The Effect of Haematin and Catalase on *Streptococcus faecalis* var. *zymogenes* Growing on Glycerol

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Streptococcus faecalis var. *zymogenes* was grown aerobically on a complex medium containing glycerol as the carbon source. Addition of haematin or bovine liver catalase to the growth medium resulted in a small increment in growth yield. Suspensions of bacteria that had been grown in the presence of haematin or catalase, respectively, translocated 0.83 to 1.98 and 1.33 to 2.53 protons per oxygen atom consumed in glycerol oxidation. Bacteria grown without haematin or catalase had nil or little respiratory-induced proton translocation during glycerol oxidation. Inclusion of haematin in the growth medium caused the bacterium to form a cyanide- and azide-sensitive catalase. Superoxide dismutase activity was similar whether or not haematin was added to the growth medium.

INTRODUCTION

Although most bacteria that form cytochromes, catalase and other haemoproteins can synthesize both the apoenzymes and their haem-prosthetic groups, some organisms lack the ability to make protohaem (Knowles, 1980). Thus, they are unable to form catalase or a cytochrome-containing respiratory system unless they are provided with exogenous haematin or, in some instances, a precursor of protohaem or a haemoprotein.

The lactic acid bacteria are usually considered to conserve energy by substrate-level phosphorylation, whether growing aerobically or anaerobically. Most lactic acid bacteria are also thought to be catalase-negative. However, addition of haematin or haemoglobin to the growth medium enables a few species to form catalase and cytochromes (Whittenbury, 1960, 1964; Bryan-Jones & Whittenbury, 1969; Sijpesteijn, 1970; Van der Wiel-Korstanje & De Vries, 1973; Ritchey & Seeley, 1974, 1976; Pritchard & Wimpenny, 1978).

We have studied the effect of including haematin in the growth medium on the formation of catalase, respiratory activity and respiratory-driven proton translocation by *Streptococcus faecalis* var. *zymogenes* grown aerobically with glycerol as the carbon source.

METHODS

Organism and growth. *Streptococcus faecalis* var. *zymogenes* strain TR was provided by Professor R. Whittenbury, University of Warwick, Coventry. This is the strain used previously by Ritchey & Seeley (1974). It was maintained on a medium containing (g l⁻¹): tryptone, 10; yeast extract, 5; glucose, 4; K₂HPO₄, 3; purified agar, 15. It was grown on medium containing (g l⁻¹): tryptone, 2.5; yeast extract, 2.5; KH₂PO₄, 3.5; K₂HPO₄, 6. The medium was adjusted to pH 6.5 with H₂SO₄ prior to heat-sterilization at 121 °C. The carbon source, glycerol, was prepared as a 0.5 M stock solution, sterilized at 121 °C and added aseptically to give 8 mM in the growth medium. Haematin (5 mg ml⁻¹ in 0.02 M-KOH) was sterilized by membrane-filtration (0.45 µm pore size), stored at 4 °C and added, where indicated, to the growth medium to give 10 µg ml⁻¹. Catalase was prepared as a 3 mg ml⁻¹ stock solution, sterilized by membrane-filtration and added, where indicated, to the growth medium to give 6 µg ml⁻¹. For media containing additional trace metals, a stock solution was prepared according to Bauchop & Elsdon (1960), heat-sterilized at 121 °C and added to the medium at 5 ml l⁻¹. The organism was grown aerobically in an orbital incubator (200 rev. min⁻¹)

at 35 °C. Cultures used as inocula contained 50 ml medium in 250 ml conical flasks; growth cultures contained 500 ml medium in 2 l conical flasks. A 2 % (v/v) inoculum of a culture grown for 16 h was used to initiate growth. To establish culture purity, samples were examined by plating, microscopy, the oxidase test (negative) and the catalase test (negative, when plated in the absence of haematin).

Determination of dry weight. Duplicate cultures were grown for 16 h in a medium containing haematin and 0, 5 or 10 mM-glycerol. They were harvested, washed twice in distilled water and centrifuged (23 000 g, 10 min), then dried at 100 °C to constant weight. Growth was estimated turbidimetrically at 680 nm (1 cm light path); an absorbance of 1.0 was equivalent to 310 μg dry wt ml^{-1} .

Determination of protein. The method of Lowry was used and the absorbance was measured at 500 nm. Bovine serum albumin was used as a standard. Samples were diluted 25-fold to minimize interference by haematin.

Estimation of H_2O_2 in the growth medium. Samples of the growth medium were removed from the growth vessel, centrifuged (2500 g, 5 min) and diluted as required. H_2O_2 in the samples was assayed polarographically in a Clark-type oxygen electrode (Rank Bros., Bottisham, Cambs.) by measuring the dissolved O_2 content at 35 °C upon adding catalase, as described by Dempsey *et al.* (1975). The response of the electrode was calibrated using solutions of H_2O_2 in 60 mM- Na^+/K^+ phosphate buffer, pH 6.5 that had been standardized by titration against a KMnO_4 solution.

Oxygen uptake. Bacteria were harvested by centrifugation (23 000 g, 10 min, 4 °C) and washed twice in 50 mM- $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ buffer, pH 6.5. The bacteria were resuspended in the same buffer to a density of approx. 0.4 mg dry wt ml^{-1} for bacteria grown to the mid-exponential phase. O_2 uptake by a 3 ml sample of resuspended bacteria was determined in an oxygen electrode at 35 °C. Oxidation rates for endogenous substrates, glycerol, glucose and lactate were measured; exogenous substrates were added to give 33 mM. The solubility of O_2 was assumed to be 0.41 $\mu\text{mol O ml}^{-1}$ at 35 °C (Chappell, 1964).

Proton-pulse measurement. Proton-pulses were measured by injecting air-saturated KCl into an anaerobic suspension of bacteria in the presence of KSCN and measuring the resultant pH change. The technique used was an adaptation of that described by Mitchell & Moyle (1967*a, b*). Bacteria were harvested by centrifuging (23 000 g, 10 min, 4 °C) and washed twice in buffer (200 mM-KCl/2 mM-glycylglycine/HCl, pH 6.5) with centrifugation (38 000 g, 5 min, 4 °C). The bacteria were resuspended in 150 mM-KSCN/50 mM-KCl/1 mM-glycylglycine/HCl, pH 6.5 to about 3 mg dry wt ml^{-1} . The KSCN concentration and buffer conditions given are those determined for optimum proton-pulses. The suspension was pipetted into a water-jacketed glass chamber maintained at 35 °C, and stirred by a magnetic stirrer and bar. A rapid-response combination glass/reference pH probe (CMAJ 72, Russell pH Ltd, Auchtermuchty, Fife) was inserted into the suspension. The completely filled chamber was sealed with Parafilm such that no air was trapped above the suspension, and a stream of O_2 -free N_2 was blown over the seal. The pH probe was connected to a Pye model 290 pH meter with scale expansion (Pye Unicam). The output was passed through a back-off circuit to a chart recorder (Kipp & Zonen BD8 multirange recorder). A preincubation period of 45 to 60 min was required for minimal pH drift. Substrates (see Table 2) were added to give 8 mM, and a further 10 min was allowed for pH stabilization. Pulses of air-saturated 200 mM-KCl (5 to 50 μl) were added through the seal by means of a 50 μl micro-syringe and the change in pH was measured. Injections of 5 μl anaerobic 4 mM-NaOH were used to calibrate the pulses. The maximum observed change in pH was used to determine the $\rightarrow\text{H}^+/\text{O}$ ratio. Correction by extrapolation back to zero time for the proton-pulses (Mitchell & Moyle, 1967*a, b*) and the anaerobic NaOH injections had only a negligible effect on the measured $\rightarrow\text{H}^+/\text{O}$ ratios. Dissipation of proton pulses was examined by addition of carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) in ethanol to give 10 μM .

Preparation of cell-free extracts. Bacteria grown to the mid-exponential phase were harvested by centrifugation (23 000 g, 10 min, 4 °C) and washed once in the buffer used for the enzyme measurements. Cells were disrupted ultrasonically (8 $\times$ 30 s using an 150 W MSE ultrasonic disintegrator at maximum power), and cell debris was removed by centrifugation (12 000 g, 10 min, 4 °C).

Determination of catalase activity (EC 1.11.1.6). Cell-free extract (0.2 ml) was incubated at 35 °C with 1.8 ml 50 mM-morpholinopropanesulphonic acid buffer (MOPS), pH 7.5 containing 70 mM- H_2O_2 for 0, 15, 30 and 60 s. The reaction was stopped by adding 0.25 ml $\text{Ti}(\text{SO}_4)_2$ reagent. This reagent was prepared by diluting $\text{Ti}(\text{SO}_4)_2$ solution [15 % (w/v) in 23 % (w/v) H_2SO_4] with 1 M- H_2SO_4 (1:1, v/v). Precipitated protein was removed by centrifuging at 2500 g for 5 min. The solutions were diluted as appropriate with 1 M- H_2SO_4 , and the absorbance was measured at 420 nm. A calibration curve for the $\text{Ti}(\text{SO}_4)_2$ reagent was prepared using H_2O_2 in 50 mM-MOPS, pH 7.5 (0 to 1250 nmol $\text{H}_2\text{O}_2 \text{ ml}^{-1}$) standardized by titration against a KMnO_4 solution (Snell & Snell, 1949). Catalase activity was expressed as the initial rate of H_2O_2 cleavage.

Determination of superoxide dismutase activity (EC 1.15.1.1). The method of McCord & Fridovich (1969) was used. The cytochrome *c* concentration was increased to 10 μM to increase reproducibility, and the reaction was assayed at 35 °C. The assay mixture contained 50 mM- $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ buffer, pH 7.8, 10 μM -

Fig. 1. Effect of the inclusion of haematin in the medium on growth and H_2O_2 accumulation. *Streptococcus faecalis* var. *zymogenes* was grown in a complex medium containing 8 mM-glycerol. Growth (○) and H_2O_2 accumulation in the medium (●) are shown for cultures grown in the absence of haematin (a, c), and in the presence of 10 μg haematin ml^{-1} (b, d). Additional trace metals (see Methods) were present in (c) and (d).

ferricytochrome *c*, 50 μM -xanthine (dissolved by adding a minimum of KOH) and sufficient xanthine oxidase to result in an increase in absorbance at 550 nm of 0.025 min^{-1} . Cell-free extract (0 to 200 μg protein) was added to the assay mixture and a graph of the activity (%) against protein concentration was plotted. One unit of superoxide dismutase activity was defined as the concentration of enzyme required to inhibit the reduction of ferricytochrome *c* at 35 °C by 50 %.

Chemicals. Tryptone, yeast extract and purified agar were obtained from Oxoid. Titanium sulphate solution (15 % (w/v) in 23 % (w/v) H_2SO_4 , technical grade) was obtained from BDH. Haematin, bovine liver catalase, bovine serum albumin, ferricytochrome *c* (type III), xanthine oxidase (Grade I, from buttermilk), and all other chemicals were obtained from Sigma or Fisons. Glass-distilled water was used throughout.

RESULTS

Addition of haematin at 10 μg ml^{-1} slightly increased the growth of *S. faecalis* (Fig. 1 a, b). H_2O_2 was detectable at only very low concentrations in the medium, whether haematin was present or not. When trace metals were added to the medium, the difference in growth yields in the presence and absence of haematin was very marked (Fig. 1 c, d). This was due to a decreased growth yield in the absence of haematin and was accompanied by production of up to 400 μM - H_2O_2 by the time the late-exponential growth phase was reached. The H_2O_2 produced during growth in the presence of trace metals and in the absence of haematin

Table 1. *Activities of substrate oxidases for bacteria grown to the mid-exponential phase on 8 mM-glycerol in the presence and absence of haematin or catalase*

Substrate (0.1 ml of a 1 M solution) was added to 2.9 ml resuspended bacteria in 50 mM-phosphate buffer, pH 6.5. Oxidase activities are shown as the mean $\pm$ S.D. (no. of observations).

Substrate oxidized	Oxidase activity [nmol O min ⁻¹ (mg dry wt) ⁻¹]		
	Bacteria grown without haematin or catalase	Bacteria grown with haematin (10 μ g ml ⁻¹)	Bacteria grown with catalase (6 μ g ml ⁻¹)
Endogenous	9 $\pm$ 12 (10)	10 $\pm$ 11 (12)	4 $\pm$ 3 (12)
Glycerol	411 $\pm$ 60 (10)	509 $\pm$ 101 (12)	376 $\pm$ 161 (12)
Glucose	148 $\pm$ 67 (10)	219 $\pm$ 28 (12)	175 $\pm$ 46 (12)
Lactate	231 $\pm$ 96 (10)	241 $\pm$ 93 (12)	129 $\pm$ 65 (12)

Table 2. *Oxygen-induced proton-pulses by bacteria grown to the mid-exponential phase*

The results are expressed as $\rightarrow$ H⁺/O ratios and are shown as the mean $\pm$ S.D. (no. of observations) for each batch of bacteria.

Incubation substrate	$\rightarrow$ H ⁺ /O ratio		
	Bacteria grown without haematin or catalase	Bacteria grown with haematin (10 μ g ml ⁻¹)	Bacteria grown with catalase (6 μ g ml ⁻¹)
Endogenous	0 (5)	0 (5)	1.67 $\pm$ 0.20 (7)
	0 (5)	0 (5)	2.27 $\pm$ 0.39 (10)
	0 (5)	0 (5)	0 (5)
			0 (5)
Glycerol	0 (5)	1.05 $\pm$ 0.09 (10)	1.33 $\pm$ 0.04 (8)
	0 (5)	0.83 $\pm$ 0.09 (11)	2.53 $\pm$ 0.56 (6)
	0.31 $\pm$ 0.26 (10)	1.98 $\pm$ 0.16 (10)	1.71 $\pm$ 0.10 (11)
			2.00 $\pm$ 0.19 (12)
Glucose	0 (5)	0 (5)	1.06 $\pm$ 0.10 (10)
	0 (5)		
Lactate	0 (5)	0 (5)	2.28 $\pm$ 0.37 (7)
	0.15 $\pm$ 0.23 (10)		

was somewhat variable but was much greater than found under the other growth conditions. When catalase (6 μ g ml⁻¹) was added to cultures as an alternative haem source which would also be able to remove H₂O₂, the growth yields were similar to those obtained with haematin, with nil or very low concentrations of H₂O₂ in the medium.

Respiratory activities were low with endogenous substrates for bacteria cultured with or without a haem source and harvested in the mid-exponential phase of growth (Table 1). Compared with bacteria grown without a haem source, haematin-grown bacteria showed increased oxidation rates for glycerol and glucose as substrates. Bacteria grown in the presence of catalase had increased rates of oxidation of glucose but a lower rate of oxidation of lactate. For bacteria harvested in the stationary phase (16 h) nil or only a very low oxidase activity was observed for glycerol 3-phosphate, pyruvate, succinate, malate, citrate, isocitrate, fructose 1,6-bisphosphate, ascorbate plus *N,N,N',N'*-tetramethyl-*p*-phenylenediamine, and ascorbate plus phenazine methosulphate.

The results obtained for oxygen-induced proton-pulses are shown in Table 2. Oxygen-induced proton extrusion was detected with glycerol as substrate for bacteria grown in the presence of haematin or catalase. Proton-pulses were also observed for oxidation of endogenous substrates, glucose and lactate by bacteria grown in the presence of catalase. Where proton-pulses were detected, additions of anaerobic KCl did not cause any acidification of the medium, and addition of 10 μ M-CCCP collapsed the proton-pulses ($\rightarrow$ H⁺/O = 0). Low

Table 3. Catalase activity of cell-free extracts from bacteria grown to the mid-exponential phase

Catalase activities are expressed as the initial rate of H_2O_2 cleavage and are shown as the mean $\pm$ S.D. (no. of observations) for each batch of bacteria.

	Catalase activity [$\mu\text{mol H}_2\text{O}_2$ cleaved min^{-1} (mg protein) $^{-1}$]	
	Bacteria grown without haematin	Bacteria grown with haematin (10 $\mu\text{g ml}^{-1}$)
Bacteria grown without additional trace metals	0 (5)	69.5 $\pm$ 14.5 (5)
	5.5 $\pm$ 3.5 (5)	108.5 $\pm$ 9.0 (5)
		105.0 $\pm$ 5.5 (5)
Bacteria grown with additional trace metals	4.0 $\pm$ 6.5 (5)	62.5 $\pm$ 2.5 (5)
	5.0 $\pm$ 2.0 (5)	58.5 $\pm$ 4.5 (5)
		53.5 $\pm$ 4.5 (5)

Fig. 2. Catalase activity in cell-free extracts of *Streptococcus faecalis* var. *zymogenes* grown to the stationary phase (16 h) in the presence of 10 μg haematin ml^{-1} , showing the relationship between catalase activity (initial rate for samples of cell-free extract containing 1 mg protein) and H_2O_2 concentration.

$\rightarrow\text{H}^+/\text{O}$ values were detected for one sample of non-haem-grown cells with glycerol and lactate as substrates. These may have been due to traces of haem in the growth medium, although in spectra of concentrated medium no haem could be detected.

The mean $\rightarrow\text{H}^+/\text{O}$ ratios for different batches of haematin-grown bacteria were 0.83 to 1.98 for glycerol oxidation. The range of mean ratios for glycerol oxidation by catalase-grown bacteria was higher (1.33 to 2.53). Two batches of catalase-grown bacteria had mean $\rightarrow\text{H}^+/\text{O}$ ratios of 1.67 and 2.27 for oxidation of endogenous substrates whereas with two other batches no oxygen-induced pulses were observed. The mean $\rightarrow\text{H}^+/\text{O}$ ratio for a single batch of catalase-grown bacteria for oxidation of glucose was 1.06 and for lactate oxidation was 2.28. A suspension of bacteria grown without haematin or catalase did not show proton-pulses with glycerol or endogenous substrates when catalase (6 $\mu\text{g ml}^{-1}$) was added to the incubation mixture.

Cell-free extracts from bacteria grown without haematin showed a very low catalase activity compared with those grown in the presence of haematin (Table 3). Concentrations of catalase were slightly higher in haematin-containing cultures of bacteria grown without added trace metals. The catalase activity had a wide pH range, with a broad optimum at pH 6 to 8, and was sensitive to cyanide and azide inhibition (50% inhibition by 10 to 30 μM cyanide or azide). The enzyme showed increasing activity with H_2O_2 up to approx. 100 mM, above which it was inactivated (Fig. 2). To compare catalase activity under the different

growth conditions, the initial H_2O_2 concentration used was 70 mM, which was that required to give half-maximal activity.

Similar activities of superoxide dismutase were present in bacteria that had been grown to the mid-exponential phase in the presence and absence of haematin. The activities were $10.1 \pm \text{s.d. } 1.5$ (3) and $8.8 \pm \text{s.d. } 0.6$ (3) units (mg protein^{-1}), respectively.

DISCUSSION

During aerobic growth of *S. faecalis* the major pathway of glycerol assimilation is via conversion to glycerol 3-phosphate by glycerol kinase. Dihydroxyacetone phosphate and H_2O_2 are then formed by glycerophosphate oxidase (Jacobs & VanDemark, 1960*a, b*; Esders & Michina, 1979). The formation of this pathway as the major aerobic route of glycerol assimilation appears to be unaffected by inclusion of haematin in the growth medium (S. Y. R. Pugh & C. J. Knowles, unpublished observations). Therefore, the detection of nil or little H_2O_2 in the medium during growth in the absence of extra trace metals (in the presence or absence of exogenous haematin) was presumably due to degradation of H_2O_2 by the bacterium. When haematin was not included in the medium little catalase was formed and the H_2O_2 must have been degraded by another route. The most likely method was by NADH peroxidase (Dolin, 1953, 1975, 1977; Hoskins *et al.*, 1962):

When haematin was present in the growth medium catalase was formed and H_2O_2 could have been removed by NADH peroxidase and/or catalase. Under these growth conditions *S. faecalis* forms a cytochrome-linked membrane-bound respiratory system which is able to oxidize NADH (Bryan-Jones & Whittenbury, 1969; Ritchey & Seeley, 1974, 1976; Pritchard & Wimpenny, 1978). It is therefore possible that H_2O_2 was detoxified primarily by catalase and NADH was oxidized preferentially by the respiratory system.

Addition of trace metals to the growth medium resulted in H_2O_2 accumulation. Furthermore, growth was depressed, which could have been due to the toxic effects of the H_2O_2 . The reasons for the appearance of H_2O_2 in the medium are not clear. One possibility is that one or more of the trace metals inhibited the NADH peroxidase activity. Metabolism could have been altered such that insufficient NADH was available for removal of H_2O_2 via the peroxidase.

Whereas most lactic acid bacteria do not exhibit any catalase-like activity, a few species form a 'pseudocatalase' (Delwiche, 1961; Johnston & Delwiche, 1962; Whittenbury, 1960, 1964). Unlike normal haem-containing catalases, pseudocatalases have none of the absorbance peaks associated with haemoproteins. They are insensitive to azide and cyanide and are not inactivated by prolonged exposure to H_2O_2 . The development of catalase activity by *S. faecalis* var. *zymogenes* on addition of haematin to the growth medium and its sensitivity to azide and cyanide suggests that it is a haem-containing enzyme.

Gallin & VanDemark (1964) obtained a P/O ratio of 0.24 for oxidation of NADH by cell-free extracts of *S. faecalis* 10C1 grown aerobically in the absence of haematin and suggested that there was coupling of ATP synthesis to electron transport at the site 1 region. This was supported by growth yield studies (Smalley *et al.*, 1968), but no corrections were made for the effects of changes in growth rate or maintenance energy requirements (cf. Tempest & Neijssel, 1980). In contrast, neither Ritchey & Seeley (1974) nor Bryan-Jones & Whittenbury (1969) obtained evidence for oxidative phosphorylation by *S. faecalis* var. *zymogenes* or *S. faecalis* NCDO 581, respectively, growing in haematin-free media. A possible reason for this discrepancy is that traces of haematin or a haemoprotein could have been present in the medium or on the glassware used by Gallin & VanDemark (1964) and Smalley *et al.* (1968) (see Knowles, 1980).

In order to determine if energy conservation coupled to respiration occurs in haematin-

grown *S. faecalis* we have measured proton translocation using the technique of Mitchell & Moyle (1967*a, b*). Respiratory-driven acidification of the suspension medium did not usually occur for bacteria grown in the absence of added haematin (Table 2). The low $\rightarrow\text{H}^+/\text{O}$ ratios obtained in two batches of bacteria could have been due to traces of haematin or a haemoprotein present in the complex growth medium. When the bacteria had been grown in the presence of haematin, $\rightarrow\text{H}^+/\text{O}$ ratios of 0.83 to 1.98 were obtained for oxidation of glycerol. This suggests that there was a single proton translocating loop during oxidation of glycerol. Assuming an $\rightarrow\text{H}^+/\sim\text{P}$ ratio of 2, a single ATP molecule was formed per oxygen atom reduced (Haddock & Jones, 1977). However, if the $\rightarrow\text{H}^+/\sim\text{P}$ ratio is higher (Brand, 1977) there would be a lower efficiency of transduction. Alternatively, the observed $\rightarrow\text{H}^+/\text{O}$ ratio could be an underestimate due to ion movements, especially of potassium (Wilson *et al.*, 1976; Reynafarge *et al.*, 1976).

When exogenous bovine liver catalase was used as a source of protohaem, slightly higher $\rightarrow\text{H}^+/\text{O}$ ratios were observed for glycerol oxidation. In addition, proton ejection was observed in response to oxidation of endogenous substrates by some batches of bacteria, and for oxidation of glucose or lactate. The rates of oxidation of endogenous substrates were extremely low for suspensions of bacteria grown under all three growth conditions (Table 1) and considerable difficulties were experienced in attaining complete anaerobiosis in the reaction chamber used to assay proton-pulses. In addition, in anaerobic suspensions containing lactate or glucose there was continuous acidification of the incubation mixture, making reliable measurements of the $\rightarrow\text{H}^+/\text{O}$ ratios difficult. It was only with glycerol as substrate that reasonable stability was attained.

That haematin-induced respiratory-driven energy transduction occurs in *S. faecalis* has also been shown recently by Pritchard & Wimpenny (1978). They grew *S. faecalis* var. *zymogenes* in continuous culture on lactate, and obtained an $\rightarrow\text{H}^+/\text{O}$ ratio of 1.37 for oxidation of lactate.

Additional energy conservation by the bacterium when it was grown in the presence of haematin or catalase was also suggested by the small but definite increase in growth yield (Fig. 1). Because of other factors (Tempest & Neijssel, 1980), interpretation of growth yield values requires more sophisticated experimentation to determine the extra energy transduction.

During aerobic growth of living cells, toxic superoxide is produced which must be removed by superoxide dismutase (Fridovich, 1978). Superoxide dismutase activity has been found in aerobically grown *S. faecalis* (Gregory & Fridovich, 1973; Britton *et al.*, 1978). Since the development of a respiratory system by *S. faecalis* was likely to result in formation of extra superoxide (Fridovich, 1978), it was possible that greater synthesis of superoxide dismutase was necessary. In fact, no overall increase in superoxide dismutase activity was found on inclusion of haematin in the growth medium, indicating that sufficient activity was already present to successfully scavenge any increases in superoxide formation.

Unlike *Lactobacillus plantarum* (Götz *et al.*, 1980) the superoxide dismutase activity of *S. faecalis* var. *zymogenes* is probably enzymic since boiling extracts caused loss of activity. Moreover, Britton *et al.* (1978) have purified the enzyme from another strain of *S. faecalis*.

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REFERENCES

- BAUCHOP, T. & ELSDEN, S. R. (1960). The growth of micro-organisms in relation to their energy supply. *Journal of General Microbiology* **23**, 457-469.
- BRAND, M. D. (1977). The stoichiometric relationships between electron transport, proton translocation and adenosine triphosphate synthesis and hydrolysis in mitochondria. *Biochemical Society Transactions* **5**, 1615-1620.
- BRITTON, L., MALINOWSKI, D. P. & FRIDOVICH, I. (1978). Superoxide dismutase and oxygen metabolism in *Streptococcus faecalis* and comparisons with other organisms. *Journal of Bacteriology* **134**, 229-236.
- BRYAN-JONES, D. G. & WHITTENBURY, R. (1969). Haematin-dependent oxidative phosphorylation in *Streptococcus faecalis*. *Journal of General Microbiology* **58**, 247-260.
- CHAPPELL, J. B. (1964). The oxidation of citrate, isocitrate and *cis*-aconitate by isolated mitochondria. *Biochemical Journal* **90**, 225-237.
- DELWICHE, E. A. (1961). Catalase of *Pediococcus cerevisiae*. *Journal of Bacteriology* **81**, 416-418.
- DEMPEY, P. M., O'LEARY, J. & CONDON, S. (1975). Polarographic assay of hydrogen peroxide accumulation in microbial cultures. *Applied Microbiology* **29**, 170-174.
- DOLIN, M. I. (1953). The oxidation and peroxidation of DPNH₂ in extracts of *Streptococcus faecalis* 10C1. *Archives of Biochemistry and Biophysics* **46**, 483-484.
- DOLIN, M. I. (1975). Reduced diphosphopyridine nucleotide peroxidase. Intermediates formed on reduction of the enzyme with dithionite or reduced diphosphopyridine nucleotide. *Journal of Biological Chemistry* **250**, 310-317.
- DOLIN, M. I. (1977). DPNH peroxidase: effector activities of DPN⁺. *Biochemical and Biophysical Research Communications* **78**, 393-400.
- ESDERS, T. W. & MICHINA, C. A. (1979). Purification and properties of L- α -glycerophosphate oxidase from *Streptococcus faecium* ATCC 12755. *Journal of Biological Chemistry* **254**, 2710-2715.
- FRIDOVICH, I. (1978). The biology of oxygen radicals. *Science* **201**, 875-880.
- GALLIN, J. I. & VANDEMARK, P. J. (1964). Evidence for oxidative phosphorylation in *Streptococcus faecalis*. *Biochemical and Biophysical Research Communications* **17**, 630-635.
- GÖTZ, F., ELSTNER, E. F., SEDEWITZ, B. & LENGELDER, E. (1980). Oxygen utilisation by *Lactobacillus plantarum*. II. Superoxide and superoxide dismutation. *Archives of Microbiology* **125**, 215-220.
- GREGORY, E. M. & FRIDOVICH, I. (1973). Induction of superoxide dismutase by molecular oxygen. *Journal of Bacteriology* **114**, 543-548.
- HADDOCK, B. A. & JONES, C. W. (1977). Bacterial respiration. *Bacteriological Reviews* **41**, 47-99.
- HOSKINS, D. D., WHITELEY, H. R. & MACKLER, B. (1962). The reduced diphosphopyridine nucleotide oxidase of *Streptococcus faecalis*: purification and properties. *Journal of Biological Chemistry* **237**, 2647-2653.
- JACOBS, N. J. & VANDEMARK, P. J. (1960a). Comparison of the mechanism of glycerol oxidation in aerobically and anaerobically grown *Streptococcus faecalis*. *Journal of Bacteriology* **79**, 532-538.
- JACOBS, N. J. & VANDEMARK, P. J. (1960b). The purification and properties of α -glycerophosphate-oxidising enzyme of *Streptococcus faecalis* 10C1. *Archives of Biochemistry and Biophysics* **88**, 250-255.
- JOHNSTON, M. A. & DELWICHE, E. A. (1962). Catalase of *Lactobacillaceae*. *Journal of Bacteriology* **83**, 936-938.
- KNOWLES, C. J. (1980). Heme-requiring bacterial respiratory systems. In *The Diversity of Bacterial Respiratory Systems*, vol. 2 (in the Press). Edited by C. J. Knowles. Boca Raton, Florida: CRC Press.
- MCCORD, J. M. & FRIDOVICH, I. (1969). Superoxide dismutase, an enzymic function of erythrocuprein (hemocuprein). *Journal of Biological Chemistry* **244**, 6049-6055.
- MITCHELL, P. & MOYLE, J. (1967a). Acid-base titration across the membrane systems of rat-liver mitochondria. *Biochemical Journal* **104**, 588-600.
- MITCHELL, P. & MOYLE, J. (1967b). Respiration-driven proton translocation in rat-liver mitochondria. *Biochemical Journal* **105**, 1147-1162.
- PRITCHARD, G. G. & WIMPENNY, J. W. T. (1978). Cytochrome formation, oxygen-induced proton extrusion and respiratory activity in *Streptococcus faecalis* var. *zymogenes* grown in the presence of haematin. *Journal of General Microbiology* **104**, 15-22.
- REYNAFARGE, B., BRAND, M. D. & LEHNINGER, A. L. (1976). Evaluation of the H⁺/site ratio of mitochondrial electron transport from rate measurements. *Journal of Biological Chemistry* **251**, 7442-7451.
- RITCHEY, T. W. & SEELEY, H. W. (1974). Cytochromes in *Streptococcus faecalis* var. *zymogenes* grown in a haematin-containing medium. *Journal of General Microbiology* **85**, 220-228.
- RITCHEY, T. W. & SEELEY, H. W. (1976). Distribution of cytochrome-like respiration in streptococci. *Journal of General Microbiology* **93**, 195-203.
- SIJPESTEIJN, A. K. (1970). Induction of cytochrome formation and stimulation of oxidative dissimilation by hemin in *Streptococcus lactis* and *Leuconostoc mesenteroides*. *Antonie van Leeuwenhoek* **36**, 335-348.
- SMALLEY, A. J., JAHLLING, P. & VANDEMARK, P. J. (1968). Molar growth yields as evidence for oxidative phosphorylation in *Streptococcus faecalis* 10C1. *Journal of Bacteriology* **96**, 1595-1600.
- SNELL, F. D. & SNELL, C. T. (1949). *Colorimetric Methods of Analysis Including Some Turbidimetric and Nephelometric Methods*, 3rd edn, vol. 2, pp. 882-883. Princeton, New Jersey: Van Nostrand.
- TEMPEST, D. W. & NEIJSSSEL, O. M. (1980). Growth yield values in relation to respiration. In *Diversity of Bacterial Respiratory Systems*, vol. 1 (in the Press). Edited by C. J. Knowles. Boca Raton, Florida: CRC Press.

- VAN DER WIEL-KORSTANJE, J. A. A. & DE VRIES, W. (1973). Cytochrome synthesis by *Bifidobacterium* during growth in media supplemented with blood. *Journal of General Microbiology* **75**, 417–419.
- WHITTENBURY, R. (1960). Two types of catalase-like activity in lactic acid bacteria. *Nature, London* **187**, 433–434.
- WHITTENBURY, R. (1964). Hydrogen peroxide formation and catalase activity in lactic acid bacteria. *Journal of General Microbiology* **35**, 13–26.
- WILSON, D. M., ALDERETE, J. F., MALONEY, P. C. & WILSON, T. H. (1976). Protonmotive force as the source of energy for adenosine 5'-triphosphate synthesis in *Escherichia coli*. *Journal of Bacteriology* **126**, 327–337.