacteria per mol of monosaccharide consumed. Glucose, lactose, acetate, and lactate were determined according to the method of Bergmeyer (1). Lactate dehydrogenases, NADH oxidase, and pyruvate oxidase were measured as previously described (5, 6, 9, 10).

The effect of anaerobic and aerobic growth conditions upon the utilization of glucose and lactose and the production of lactic acid and acetic acid was investigated (Fig. 1 and 2). It was found that under anaerobic growth conditions only D- and L-lactic acids were produced; acetic acid was detectable only in trace amounts or not at all. Under aerobic conditions, in addition to lactic acid, acetic acid was also produced. The amount of acetic acid formed depended on the sugar compound. With glucose only small amounts of acetic acid (4 to 8%) were produced, whereas with lactose more than 50% of the excreted acids consisted of acetic acid. Under aerobic growth conditions the amount of excreted lactic acid reached a maximum at the beginning of the stationary phase. Subsequently, the lactic acid was gradually consumed, and almost simultaneously an equivalent amount of acetic acid was produced. This effect was again much more pronounced with lactose than with glucose.

L. plantarum metabolized glucose much more rapidly than lactose. Under aerobic growth on glucose the mean doubling time was 2.85 h; during growth on lactose the doubling time increased to 4.75 h. Under anaerobic growth the difference in the doubling time between glucose (2.7 h) and lactose (3.4 h) cells was less pronounced.

The dry weight was determined at intervals throughout the stationary phase. The maximal cell yield per liter of medium is given in Table 2. The cell yield of *L. plantarum* was always significantly higher during aerobic growth than during anaerobic growth. The Y values under anaerobic growth conditions were relatively constant. Under aerobic growth conditions the mean values for the molar growth yield for glucose

TABLE 1. Composition of medium

Component[a]	Amt per liter
Glucose or lactose	2.0 g
Hydrolyzed casein (E. Merck AG)	2.5 g
KH_2PO_4	2.0 g
$MgSO_4 \cdot 7H_2O$	100 mg
$MnSO_4 \cdot H_2O$	20 mg
L-Asparagine	150 mg
L-Cystine	150 mg
D- and L-Tryptophan	100 mg
D- and L-Methionine	0.5 mg
Thiamin	0.5 mg
Riboflavin	0.5 mg
Flavin adenine mononucleotide	0.5 mg
Calcium pantothenate	0.5 mg
L-Ascorbic acid	0.5 mg
Nicotinic acid	0.5 mg
Pyridoxal	0.1 mg
p-Aminobenzoic acid	0.1 mg
Biotin	5.0 μg
Folic acid	5.0 μg

[a] Sugar, salts, amino acids, and vitamins were separately sterilized.

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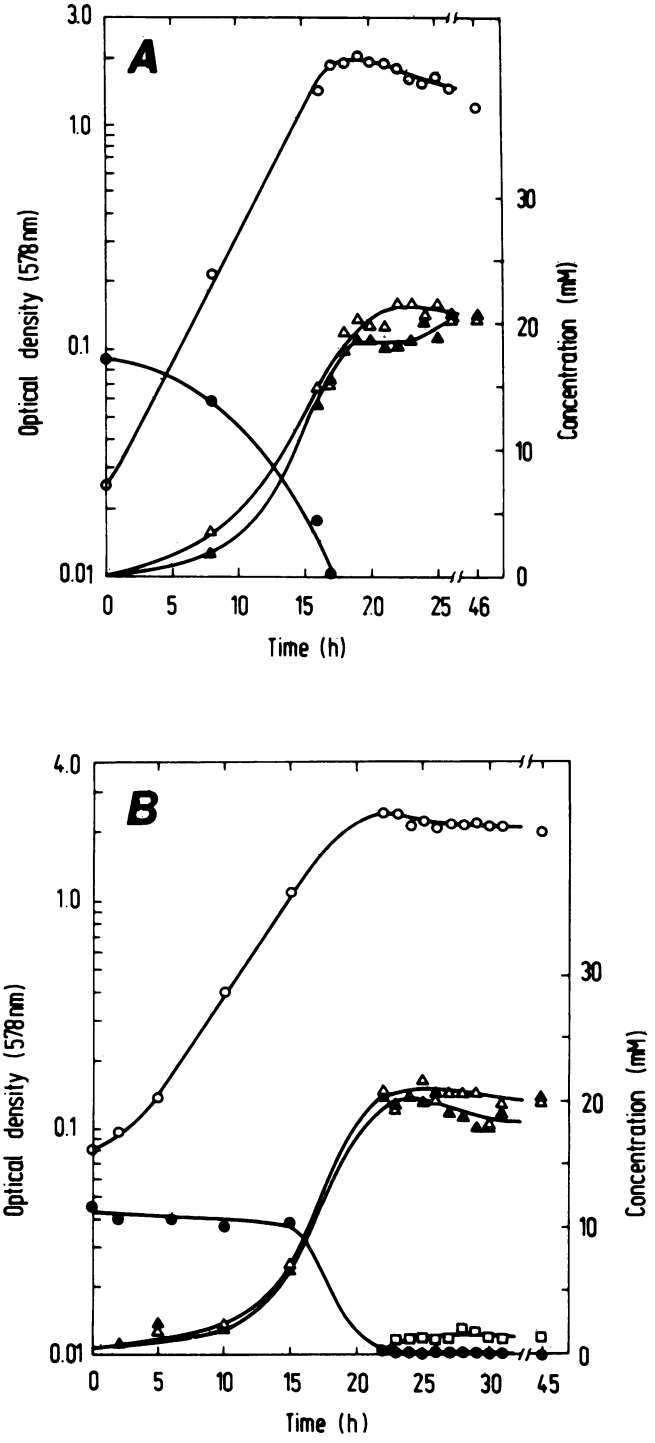

FIG. 1. Growth curves for *L. plantarum* L809. Cells were grown in the semisynthetic medium under anaerobic conditions with (A) 0.4% glucose and (B) 0.4% lactose. Symbols: ○, optical density; ●, sugar concentration in the medium; ▲, D-lactic acid; △, L-lactic acid; □, acetic acid.

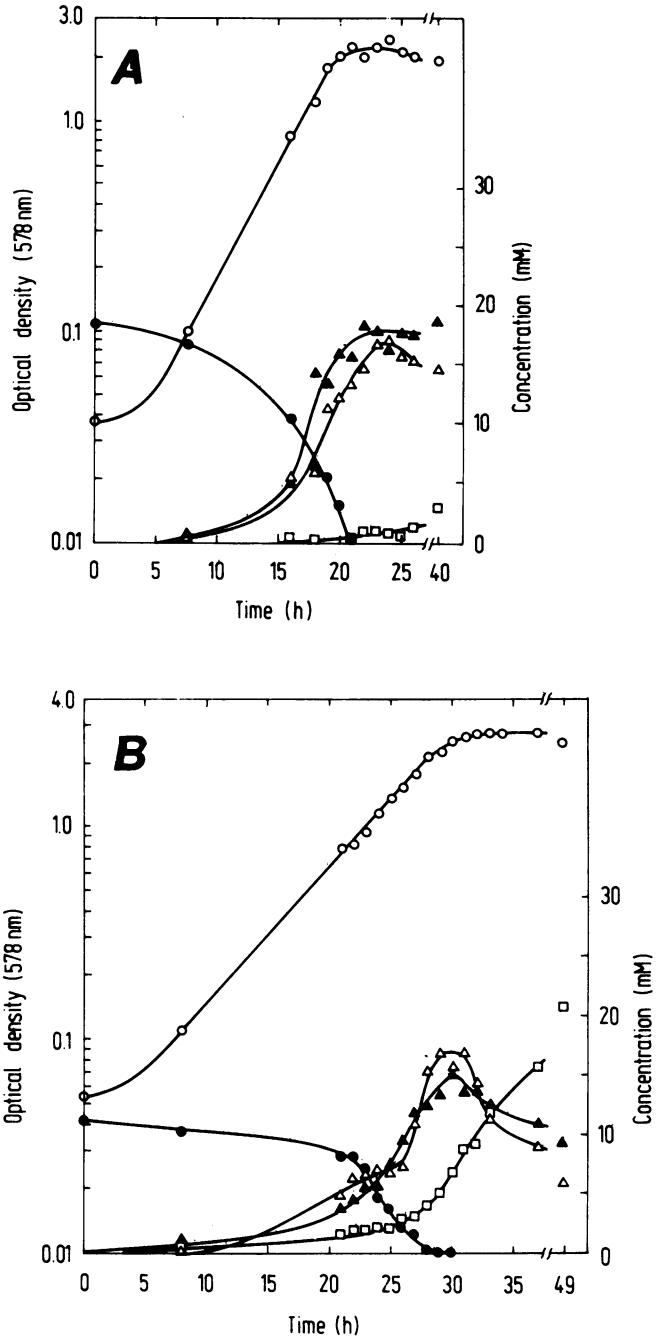

FIG. 2. Growth curves for *L. plantarum* L809. Cells were grown in the semisynthetic medium under aerobic conditions with (A) 0.4% glucose and (B) 0.4% lactose. Symbols are as shown in the legend to Fig. 1.

and lactose were 25.1 and 32.6, respectively. These results show that the *Y* values under aerobic growth conditions were significantly higher than those under anaerobic growth conditions. Higher molar growth yield was coupled to an increase in acetic acid production.

Under aerobic conditions the specific activity of pyruvate oxidase was found to be five- to sixfold greater with lactose than with glucose (Table 2), indicating that the synthesis of pyruvate oxidase under aerobic growth conditions was repressed during growth on glucose. The NADH oxidase and lactate dehydrogenase activities were not essentially affected, either by the sugar compound or by oxygen. The specific activity of the NADH oxidase was more or less constant during growth, whereas that of pyruvate oxidase increased

TABLE 2. The influence of oxygen, sugar compound, and sugar concentration on the fermentation pattern, growth yield, and enzyme activity of *L. plantarum*[a]

Growth conditions	Actual sugar concn[b] (mM)	Maximum yield[c] (mg [dry weight]/liter)	Time[d] (h)	Fermentation products[e]			Molar growth yield (*Y*) (g [dry weight]/mol of monosaccharide)	Enzyme sp act (mU/mg [dry weight])		
				Lactic acid (mM)		Acetic acid (mM)		Pyruvate oxidase	NADH oxidase	D- and L-lactate dehydrogenase
				D	L					
Aerobic										
O_2 + glucose (0.2%)	10.7	261	24	8.6	8.0	1.8	24.3	9.2	25.7	5,689
O_2 + glucose (0.4%)	18.6	481	25	16.3	17.1	3.0	25.9	6.6	42.1	6,914
O_2 + lactose (0.2%)	5.4	350	32	5.9	4.6	13.7	32.4	67.7	38.5	4,507
O_2 + lactose (0.4%)	11.2	610	37	10.8	8.9	15.7	27.1	38.4	21.1	6,050
Anaerobic										
N_2 + glucose (0.2%)	9.2	190	22	10.4	9.8	0	20.9	3.3	28.1	7,729
N_2 + glucose (0.4%)	17.1	325	22	19.3	18.9	0	19.0	3.2	21.2	6,101
N_2 + lactose (0.2%)	5.4	217	27	9.1	11.8	0.1	22.1	9.7	43.3	4,021
N_2 + lactose (0.4%)	11.8	483	29	17.9	20.8	1.3	20.4	7.9	50.7	7,089

[a] Data are derived from the growth curves.
[b] The actual sugar concentration in the fermentor before inoculation.
[c] The maximal dry weight which was achieved in the stationary-growth phase.
[d] The time at which the sample was taken.
[e] Minor amounts of acetoin (<1%) are produced by aerobic lactose degradation.

during the course of the log phase, reached an optimum in early-stationary phase, and declined later (Fig. 3). These results show that pyruvate oxidase was induced or derepressed by a particular compound, which exerted its maximal effect at the end of the log phase. The question remains as to why glucose and not lactose-derived glucose represses pyruvate oxidase. An explanation could be that a much lower growth rate was obtained with lactose than with glucose. The slowdown in the growth may decrease the size of the intermediate pools and may thereby effect the derepression of pyruvate oxidase.

In the semisynthetic medium with and without 200 mM D- and L-lactate, growth of *L. plantarum* was studied at various pH values and under aerobic and anaerobic growth conditions. Growth of *L. plantarum* in this medium could only be observed in the presence of lactate and under aerobic conditions (Fig. 4A). Growth was optimal within a pH range

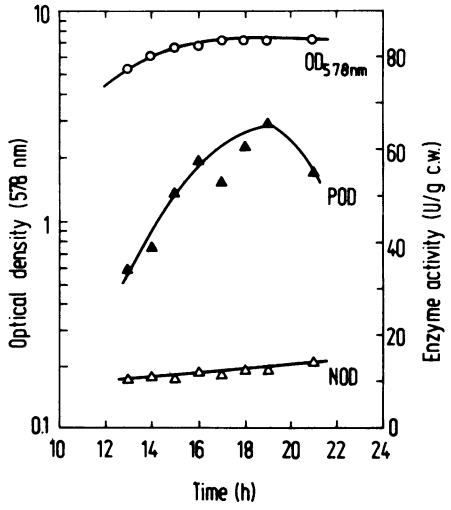

FIG. 3. Influence of the growth phase of *L. plantarum* L809 upon pyruvate oxidase and NADH oxidase. Cells were grown under aerobic conditions in a complex medium (10). Symbols: ○, optical density; ▲, pyruvate oxidase activity; △, NADH oxidase activity. Enzyme activity is expressed in units per gram of cells (wet weight).

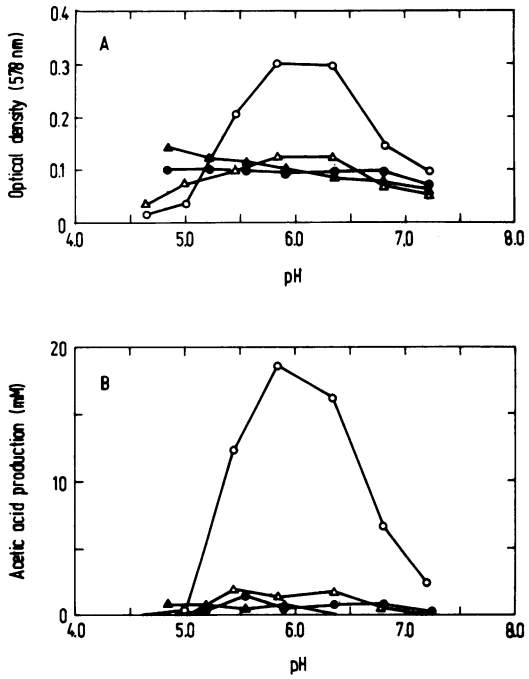

FIG. 4. Influence of oxygen and pH upon the growth of *L. plantarum* L809 on lactate. Cells were cultivated in the semisynthetic medium at various pH values in the presence and absence of D- and L-lactate (200 mM) and in the presence and absence of oxygen. The pH of the medium was adjusted with H_2SO_4 or NaOH. Cells were cultivated for 48 h at 30°C, and subsequently the optical density (A) and the excreted acetic acid (B) were determined. Symbols: ○, aerobic cultivation with lactate; ●, anaerobic cultivation with lactate; △, aerobic cultivation without lactate; ▲, anaerobic cultivation without lactate.

of 5.5 to 6.5. Little or no growth was observed under anaerobic conditions and in the absence of lactate. Growth on lactate also correlated well with the excretion of acetate (Fig. 4B). The aerobic metabolism of lactate, however, only supported limited growth of *L. plantarum*. This may be due to increased pyruvate oxidase activity and a reduced NADH pool, leading to an inability to degrade H_2O_2 and to carry out sufficient gluconeogenesis. The regulation of the activities of lactate dehydrogenase, NADH oxidase, NADH peroxidase, and pyruvate oxidase appears to be important in the aerobic lactate metabolism of *L. plantarum* since the various enzymes influence the intracellular concentrations of pyruvate and lactate and NADH + H^+ and NAD.

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