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Growth and Antioxidant Activity of *Desulfotomaculum acetoxidans* DSM 771 Cultivated in Acetate or Lactate Containing Media

LUCYNA PAWŁOWSKA-ĆWIĘK* and RYSZARD PADO

Pedagogical University of Cracow, Departament of Microbiology, Kraków, Poland

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Abstract

Three independent 28 or 32-day stationary cultures of *Desulfotomaculum acetoxidans* DSM 771 strain were carried out under anoxic conditions in acetate or lactate-containing media. The acids were the sole carbon and energy sources in these media. During cultivation the turbidity (for calculation of cell division index) and hydrogen sulfide contents were determined in culture broth and reduced glutathione and protein concentrations were assayed in culture broth supernatant. In these three successive cultures, the bacterium initially grew much faster on lactate than on acetate. However, after two weeks of culture this difference disappeared and in fact the growth rate was higher on acetate than on lactate. The level of H_2S formed (product of the dissimilatory pathway of sulfate reduction) demonstrated that this pathway was more effective when lactate was a carbon source and the average H_2S concentration was from over 3-fold to about 9-fold greater in lactate than in acetate cultures. Also GSH (glutathione, product of the assimilatory sulfate reduction pathway) average level was about 2-fold higher in lactate-grown cultures. The high negative values of the correlation coefficients between GSH and O_2 levels, especially during the first 4 days of cultivation, indicate that GSH is a very important antioxidizing extracellular agent of *D. acetoxidans*. The rapid increase in GSH level, preceding the release of H_2S, indicates the metabolic priority of the assimilation pathway of sulfate reduction. For both carbon sources the highest coefficient of correlation was found between protein and H_2S levels. These results suggest that hydrogen sulfide is bound by proteins (which contain cysteinyl residues) secreted by *D. acetoxidans* cells. Indicated way of H_2S bounding could result in its acccumulation. This coefficient of correlation increased gradually in the successive cultures. The ratio of H_2S concentration to protein concentration increased gradually in the successive cultures, too.

Key words: growth of *D. acetoxidans* on acetate and on lactate, antioxidant activity

Introduction

The sulfate-reducing bacteria (SRB – group 7) are capable not only of assimilative sulfate reduction but also of dissimilatory reduction of sulfate or sulfur. The dissimilatory pathway is the source of energy for SRB. The reductive character of metabolism, especially of the dissimilatory pathway requires strictly anaerobic conditions for SRB growth. These pathways are coupled to the utilization of hydrocarbon derivatives; lactate is a very good substrate for most SRB. However, Widdel and Pfennig (1981) postulated that the Gram-positive strains of *Desulfotomaculum acetoxidans* never utilized lactate as an electron donor and sporulated only when acetate was the organic substrate. Consequently, Campbell and Singleton (in Bergey's Manual of Systematic Bacteriology) described this species as growing on media with acetate, but not with lactate (Campbell and Singleton, 1986). In contrast, we found that *D. acetoxidans* DSM 771 consumed lactate, too (Pado and Pawłowska-Ćwięk, 2004). Because the ability of this species to grow on medium with lactate remains controversial (Holt *et al*., 1994), we have attempted to thoroughly investigate the growth of *D. acetoxidans* DSM 771 on acetate and on lactate. We also determined the effects of these carbon sources on the antioxidative activity of this bacterium.

Experimental

Material and Methods

Material. *Desulfotomaculum acetoxidans* strain DSM 771 was grown at room temperature (19–23°C). The primary inoculum was 1 ml active culture from Deutsche Sammlung von Mikroorganismen. Half volume of the inoculum was used immediately

* Corresponding author: L. Pawłowska-Ćwięk, Pedagogical University of Cracow Department of Microbiology, ul. Podbrzezie 3, 31-054 Kraków, Poland; fax: (48) 12 6626709; **e-mail; lpc@ap.krakow.pl**

to inoculate 50 ml of medium with 42 mM acetate and the other half to inoculate 50 ml of medium with 42 mM lactate. After 3 weeks both cultures were supplemented with 50 ml of the respective fresh medium and further kept in the dark at room temperature. After the next 3 weeks these cultures broths were used as the inocula (45 ml) for the first 32-day culture (culture **I**). Each inoculum was supplemented with the respective fresh medium 3 weeks before the next culture (culture **II** – strictly on completion of the first cultures and culture **III** – six months after the first culture was completed). All the cultures were conducted in parallel for acetate or lactate-supplemented media in 500 ml Erlenmayer flasks each containing 450 ml of the culture medium. The lactate concentration was determined on the basis of the results of earlier cultures (Pado and Pawłowska-Ćwięk, 2004). In the first series of cultures, also the culture in lactate-supplemented medium but inoculated with the acetate-containing inoculum was executed. After fixing oxygen detectors and inoculation, the media were immediately covered with a liquid paraffin layer (about 5 mm thick), which was maintained throughout the culture. This paraffin layer made easier monitoring, particularly of oxygen level in the culture, without the risk of the culture being exposed to air.

Other medium components were as follows: 21.12 mM Na_2SO_4, 1.15 mM KH_2PO_4, 4.02 mM KCl, 5.61 mM NH_4Cl, 1.13 mM $CaCl_2$, 1.97 mM $MgCl_2$, 85.55 mM NaCl and trace elements (according to DSM-bank instruction) (Pado and Pawłowska-Ćwięk, 2004, Pawłowska-Ćwięk and Pado, 2005).

Growth. The classic Monod's method (Monod, 1949) of graphical representation of bacterial growth in continuous cultures (bacterial growth curve) consists in plotting the number of living cells in 1 ml of culture broth as a function of cultivation time but this assay is very time-consuming. Therefore it is frequently replaced by a simpler method, based on culture turbidity measurements (nephelometry) used for cell number evaluation (Gottschal, 1992). On the basis of the results of our earlier study the high correlation coefficient (0.6295) between the Monod's and nephelometry methods was found. The culture turbidity measurements facilitated much faster determination of the cell division index (CDI). The turbidity of culture broth was measured throughout the cultures (as shown in Table I) at 580 nm using a Specol 11 colorimeter with a TK attachment (Carl Zeiss Jena). Each result in the table is an arithmetic mean from five measurements with standard deviation ranging from 4 to 11%. Prior to sampling the flasks were gently manually agitated for 10 min. On the basis of turbidity values the cell division index (CDI) was calculated:

$$CDI = \tau_x / \tau_0$$

where: τ_0 – turbidity at τ_0 (CDI on the day of inoculation is 1.00), τ_x – turbidity in successive days of the culture (τ_x).

Chemical analysis. Proteins and reduced glutathione contents were assayed (without using any cell membrane disrupting agents) in culture broth supernatant after centrifugation at 6000 × *g* for 15 min. The amount of proteins was estimated by the Lowry's method (Lowry *et al*., 1951). Reduced glutathione concentration was determined by the method described by Akerboom and Sies (1981). The concentration of hydrogen sulfide was measured in culture broth by the methylene blue method of Fago and Popowsky (1949) but the samples with reagents were left overnight.

The colorimetric analyses and spectrum scanning were performed using the CECIL 8020 spectrophotometer. The standard curves obtained for known concen-

Table I
Average protein levels (mg/ml) in culture broth supernatant during successive cultures

Series		0/[a]	Culture day											
			1	2	3	4	7	10	14	17	21	24	28	31
I	acetate	10.5 4.8/[b]		13.3 7.6/[b]	14.8 7.5/[b]	15.0 7.7/[b]	29.35 8.2/[b]	28.7 6.3/[b]	31.2 6.5/[b]	45.8 11.8/[b]	31.8 12.2/[b]	92.1 14.5/[b]	29.95 15.1/[b]	26.85 13.1/[b]
	lactate	11.5 4.8/[b]		25.8 8.5/[b]	54.7 12.8/[b]	102.5 17.2/[b]	175.65 18.6/[b]	157.1 14.7/[b]	108.15 13.3/[b]	100.9 12.2/[b]	108.8 11.9/[b]	133.6 15.2/[b]	88.5 16.2/[b]	69.6 15.8/[b]
II	acetate	21.3 8.7/[b]	26.1 8.1/[b]	23.0 7.9/[b]	26.2 7.3/[b]	26.0 8.5/[b]	10.8 8.5/[b]	26.0 6.8/[b]	23.9 5.9/[b]	16.7 5.8/[b]	36.7 8.7/[b]	46.1 9.3/[b]	29.7 7.9/[b]	
	lactate	38.5 9.1/[b]	49.0 9.9/[b]	54.8 10.2/[b]	67.8 13.1/[b]	59.7 9.2/[b]	53.2 11.3/[b]	43.4 10.5/[b]	59.7 9.9/[b]	61.1 10.3/[b]	61.5 11.5/[b]	92.4 12.9/[b]	66.9 9.6/[b]	
III	acetate	20.6 8.4/[b]	13.0 5.2/[b]	11.0 5.6/[b]	10.0 5.0/[b]	6.8 6.9/[b]	12.8 7.2/[b]	26.8 6.4/[b]	40.0 6.6/[b]	19.2 6.5/[b]	11.0 5.0/[b]	37.0 10.1/[b]	17.0 10.3/[b]	40.1 12.3/[b]
	lactate	40.8 9.6/[b]	35.7 9.9/[b]	34.2 9.8/[b]	74.7 13.1/[b]	80.8 12.8/[b]	88.7 13.5/[b]	70.5 11.8/[b]	26.65 8.9/[b]	17.0 7.0/[b]	19.85 9.1/[b]	16.8 10.5/[b]	16.2 10.8/[b]	13.5 11.6/[b]

/[a] – inoculation day; /[b] – standard deviation [%]

trations of respective standards. All the reagents (including standards: albumin for protein, Na_2S for hydrogen sulfide and GSH) were of analytical grade from Merck or Fluka. The results of triplicate assays are presented as arithmetic mean ± the standard deviation (the latter was similar for protein, GSH and H_2S) (Table I).

The oxygen level in culture broth was measured before mixing the cultures to avoid an error caused by air diffusion (the differences of oxygen concentration before and after mixing were 2.6–4.3 μM O_2). Because between the measurements, the cultures were kept without mixing, these measurements were made very gently for five different positions of the culture CTN-980 R oxygen detector (ELSENT Poland) coupled to a CX-315 microcomputer pH/oxygenmeter (ELMETRON Poland).

Results and Discussion

Cell division index. The obtained values of CDI revealed that the bacterium grew faster on lactate (about 2-fold higher turbidity) than on acetate within the first two weeks of the culture (Fig. 1). After this time, in the first and the third cultures, the CDI was slightly higher on acetate. After 24 days CDI was again higher (about 3-fold) for the lactate containing culture medium (in culture **III**). However, the culture on lactate medium but inoculated with acetate inoculum showed increase of neither cell division index nor hydrogen sulfide level. On the other hand, the lack of an increase in CDI values when the lactate medium was inoculated with acetate inoculum indicates that the adaptation process requires a relatively long time for the changeover of metabolic pathways, essential for switching on lactate catabolism. The necessity of a changeover of metabolic pathways was confirmed by an earlier observation regarding the synthesis of different redox proteins in cultures of three *Desulfovibrio* strains grown on hydrogen or lactate (Steger *et al*., 2002).

The cell division is related to biosynthesis processes, particularly the production of proteins. According to some authors (Hancock and Poxton, 1988; Russel, 1988) "free" wall-associated proteins will continue to be synthesized and will be released directly into the culture supernatant. The obtained average content of determined protein in supernatant was at least 2-fold higher in lactate cultures and in culture **I** (when the cultures were inoculated with the youngest inoculum) were even 3-fold higher than in acetate cultures (Table I and II). Interestingly, as the inoculum grew older the average protein level was progressively reduced in both cultures, but more in the cultures on lactate. Thus, the above – mentioned decrease in the protein content in cultures inoculated with aged

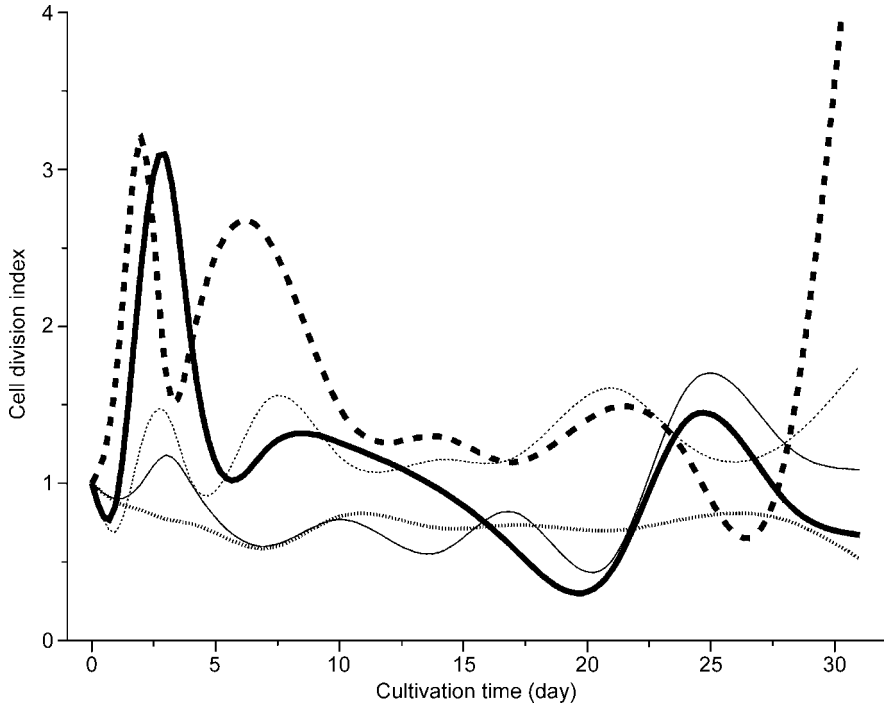

Fig. 1. Cell division index during cultivation of cultures on acetate (thin lines) or lactate (thick lines): culture **I** – solid lines; culture **III** (six months after the first series was completed) – dashed lines; culture on a medium with lactate but inoculated with an acetate inoculum – pointed line. For more clear illustration culture **II** (after the first series was completed) is not presented, since it was similar to series I. Student's t-test values for lactate to acetate culture in successive cultures: **I** – 1.967 (statistically insignificance where $p>0.05$); **II** – 3.969 (0.01 significance level); **III** – 2.588 (0.05 significance level).

inoculum suggests that the age of the inoculum compromises the capability of cells to synthesis and secretion of proteins. The highest coefficient of correlation between CDI and protein level was found for culture **I** (Table III). However, this correlation coefficient in culture **III** (with 7-month-old inoculum) was much higher for the lactate than acetate culture.

Reports on *D. acetoxidans* are rather scarce. This species has not been grown earlier on lactate (Stackebrandt *et al*., 1997; Hristova *et al*., 2000; Scholten and Stams, 2000; Boschker *et al*., 2001; Londry and Des Marais, 2003; Londry *et al*., 2004), because it was commonly believed to be unable to grow on media containing lactate as a sole carbon source (Widdel and Pfenning, 1981; Campbell and Singleton, 1986; Holt *et al*., 1994). Our experience showed that *D. acetoxidans* DSM 771 was also capable of catabolic utilization of lactate (Pado and Pawłowska-Ćwięk, 2004; Pado and Pawłowska-Ćwięk, 2005; Pawłowska-Ćwięk and Pado, 2005). However, in agreement with earlier findings (Widdel and Pfenning, 1981; Campbell and Singleton, 1986), we did not observe sporulation, even after 80 days of culturing in the presence of lactate (Pado and Pawłowska-Ćwięk, 2004).

The obtained relationship between the secreted protein level and CDI is reflected in the correlation coefficient but only in the first series, especially in the acetate culture (Table III). These results are in accordance with the data of Londry and Des Marais (2003) who used ^{13}C acetate. Those authors proved that *D. acetoxidans* (unlike three other species of SRB) effectively incorporated acetate into biomass *via* acetyl-CoA. Moreover, they observed that this species was capable of lithotrophic growth using carbonate and gaseous CO_2. This lithotrophic growth capability could explain the better growth of *D. acetoxidans* in acetate culture but only after two weeks of the cultivation (in the first and second cultures), when carbonate (including dissolved CO_2) accumulated as a consequence of acetate catabolism (Fig. 1).

Reduced glutathione. Also the GSH level was higher in cultures with lactate than in those with acetate (Fig. 2–4). As the cultures with lactate produced slightly higher levels of both GSH and H_2S it suggests that lactate is more advantageous for the assimilatory and dissimilatory sulfate reduction pathways too. It is known that lactate contains more hydrogen atoms than acetate and this is very important in sulfate reduction processes. Since cell membranes were not disrupted prior to GSH determination, the measured GSH was extracellular. The results obtained in all three cultures (designated **I**, **II** and **III**) indicate that GSH biosynthesis and secretion began immediately after inoculation and in early cultures the GSH level increased more rapidly than that of H_2S (Fig. 2–4). The early GSH domination over H_2S suggests priority of the assimilatory over the dissimilatory pathway.

The initial sulfate concentration in the media was 21 mM. On the basis of the highest GSH levels (always during the first four days of the cultures), sulfur incorporation from sulfate into GSH was counted: it ranged from 0.35 to 2.55‰ in acetate cultures and from 0.68 to 3.74‰ in lactate cultures. Contrary to

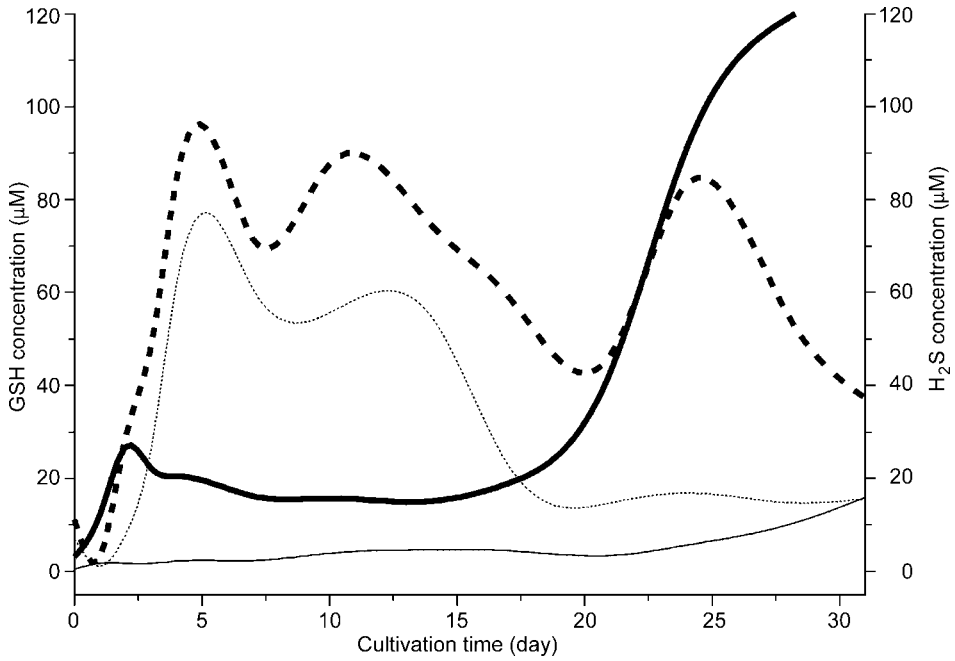

Fig. 2. Hydrogen sulfide (solid lines) and glutathione (dotted lines) levels within culture **I**: acetate cultures (thin lines) or lactate cultures (thick lines).

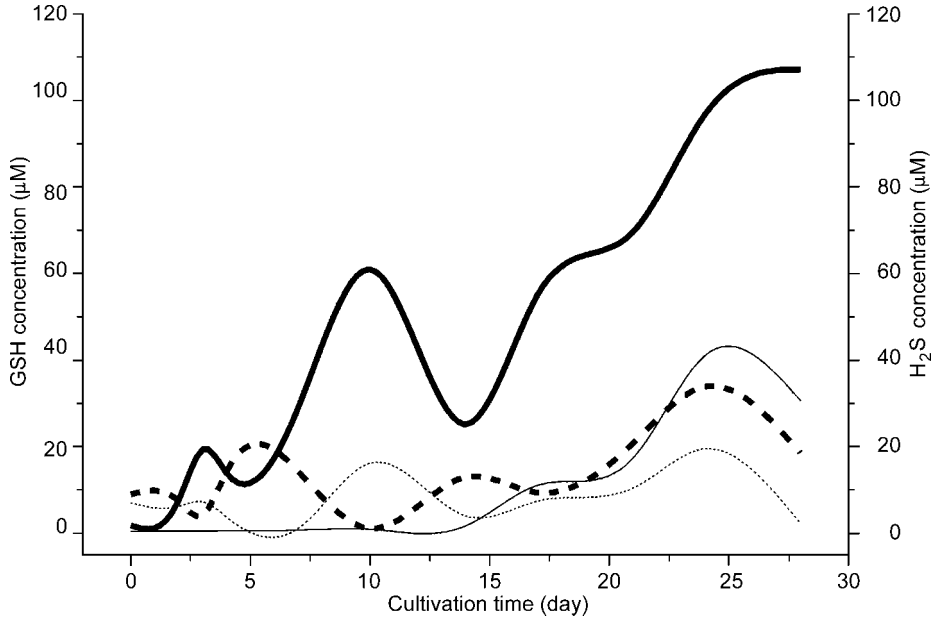

Fig. 3. Hydrogen sulfide (solid lines) and glutathione (dotted lines) levels within culture **II**: acetate cultures (thin lines) or lactate cultures (thick lines).

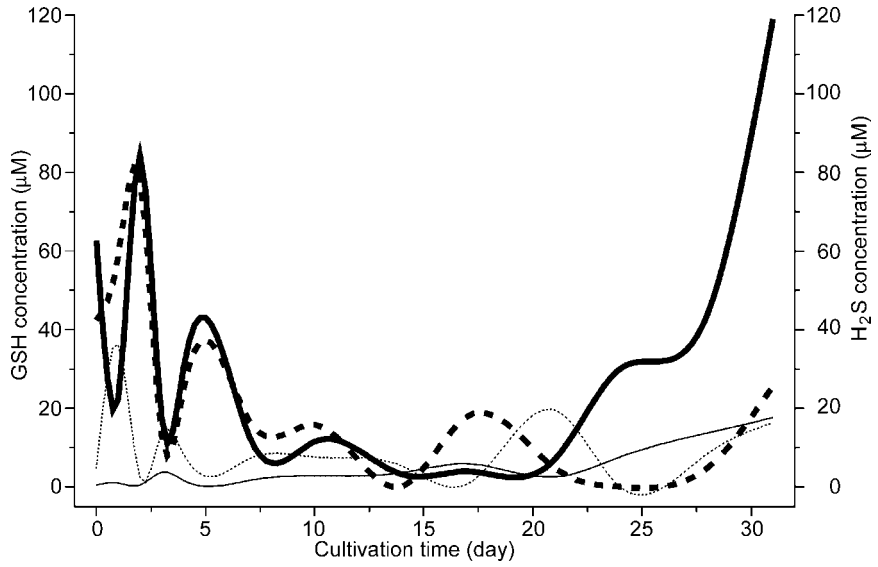

Fig. 4. Hydrogen sulfide (solid lines) and glutathione (dotted lines) levels within culture **III**: acetate cultures (thin lines) or lactate cultures (thick lines).

our expectation we did not find any correlation between GSH and H_2S levels (Table III). The absence of such correlation can explain different metabolic requirements of bacterial cells during of the cultivation. The observed regular, significant and negative values of correlation coefficients between GSH and oxygen (the more GSH the less oxygen) prove that the reduced glutathione performs the role of an antioxidant, as expected (Table III, compare Fig. 2–4 and 5). The antioxidant role of GSH was especially clear at the beginning of cultivation (the first 4 days), because within this period the greatest decreased in oxygen level was observed (by about 100 μM).

As it is well known, GSH is the major antioxidant agent (both extra- and intracellular) in all live organisms (Poot *et al*., 1995; Deneke 2000; Hand and Honek, 2005). The obtained results (the ratio of GSH and protein concentration) showed that lactate stimulated the production of GSH, so thus increasing the antioxidant activity of the examined strain. These levels of GSH were lower (Table II) as compared to Fareleira *et al.* (2003) (1.8±0.6 nmol GSH per mg protein of *Desulfovibrio gigas* cells). However, we determined the extracellular GSH, while those authors determined the total GSH. In this work, a rapid increase in GSH level was observed at the beginning of

Table II
Average levels of GSH (μM) and protein (mg/ml) in culture broth supernatant and H_2S levels in culture broth and Student's t-test values in individual series

Series	Protein Lactate	Protein Acetate	A	H_2S Acetate	H_2S Lactate	A	GSH Acetate	GSH Lactate	A
I	30.78	94.73	3.08	4.480	40.923	9.13	27.738	53.126	1.92
			5.186	0.146/[b]	0.432/[b]	**2.496**	0.901/[d]	0.561/[d]	**3.731**
			(0.001)	0.83/[c]	6.07/[c]	(0.05)	2.55/[e]	3.37/[e]	(0.01)
II	26.04	58.97	2.26	9.579	34.622	3.61	7.719	13.249	1.72
	15.40/[a]	37.75/[a]	**11.123**	0.368/[b]	0.587/[b]	**3.352**	0.296/[d]	0.225/[d]	**3.069**
			(0.001)	1.45/[c]	4.66/[c]	(0.01)	0.35/[e]	0.68/[e]	(0.02)
III	20.41	41.18	2.02	4.813	34.458	7.16	10.040	24.117	2.40
	21.62/[a]	30,17/[a]	**2.151**	0.236/[b]	0.837/[b]	**2.050**	0.492/[d]	0.586/[d]	**2.059**
			nss	0.83/[c]	5.54/[c]	nss	0.64/[e]	3.74/[e]	nss

A – the ratio of protein or H_2S or GSH average concentration in lactate culture to in acetate culture; bold values – Student's t-test for lactate to acetate culture; ( ) – significance level; nss – statistically insignificance ($p>0.05$); /[a] – decrease relative to previous series (%); /[b] – the ratio of H_2S and protein concentration (nmol/mg protein); /[c] – sulfur incorporation from sulfate (initial concentration 21 mM) into hydrogen sulfide (on final cultivation day) (‰); /[d] – the ratio of GSH and protein concentration [nmol/mg protein]; /[e] – sulfur incorporation from sulfate (21 mM) into glutathione (in early exponential phase) (‰).

the culture (Fig. 2–4). However, Fareleira *et al.* (2003) did not observe any significant differences when oxygen concentration in the medium or the duration of the oxic period in *D. gigas* cells were increased. This phenomenon may be explained by GSH secretion initiated by O_2 and/or reactive oxygen species present in the fresh medium and taxonomic differences between these strains. Extracellular oxygen-utilizing processes are well known in eucaryotic *Deuteromycotine* (Odier and Artaud, 1992; Leonowicz *et al.*, 2001). This strategy should also apply to anaerobic bacteria because it protects the cells against the penetration of toxic radicals into the cytosol.

Hydrogen sulfide. The amounts of H_2S (or sulfide) were much greater in cultures grown on lactate than on acetate (Table II): in the first culture about 10-fold higher (during the first week of cultivation – Fig. 2); in the second culture about 30-fold higher (during the first week – Fig. 3); and in the third culture over 45-fold higher (during the first week of cultivation – Fig. 4).

The absorption spectra of 2-days samples (in the first culture) after addition of methylene blue method's reagents showed the presence of peaks at 411.6 and 665 nm (which is characteristic for product formed in this assay) only for the lactate culture (Fig 5A). Our earlier research showed that the complex of 4-hydroxybenzoate and ferrous ions (present in the reagent used for this assay) exhibited absorption maximum at 411.4 nm (Pawłowska-Ćwięk and Pado, 2005). In this culture (culture **I**) the absorption spectra of 17-days sample from lactate culture showed peak at 502.1 nm and from acetate culture only just 32-days sample (Fig. 5B). This absorption maximum was characteristic for complex ferrous ions and 4-hydroxy-3-sulfobenzoate and this ligand was product of 4-hydroxybenzoate sulfonation. The 4-hydroxy-3-sulfobenzoate as extracellular metabolite was requisite for sulfate transport processes in this strain (Pawłowska-Ćwięk and Pado, 2005). The obtained results suggest that lactate was more efficiently for sulfate transport processes than acetate in *D. acetoxidans*. The more efficiency of lactate requires further research. The incorporated sulfur index was counted: in lactate cultures it ranged from 4.66 to 6.07‰, while in acetate ones from 0.83 to 1.45‰ (Table II). Kaplan and Rittenberg (1964) observed sulfur isotope fractionation for *D. desulfuricans* increasing in the order lactate, acetate and ethanol. A correlation between sulfate reduction rates and fractionation was also confirmed by continuous culture experiments (Chambers *et al*., 1975). Detmers *et al*. (2001) examined 32 species of SRB and found that all incomplete-lactate-oxidizing sulfate reducers fractionated 2.0–17‰ of an isotope of sulfur (^{34}S), whereas all examined acetate-oxidizing species fractionated 18.0–22.0‰.

The present results show that *D. acetoxidans* is capable not only of complete acetate oxidation, but it can also utilize lactate. The incomplete oxidation of lactate to acetate by sulfate yields 3-fold more energy than the complete oxidation of acetate to CO_2 (Londry and Des Marais, 2003). So probably the examined strain in the first stage metabolizes lactate to acetate but produces less hydrogen sulfide, and in the next stage it oxidizes acetate generating more H_2S (Fig. 2–4), as follows:

$$SO_4^{2-} + 2\ CH_3CH(OH)COO^- \rightarrow\rightarrow\rightarrow 2\ \text{acetylCoA} + + 2\ HCO_3^- + HS^- \quad (1)$$

$$SO_4^{2-} + 2\ \text{acetylCoA} \rightarrow\rightarrow\rightarrow 4\ CO_2 + H_2S \quad (2)$$

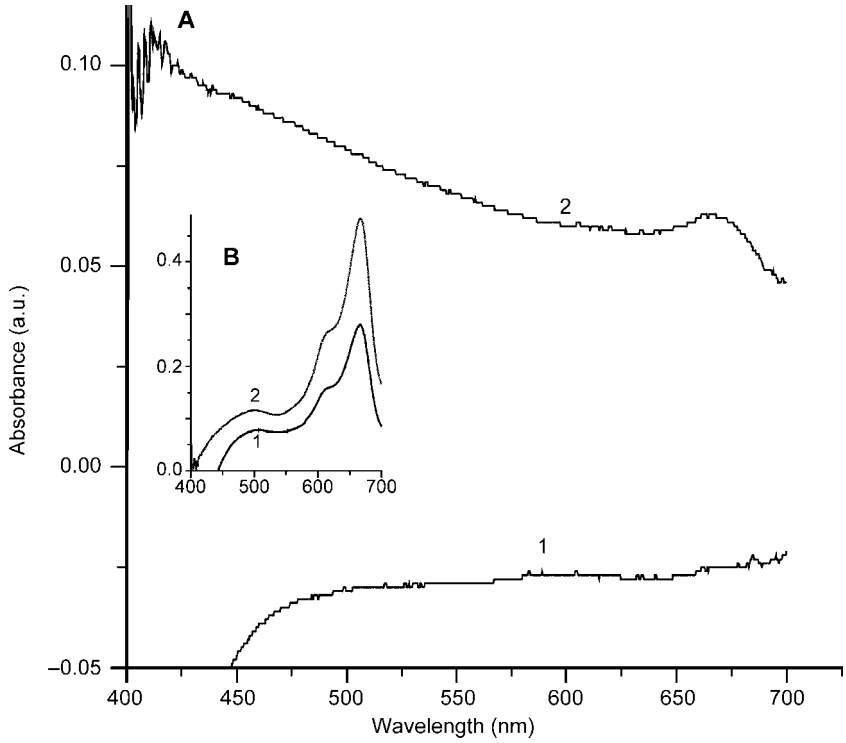

Fig. 5. Absorption spectra of culture samples: 1 – acetate culture; 2 – lactate culture after 2 days of cultivation (A) and 17 days (lactate culture) or 32 days (acetate culture) of cultivation (B) with methylene blue method's reagents.

Although the average H_2S level decreased in consecutive cultures, this decrease was smaller in the cultures with lactate than acetate (Table II). The calculated correlation coefficients show that H_2S amounts correspond to levels of determined proteins (Table III). Despite the levels of both proteins and hydrogen sulfide were reduced in the next culture, but their correlation coefficients increased in the successive cultures, both with acetate and lactate. In lactate culture, in the third culture, was found higher H_2S level in samples from immediately inoculated culture than on the next day (in contrast to other culture-compare Fig. 2, 3 and 4). The higher H_2S amount in samples from freshly inoculated lactate culture proves extracellular accumulation of hydrogen sulfide. Moreover, the average amount of H_2S per mg of protein increased in subsequent series, but only in lactate cultures (Table II). These results suggest that extracellular

Table III
Correlation coefficients

Series Culture	CDI *vs.* Protein	CDI *vs.* H_2S	GSH *vs.* CDI	GSH *vs.* Protein	GSH *vs.* O_2	GSH *vs.* H_2S	H_2S *vs.* Protein	H_2S *vs.* O_2
I acetate	0.6772/[a]	0.2647	–0.2760	–0.5801	–0.4386/[a]	–0.6049	0.1223/[a]	–0.1394/[a]
	0.7105/[b]				–0.5426/[c]		0.1278/[d]	0.5968/[e]
I lactate	0.2033/[a]	–0.3107	0.8451	0.3093	–0.5916/[a]	0.0382	0.1275/[a]	–0.1326/[a]
	0.3716/[b]				–0.5856/[c]		0.1295/[d]	0.4821/[e]
II acetate	–0.7571/[a]	–0.7209	–0.3848	0.7549	–0.1341/[a]	0.5754	0.4226/[a]	0.0516/[a]
	–0.7690/[b]				–0.5587/[c]		0.4016/[d]	0.8278/[e]
II lactate	–0.3301/[a]	–0.6095	–0.6221	0.8162	–0.2531/[a]	0.7020	0.5886/[a]	–0.2393/[a]
	–0.7415/[b]				–0.6826/[c]		0.6046/[d]	–0.0346/[e]
III acetate	0.0407/[a]	0.2010	–0.1443	–0.1440	0.1863/[a]	–0.0049	0.7449/[a]	–0.5951/[a]
	–0.0199/[b]				–0.7210/[c]		0.7875/[d]	–0.5105/[e]
III lactate	0.2181/[a]	0.0981	0.2761	0.0748	0.2286/[a]	0.4897	0.6760/[a]	0.4392/[a]
	0.3623/[b]				–0.5356/[c]		0.9357/[d]	0.2519/[e]

CDI – cell division index; /[a] – whole period of cultivation; /[b] – from the fourth day to the end; /[c] – for the first 4 days of cultivation; /[d] – from inoculation day to 21-th day; /[e] – from the second day to the end of cultivation.

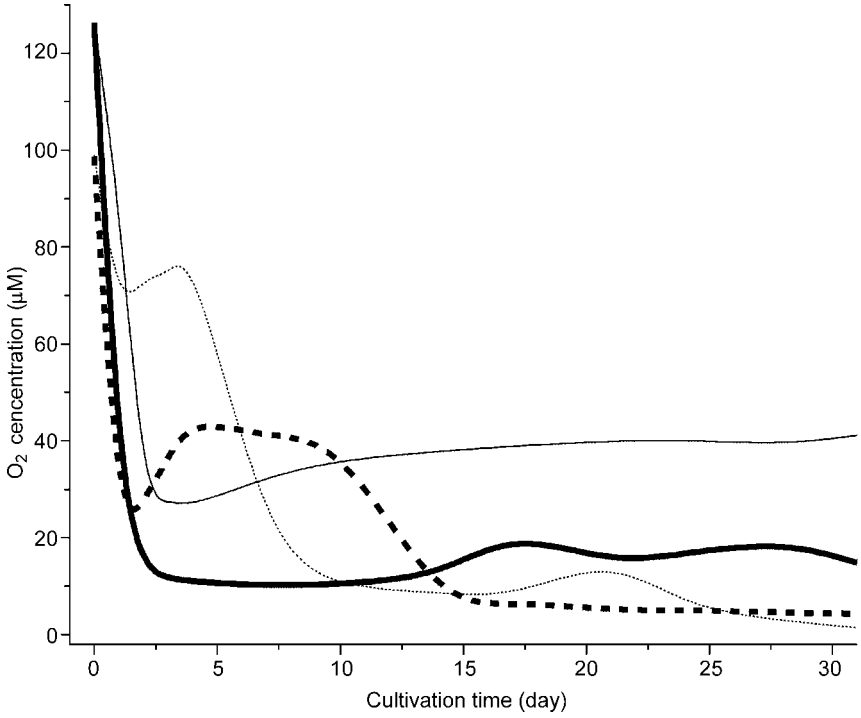

Fig. 6. Oxygen levels during cultivation of cultures on acetate (thin lines) or lactate (thick lines): culture **I** – solid lines; culture **III** (six months after the first series was completed) – dashed lines. For more clear illustration culture **II** (after the first series was completed) is not presented, since it was similar to culture **I**. Student's t-test values for lactate to acetate culture in successive cultures: **I** – 5.886 (0.001 significance level); **II** – 2.795 (0.05 significance level); **III** – 1.424 (statistically insignificance where $p>0.05$).

proteins bound dissimilated H_2S. Our earlier research showed H_2S accumulation by proteins linked to the cell wall this strain, because hydrogen sulfide levels were much higher in the lysozyme-treated samples than in the untreated samples (Pado and Pawłowska-Ćwięk, 2004). Hydrogen sulfide can be bound by cysteinyl residues of proteins forming disulfides, which release the so-called labile sulfur in acidic environment (Ogasawara *et al.*, 1994). However, the results obtained in this work show a decrease of the amount of extracellular proteins with the age of used inoculum, but may be, the copies number of protein containing of Cys residues increased (*e.g.* proteins including in the transport processes). According to Russell (1988), these proteins could be associated with the cell wall, but not covalently linked.

Surprising was the fact that, contrary to expectation, O_2 did not decrease H_2S level; in acetate cultures, in the first and the second series oxygen presence was even advantageous, especially from the second day until the end of cultivation (compare Fig. 2–4 and 6). Also the high values of correlation coefficients for these cultures (Table III) show that oxygen could even be a positive factor for the dissimilatory sulfate reduction pathway (*e.g.* through the influence on sulfate transport processes) (Pawłowska-Ćwięk and Pado, 2005). Also Johnson *et al*. (1997) found a positive influence of oxygen (48 μM) on the growth of *Desulfovibrio vulgaris* if 250 μM hydrogen sulfide was added to the medium.

Conclusion. Although growth of *D. acetoxidans* DSM 771 on lactate requires at least two successive passages on this medium the species grows better on lactate than on acetate, which is contrary to earlier observations of other researches. *D. acetoxidans* lactate cultures produced higher levels of both GSH and H_2S than cultures with acetate, so lactate is a better substrate for metabolic processes, especially the sulfate reduction pathways. The higher level of reduced glutathione in lactate cultures results in the increase of *D. acetoxidans* antioxidant activity, which could be very important for the survival in natural environment.

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