Active Role of Oxygen and NADH Oxidase in Growth and Energy Metabolism of *Leuconostoc*

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Growth of 12 *Leuconostoc* strains in a broth medium with and without aeration was compared. In general, the aerated cultures grew faster and produced more biomass, at the expense of glucose and other sugars, than unaerated cultures. The more efficient growth correlated well with the production of acetate rather than ethanol as an end-product of metabolism in aerated cultures; unaerated cultures produced little or no acetate.

Mutants of *L. mesenteroides* X2 were isolated that had lost the capacity to be stimulated by aeration; they were completely deficient in NAD(P)H oxidase activity and did not accumulate acetate in aerobic cultures. Without NAD(P)H oxidase the mutants rely on the ethanol branch of the heterolactate pathway to regenerate NAD(P)⁺ from NAD(P)H, irrespective of the presence or absence of O₂. The presence of NAD(P)H oxidase in parental cultures allows them to utilize O₂ as a terminal electron acceptor and produce more ATP per mol of sugar utilized when O₂ is available than when it is limiting.

INTRODUCTION

The genus *Leuconostoc* is regarded as a legitimate taxonomic group of the lactic acid bacteria and as such it is generally believed that the sole energy-producing mechanism of members of this genus is an O₂-independent fermentation. By this mechanism (heterolactic fermentation pathway) hexoses are converted to equimolar quantities of lactate, ethanol and CO₂ (DeMoss *et al.*, 1951). The early work on hexose metabolism was done with *L. mesenteroides* strain 39 (ATCC 12291), which did not utilize O₂ and produced the same end products in the presence and absence of O₂. Later work indicated that this strain was unusual as several other strains of *L. mesenteroides* did react with O₂ (Johnson & McCleskey, 1957, 1958). From studies in which growth data were reported it seems that growth of several strains at the expense of hexoses was faster in the presence of O₂ than in its absence (Johnson & McCleskey, 1957; Whittenbury, 1963, 1966; Fitzgerald, 1983), indicating that O₂ must have an active rather than a passive role in the energy metabolism of these bacteria.

During aerobic growth acetate is a major end-product of hexose metabolism by *Leuconostoc* (Ito *et al.*, 1983; Johnson & McCleskey, 1957; Keenan, 1968; Yashima *et al.*, 1970). Acetate formation via acetate kinase conserves energy-rich phosphate, which is otherwise lost when acetyl phosphate is reduced to ethanol to regenerate NAD⁺ in the absence of O₂. Such a mechanism presumes an O₂-dependent system of NADH oxidation to regenerate NAD⁺ needed to dehydrogenate glucose 6-phosphate and 6-phosphogluconate; NADH oxidases have been demonstrated in strains of *Leuconostoc* (Kawai *et al.*, 1971; Fitzgerald, 1983).

In this study we compare growth rates, biomass yields, end products of hexose and some relevant enzyme activities in aerated and unaerated cultures of 12 parent strains of *Leuconostoc* and NAD(P)H oxidase negative mutants of *L. mesenteroides* X2.

METHODS

Bacteria and growth conditions. The strains used are listed in Table 1. They were grown in the medium of de Man, Rogosa and Sharpe, designated MRS (de Man *et al.*, 1960), at pH 6.8, without the acetate, citrate or Tween 80. Glucose, lactose, galactose or maltose (1%, w/v, unless otherwise stated) were the energy sources. Aerated cultures

Table 1. *Effect of aeration on growth rates*

Strain	Origin*	Ratio of specific growth rates† of cultures growing on‡:			
		Glucose	Galactose	Lactose	Maltose
<i>Leuconostoc mesenteroides</i> X2	T. Cogan	2.04	1.65	2.17	1.39
<i>L. mesenteroides</i> ATCC 12291	ATCC	1.00	NG	NG	1.00
<i>L. mesenteroides</i> 523	NCDO	2.04	1.26	1.47	1.03
<i>L. paramesenteroides</i> 7-1	T. Cogan	1.40	NG	2.18	1.13
<i>L. paramesenteroides</i> 9-1	T. Cogan	1.47	NG	2.06	1.13
<i>L. paramesenteroides</i> 803	NCDO	1.32	ND	ND	ND
<i>L. paramesenteroides</i> 871	NCDO	1.33	ND	ND	ND
<i>L. lactis</i> NCW-1	T. Cogan	1.72	NG	1.77	1.22
<i>L. lactis</i> 533	NCDO	1.80	ND	ND	ND
<i>L. lactis</i> N2	T. Cogan	1.48	NG	1.68	1.26
<i>L. cremoris</i> 543	NCDO	1.40	ND	ND	ND
<i>L. dextranicum</i> 812	NCDO	1.15	ND	ND	ND

ND, Not determined; NG, no growth.

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† Ratio of specific growth rates, k (h^{-1}), of cultures growing aerobically to those without aeration.

‡ MRS with sugar at 1% (w/v). Glucose was the only sugar used for NCDO strains.

were grown in shallow layers (e.g. 100 ml in a 500 ml Erlenmeyer flask) in shaking water baths (100 oscillations min^{-1}). Un-aerated cultures were in substantially filled unshaken flasks; anaerobic cultures had a head of N_2 . The growth temperature was 30 °C. Growth was monitored as OD_{580} and the data were converted to dry weight (mg ml^{-1}) by means of a calibration graph.

Mutant isolation. Mutants of *L. mesenteroides* X2 deficient in NAD(P)H oxidase (Nox) were isolated by treating suspensions of the parent culture in 0.1 M-sodium citrate buffer, pH 7.0, with 100 μg *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine ml^{-1} for 1 h at 30 °C, allowing for phenotypic expression and plating aerobically on MRS glucose plates. Small colonies were checked for their ability to produce equal biomass yields in MRS glucose broth cultures incubated with or without aeration, or for their inability to produce colonies on MRS mannitol plates incubated aerobically. Both classes of mutants were checked for NAD(P)H oxidase activity and deficient ones were designated Nox mutants.

End-products of sugar metabolism. Acetate, ethanol, acetoin, diacetyl and butylene glycol were measured by the gas chromatographic method of Thornhill & Cogan (1984). D(–)Lactate was assayed by the method of Gawehn & Bergmeyer (1974). Hydrogen peroxide was measured according to Dempsey *et al.* (1975).

O₂ utilization by whole cells. An O₂ electrode was used to measure O₂ uptake by washed cell suspensions in buffered substrate as described previously (Murphy & Condon, 1984).

Enzyme assays in cell-free extracts. Cell-free extracts were made from concentrated, washed cell suspensions in 50 mM-sodium phosphate buffer, pH 7.0, at 0 °C, by extrusion in a French press operated at 147 MPa. Extracts were maintained at 0 °C and assayed as quickly as possible. Alcohol dehydrogenase activities were assayed in 50 mM-sodium phosphate buffer, pH 7.0, containing 20 mM-acetaldehyde and 0.2 mM-NADH or NADPH. NADPH-dependent activities (EC 1.1.1.2) were generally two to four times those of NADH-dependent activities (EC 1.1.1.1).

The other enzyme activities were assayed according to the following procedures: NADH oxidase and NADH peroxidase, Anders *et al.* (1970); NADPH oxidase, Kawai *et al.* (1971); phosphoketolase (EC 4.1.2.9), Goldberg *et al.* (1966); acetate kinase (EC 2.7.2.1), Lees & Jago (1976); phosphate acetyltransferase (EC 2.3.1.8), Yashima *et al.* (1971); acetaldehyde dehydrogenase (EC 1.2.1.10), Stadtman & Burton (1957); NAD⁺-dependent D(–)lactate dehydrogenase (EC 1.1.1.28), Murphy *et al.* (1985). All specific activity units are $\mu\text{mol} (\text{mg protein})^{-1} \text{min}^{-1}$. The protein concentration in cell-free extracts was determined by the Lowry method.

RESULTS

Effects of aeration on growth

Of the 12 parent *Leuconostoc* strains studied 11 generally grew more efficiently in the presence rather than in the absence of air. The greater efficiencies were expressed as shorter lag periods, higher growth rates and greater biomass yields. All cultures were grown in MRS glucose and

Fig. 1. Growth (a) and production of acetate (b), D(-)lactate (c) and ethanol (d) in MRS glucose medium by *Leuconostoc mesenteroides* X2 in steady-state aerated (■) and un-aerated (▲) cultures and in transitional cultures (●, aeration after 4 h of un-aerated growth). Acetate was not detected in the steady-state un-aerated culture.

seven cultures in MRS with galactose, lactose or maltose as the energy source. Growth rate and yield data were quite reproducible, varying by less than 5% in duplicate experiments. With the exception of *L. mesenteroides* ATCC 12291, specific growth rates of aerated cultures were generally higher than those in un-aerated cultures (Table 1). This was especially true of glucose- or lactose- grown cultures in which aeration allowed growth rates 40–100% more than those in un-aerated cultures. The higher growth rates were not due to diminished rates of acid accumulation, as the pH values of aerated cultures decreased faster than those of un-aerated cultures. With maltose as the energy source the differences between specific growth rates of aerated and un-aerated cultures were not as great as those at the expense of glucose or lactose, but in most cases the absence of aeration induced longer lag periods than those in the corresponding aerated cultures, even though the inocula for all cultures were grown without aeration.

L. mesenteroides X2 (Fig. 1) and *L. lactis* NCW-1 (data not shown) were grown with glucose or lactose as the energy source without aeration to mid exponential phase and then aerated. New growth rates characteristic of those of balanced aerated cultures were established within 15–30 min. Sudden aeration of *L. mesenteroides* ATCC 12291 had no effect on growth rate.

The final culture densities were generally substantially greater in aerated than in un-aerated cultures. In a detailed analysis of three strains growing in MRS medium with limiting glucose or lactose (Table 2), biomass yields of aerated *L. mesenteroides* X2 or *L. lactis* NCW-1 cultures were substantially greater than those of un-aerated cultures. Greater differences were observed at lower concentrations of sugar; in some comparisons the biomass yields from aerated cultures were at least threefold greater than those of un-aerated cultures. Yields of *L. mesenteroides* ATCC 12291 were not affected by aeration.

Some cultures were tested for H_2O_2 production (Table 3). It was not detected in cultures of *L. mesenteroides* X2 or *L. mesenteroides* ATCC 12291. The latter did not utilize O_2 and the enzyme system in the former, responsible for O_2 utilization [NAD(P)H oxidase; see Tables 4 and 5], catalysed formation of H_2O rather than H_2O_2 . All strains tested accumulated H_2O_2 in aerated but not in un-aerated cultures. Accumulation did not exceed 0.1 mM until the late exponential or early stationary phase and eventually reached 0.5 to 1.0 mM after 24 h. The presence of catalase

Table 2. *Effect of aeration on growth yields*

Strain	Incubation conditions	Growth yields (mg l ⁻¹) at the expense of:							
		Glucose (mM)				Lactose (mM)			
		5	10	20	<i>Y</i> _{Glc} *	5	10	20	<i>Y</i> _{Lac} *
<i>L. mesenteroides</i> X2	Aerated	270	462	642	46	420	630	870	88
	Un aerated	68	147	355	18	110	290	452	29
<i>L. mesenteroides</i> Nox 1	Aerated	80	ND	340	17	ND	ND	ND	ND
	Un aerated	70	ND	330	16.3	ND	ND	ND	ND
<i>L. mesenteroides</i> ATCC 12291	Aerated	98	168	405	19.3	NG	NG	NG	NG
	Un aerated	108	160	385	18.5	NG	NG	NG	NG
<i>L. lactis</i> NCW-1	Aerated	185	310	720	37	440	650	710	88
	Un aerated	80	150	304	14.5	150	290	330	31

ND, Not determined; NG, no growth.

* Molar growth yields (g mol⁻¹).Table 3. *Comparison of the end-products of metabolism accumulated during aerated or un aerated growth*

Cultures of strains X2, Nox 1 and ATCC 12291 were assayed after 8 h and all others after 24 h growth in MRS glucose (56 mM) medium.

Strain	Metabolic end-product (mM)*							
	Aerated culture				Un aerated culture			
	D(-)Lactate	Acetate	Ethanol	H ₂ O ₂	D(-)Lactate	Acetate	Ethanol	
<i>L. mesenteroides</i> X2	27.5	18.4	7.8	0†	23.5	0	25.4	
<i>L. mesenteroides</i> Nox 1	27.2	0.52	28.4	0†	38	0.96	34.3	
<i>L. mesenteroides</i> ATCC 12291	54	0	32.5	0†	41	0	30.6	
<i>L. mesenteroides</i> 523	22.5	20.4	3.6	0.88	30	4.4	29.1	
<i>L. paramesenteroides</i> 7-1	30	17.9	6.3	0.60	30	2.9	31.5	
<i>L. paramesenteroides</i> 9-1	27.5	15.7	7.9	0.68	32.5	2.05	29.1	
<i>L. lactis</i> NCW-1	25	22.9	4.2	0.95	40	3.2	26.8	
<i>L. lactis</i> N2	20	18.3	6.7	0.83	27.5	2.0	32.5	

* Acetoin, diacetyl or butylene glycol were not detected in these cultures.

† H₂O₂ was not detected in these cultures irrespective of incubation time.

(860 Sigma units ml⁻¹) in aerobic cultures of *Leuconostoc* capable of H₂O₂ accumulation prevented H₂O₂ accumulation, but did not affect growth rates or yields, indicating that H₂O₂ accumulation did not inhibit these strains. NADH peroxidase activity was not detected in cell-free extracts of any of these strains.

End-products of sugar metabolism

Filtrates of aerated or un aerated cultures grown in MRS media were analysed for end-products characteristic of *Leuconostoc* metabolic pathways. Approximately similar data were obtained with glucose (56 mM), lactose (28 mM) or maltose (28 mM) as the energy source; those in Table 3 refer to metabolism of glucose. Diacetyl, acetoin or butylene glycol were not produced in any of the cultures tested. Both aerated and un aerated cultures had substantial amounts of D(-)lactate. Un aerated cultures produced substantial amounts of ethanol and little acetate whereas the aerated cultures produced much more acetate and much less ethanol. An exception to this observation was *L. mesenteroides* ATCC 12291, which produced D(-)lactate and ethanol whether or not cultures were aerated.

The patterns of end-product accumulation during aerated or anaerobic growth of *L. mesenteroides* X2 in MRS glucose (56 mM) are shown in Fig. 1. Acetate did not accumulate in the

Table 4. *NADH and NADPH oxidase activities in cell-free extracts*

Extracts were made from late exponential phase cultures growing aerobically in MRS glucose medium. The sensitivity of the assay was 0.002 units (mg protein)⁻¹.

Strain	Enzyme activity [units (mg protein) ⁻¹]	
	NADH oxidase	NADPH oxidase
<i>L. mesenteroides</i> X2	0.70	0.07
<i>L. mesenteroides</i> X2 Nox 1*	0	0
<i>L. mesenteroides</i> ATCC 12291	0	0
<i>L. mesenteroides</i> 523	1.01	0.05
<i>L. paramesenteroides</i> 7-1	0.79	0.04
<i>L. paramesenteroides</i> 9-1	0.76	0.03
<i>L. paramesenteroides</i> 803	0.22	ND
<i>L. paramesenteroides</i> 871	0.30	ND
<i>L. lactis</i> NCW-1	0.86	0.01
<i>L. lactis</i> N2	0.95	0.04
<i>L. lactis</i> 533	2.93	ND
<i>L. cremoris</i> 543	2.12	ND
<i>L. dextranicum</i> 812	0.46	ND

ND, Not determined.

* Ten other mutant strains (*L. mesenteroides* X2 Nox 2 to 11) were also without NADH oxidase activity.

unaerated culture throughout the growth period (Fig. 1*b*) and ethanol did not accumulate in the aerated culture to any substantial extent until growth slowed down (Fig. 1*d*). D(-)Lactate accumulated throughout, in aerated or unaerated cultures but at lower rates in the latter (Fig. 1*c*). In transitional cultures, after sudden aeration of anaerobic cultures, acetate accumulated rapidly coincident with the increase in growth rate; these changes were accompanied by a more gradual decrease in ethanol and an increase in D(-)lactate accumulation. Similar data were obtained with *L. lactis* NCW-1 in MRS glucose and with both strains in MRS lactose. Ethanol accumulation in aerated MRS glucose cultures of *L. mesenteroides* X2 was dependent on glucose concentration. Only traces of ethanol accumulated in cultures with less than 20 mM-glucose, the end products being D(-)lactate and acetate exclusively.

Key role of NADH oxidase

The coincidence of acetate accumulation, higher growth rates and higher yields in aerated cultures can be explained if acetyl phosphate is normally converted to acetate during aerobic metabolism and is diverted to ethanol only when O₂ becomes limiting. This proposal depends on an alternative mechanism to hydrogenation of acetyl-CoA and acetaldehyde for NAD⁺ regeneration in aerobically growing cells. The most logical alternative is NADH oxidase. Cell-free extracts of late exponential phase cultures of 11 of the 12 parent cultures tested had substantial NADH oxidase activity and traces of NADPH oxidase activity; the exception was *L. mesenteroides* ATCC 12291, which had neither (Table 4). These data suggest that the absence of NADH oxidase activity makes *L. mesenteroides* ATCC 12291 reliant on the ethanol branch of the heterolactate pathway to regenerate NAD⁺ and this limits its capacity for ATP generation via acetate kinase. If this explanation is true, mutants deficient in NADH oxidase, obtained from a parent strain which grows better aerobically than anaerobically, should lose the capacity for better aerobic growth.

Several mutants of *L. mesenteroides* X2 were isolated on the basis that their growth rates and yields were similar in aerated and unaerated MRS glucose (56 mM) cultures. Five such mutants (designated Nox 1 to 5) were analysed for NADH oxidase activity and all were totally deficient (Table 4). Since the parent *L. mesenteroides* X2 utilizes mannitol aerobically but not anaerobically, it can be argued that mannitol utilization depends on NADH oxidase activity. A

Table 5. *Comparison of heterolactate pathway enzyme activities in cell-free extracts of Leuconostoc mesenteroides X2 growing in aerated and unaerated cultures*

Extracts were made from late exponential phase cultures.

Enzyme	Specific activity [units (mg protein) ⁻¹]	
	Aerated	Unaerated
NADH oxidase	0.72	0.086
Phosphoketolase	2.72	2.77
Acetate kinase	2.61	1.14
Phosphate acetyltransferase	0.27	1.41
Acetaldehyde dehydrogenase	0.041	0.049
Alcohol dehydrogenase	0.51	1.40
D(–)Lactate dehydrogenase	7.35	6.55

second group of mutants were isolated that lost the ability to grow aerobically on mannitol. Six such mutants (designated Nox 6 to 11) were analysed for NADH oxidase activity; they were again totally deficient (Table 4) and their growth rates and yields were similar whether growing aerobically or anaerobically.

One of the mutants, *L. mesenteroides* X2 Nox 1, was characterized further. Growth rates and yields (Table 2) in aerated or unaerated cultures were identical. Buffered washed whole cells were incapable of utilizing O₂ when supplied with glucose whereas cells from a parent culture utilized 0.09 µmol (mg dry wt)⁻¹ min⁻¹. Cell-free extracts of the mutant had neither NADH nor NADPH oxidase activities (Table 4) but were not deficient in acetate kinase, phosphate acetyltransferase, acetaldehyde dehydrogenase, alcohol dehydrogenase or lactate dehydrogenase activities. Aerobic cultures in MRS glucose did not accumulate acetate to concentrations greater than 1 mM and aerobic or anaerobic cultures in MRS glucose accumulated D(–)lactate and ethanol in approximately equimolar quantities (Table 3). The Nox 1 mutant was similar to but distinguishable from *L. mesenteroides* ATCC 12291.

Comparison of heterolactate pathway enzyme activities in aerated and unaerated cultures

Whether or not acetyl phosphate is metabolized to acetate or ethanol depends on the availability of the enzymes relevant to the alternative branches of the heterolactate pathway. These are NADH oxidase (when O₂ is available as substrate) and acetate kinase for acetate formation, and phosphate acetyltransferase, acetaldehyde dehydrogenase and alcohol dehydrogenase for ethanol formation. Activities of these enzymes, together with those of phosphoketolase and NAD⁺-dependent D(–)lactate dehydrogenase [D(–)LDH] of the main trunk pathway were assayed in cell-free extracts of MRS glucose cultures of *L. mesenteroides* X2 growing with or without aeration. The specific activities of NADH oxidase (especially) and acetate kinase were substantially higher and the specific activities of phosphate acetyltransferase (especially) and alcohol dehydrogenase were substantially lower in aerated than in unaerated cultures (Table 5). Little or no effect of aeration was noted on the specific activities of acetaldehyde dehydrogenase, phosphoketolase or D(–)LDH.

DISCUSSION

The growth data in this report confirm and extend previous observations (Johnson & McCleskey, 1957; Whittenbury, 1963, 1966) that many strains of *Leuconostoc* grow better in aerated culture than without O₂. Though H₂O₂ accumulated in most aerated cultures because of inadequate H₂O₂ dismutation systems, such as NADH peroxidase, the concentration of H₂O₂ accumulated did not reduce growth rates or yields of aerated cultures. When grown in the absence of O₂ the ability of leuconostocs to obtain energy from the metabolism of glucose and some other hexose sugars is restricted. The loss of the ability to grow better, by mutations which

caused the abolition of NADH oxidase activity, strongly suggests that this enzyme plays an essential role in the aerobic energy metabolism of these bacteria. The failure of *L. mesenteroides* ATCC 12291, which was also NADH oxidase negative, to grow better in the presence of air than in its absence, confirms this view. In an earlier study (Garvie, 1969) significant NADH oxidase activity in *L. paramesenteroides* 803 or 871, *L. lactis* 533, *L. cremoris* 543 or *L. dextranicum* 812 was not detected. In the present study aerated cultures of these five strains had substantial NADH oxidase activity. It is unlikely that the earlier work used aerated cultures and the electrophoresis detection system may not have been as sensitive as the enzyme assay used here. The substitution of acetate for ethanol during aerobic growth of leuconostocs on hexoses has been observed often (Ito *et al.*, 1983; Johnson & McCleskey, 1957; Keenan, 1968; Yashima *et al.*, 1970). In the present study better growth of the leuconostocs which responded to aeration was invariably associated with accumulation of acetate rather than ethanol in the culture media. The NADH oxidase mutants and *L. mesenteroides* ATCC 12291 accumulated little or no acetate when growing aerobically. Cell-free extracts of *L. mesenteroides* strains have acetate kinase activity (Yashima *et al.*, 1971; Table 5) which catalyses the formation of acetate from acetyl phosphate, a reaction that increases the availability of ATP in aerobic cultures. It is reasonable therefore to conclude that because of their better ability to produce ATP the aerobic cultures of many *Leuconostoc* strains grow faster and produce more biomass from hexoses than anaerobic cultures. The essential function of NADH oxidase is that it allows O₂ to act as a terminal electron acceptor as in more conventional respiration systems. As a consequence, acetyl phosphate is not wasted in the formation of the alternative electron acceptors acetyl-CoA and acetaldehyde.

The regulation of synthesis of the enzymes concerned with the alternative routes of acetyl phosphate metabolism is ideally organized to take advantage of the presence or absence of O₂. When O₂ is available the specific activities of NADH oxidase and acetate kinase are high and those of phosphate acetyltransferase and alcohol dehydrogenase are low, which facilitates acetate synthesis and high energy phosphate conservation. When O₂ is not available the limitation on sugar utilization is in the anaerobic regeneration of NAD⁺ and in that situation leuconostocs respond with greater phosphate acetyltransferase and alcohol dehydrogenase activities and less NADH oxidase and acetate kinase activities. High levels of alcohol dehydrogenase in anaerobic glucose-grown *L. mesenteroides* cultures, relative to those in aerobic cultures, were noted previously (Ito *et al.*, 1974).

The nature of the heterolactate pathway in leuconostocs was established with *L. mesenteroides* ATCC 12291 (strain 39), which produced equimolar quantities of D-lactate, ethanol and CO₂ from hexoses, whether or not the cultures were aerated. Consequently, the pathway of glucose dissimilation by this strain is accepted as the standard. The data presented here confirm that strain ATCC 12291 is atypical. Since the acetate branch of the heterolactate pathway supports faster growth and greater biomass production, it is reasonable to consider that the formation of acetate is the primary route of utilization of acetyl phosphate and that the ethanol branch of the pathway is a secondary pathway that wastes high energy phosphate. The NAD(P)H oxidase allows O₂ to participate as an electron acceptor in the regeneration of oxidized pyridine nucleotides. Preliminary data (Lucey, 1985) indicate that, in the absence of O₂, compounds such as pyruvate and acetaldehyde stimulate growth rate and yields of some leuconostocs at the expense of glucose. Such stimulations can also be interpreted as the provision of suitable terminal electron acceptors (via LDH and ADH) for efficient regeneration of oxidized pyridine nucleotides and the consequent conservation of acetyl phosphate for ATP synthesis.

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