Respiratory activity in *Listeria monocytogenes*

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1. SUMMARY

*Listeria monocytogenes* possessed glucose oxidase and NADH oxidase activities in whole cells and lysed protoplasts respectively. The NADH oxidase activity sedimented with the membrane fraction and was inhibited by the respiratory inhibitors rotenone, 2-heptyl-4-hydroxyquinoline-N-oxide and cyanide, suggesting the presence of a membrane associated respiratory chain.

2. INTRODUCTION

*Listeria monocytogenes* is a Gram-positive non-spore forming bacterium which is widespread in the environment and has been recognised as the causal agent of disease in animals and man [1]. Contamination of food with *L. monocytogenes* has been recognised as the cause of epidemics of listeriosis [2] and has caused much recent concern.

The metabolism of *Listeria* sp. has received only limited attention. Glucose is utilised for growth under aerobic and anaerobic conditions. Anaerobic growth yields lactate as the major metabolic end product, whilst aerobic growth yields acetate and lactate [3,4]. The mode of oxygen utilisation has however received little attention. Oxygen consumption is stimulated by glucose, but not by TCA cycle intermediates; these also failed to stimulate growth, suggesting the absence of uptake systems [5,6]. These workers failed to detect cytochromes by reduced minus oxidised difference spectra in whole cells of *L. monocytogenes* or in cell free extracts, however the presence of cytochromes *a*, *b*, *d*, *o* have been reported [7]. This work examines the respiratory activity of *L. monocytogenes* and the effect of respiratory inhibitors.

3. MATERIALS AND METHODS

3.1. Chemicals

β-Nicotinamide adenine dinucleotide reduced form (NADH) (grade III) rotenone, HQNO and lysozyme (grade I) were obtained from Sigma Chemical Co., Poole, Dorset, U.K. All other chemicals were obtained from BDH, Poole, Dorset, U.K., and were of ANALAR grade.

3.2. Growth of bacteria

*Listeria monocytogenes* NCTC 7973 was grown on coryneform broth (Difco tryptone, 10 g l⁻¹;
Difco yeast extract 5 g l⁻¹; glucose 5 g l⁻¹; sodium chloride 5 g l⁻¹; pH 7.2) or 'Listeria salts medium' (K₂HPO₄, 8 g l⁻¹; NaH₂PO₄ 3.1 g l⁻¹; NH₄Cl 1 g l⁻¹; MgSO₄·7H₂O 0.4 g l⁻¹; FeSO₄·7H₂O, 40 mg l⁻¹; MnSO₄·2H₂O, 1.5 mg l⁻¹; (NH₄)₆ Mo₇O₂₄·4H₂O, 1.2 mg l⁻¹; Difco yeast extract 0.1% (w/v), glucose 5 g l⁻¹). For aerobic growth, cultures were incubated overnight at 37°C on an orbital shaker; 10 ml was inoculated into 400 ml broth in a 2-l baffled flask and incubated at 37°C with vigorous rotation.

For anaerobic growth, overnight cultures were inoculated into 500 ml broth in a 500 ml Duran bottle (Schott, Mainz, F.R.G.) which had been previously placed in a boiling water bath for 30 min, cooled to 37°C in a water bath and supplemented with 25 ml of a 10% sodium thioglycollate solution. Cultures were incubated without shaking in a 37°C incubator.

All cultures were harvested at mid-log phase by centrifugation at 6000 × g for 10 min, washed and resuspended in either 50 mM potassium phosphate buffer pH 7.0 (whole cells) or protoplast buffer (50 mM Tris-HCl, 10 mM NaCl, 0.5 M sucrose pH 6.7) to OD₆₀₀ = 20. (equivalent to 19 mg ml⁻¹).

3.3. Protoplast preparation
Protoplasts were prepared according to the method of Ghosh and Murray [8]. Lysozyme (0.5 ml of 3 mg/ml in protoplast buffer) was added to bacterial suspensions in protoplast buffer (2.5 ml) and incubated at 37°C in a shaking water bath for 15 min. MgCl₂ (0.1 ml of a 1 M solution in protoplast buffer) was added and incubation continued for a further 35 min; the protoplasts were then stored on ice.

3.4. Preparation of lysed protoplasts
Protoplasts prepared from cells grown aerobically in coryneform broth were centrifuged (6000 × g for 10 min) and resuspended in an equivalent volume of 50 mM potassium phosphate buffer pH 7.0, with a few grains of DNAse. Subsequent centrifugation (6000 × g for 10 min) yielded a supernatant 'cytoplasmic fraction' and a pelleted 'cell membrane' fraction which was resuspended in 2.5 ml 50 mM potassium phosphate buffer, pH 7.0.

Table 1
Glucose oxidase and NADH oxidase activity of whole cells and protoplasts of L. monocytogenes

<table>
<thead>
<tr>
<th>Cells</th>
<th>Growth media</th>
<th>Conditions</th>
<th>Glucose oxidase (mg atom 0 min⁻¹)</th>
<th>NADH oxidase (mg atom 0 min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole</td>
<td>CB a</td>
<td>Aerobic</td>
<td>128.2 ± 44.9 (7)</td>
<td>0</td>
</tr>
<tr>
<td>Protoplasts</td>
<td>CB</td>
<td>Aerobic</td>
<td>0</td>
<td>302.1 ± 120.6 (7)</td>
</tr>
<tr>
<td>Whole</td>
<td>Salts + glucose</td>
<td>Aerobic</td>
<td>100.8 ± 9 (3)</td>
<td>0</td>
</tr>
<tr>
<td>Protoplasts</td>
<td>Salts + glucose</td>
<td>Aerobic</td>
<td>116.3 ± 20.2 (3)</td>
<td>57.2 ± 13 (4)</td>
</tr>
<tr>
<td>Whole</td>
<td>CB</td>
<td>Anaerobic</td>
<td>92.3 ± 7 (4)</td>
<td>79.0 ± 19.6 (4)</td>
</tr>
<tr>
<td>Protoplasts</td>
<td>CB</td>
<td>Anaerobic</td>
<td>81.5 ± 41.9 (3)</td>
<td>15.2 ± 9 (4)</td>
</tr>
<tr>
<td>Whole</td>
<td>Salts + glucose</td>
<td>Anaerobic</td>
<td>5.6 ± 3.0 (4)</td>
<td>39.6 ± 26 (4)</td>
</tr>
<tr>
<td>Protoplasts</td>
<td>Salts + glucose</td>
<td>Anaerobic</td>
<td>27.7 ± 9 (4)</td>
<td>0</td>
</tr>
</tbody>
</table>

a Coryneform broth.
The absorbance at 340 nm was recorded on a Pye-Unicam SP8-150 spectrophotometer.

4. RESULTS AND DISCUSSION

4.1. Oxidase activities

*Listeria monocytogenes* NCTC 7973 grown in aerobic conditions in either coryneform broth or on glucose possessed substantial glucose oxidase activity in whole cells. Lysed protoplasts possessed an NADH oxidase activity but were unable to oxidise glucose (Table 1). When grown under anaerobic conditions on coryneform broth there was still a substantial glucose oxidase activity in whole cells. In addition unlike aerobically grown cells, these possessed the ability to oxidise exogeneously supplied NADH, probably indicating a greater fragility of these cells resulting in disruption during cell preparation. When grown anaerobically on glucose, the glucose oxidase activity was considerably reduced. This may have been due to increased fragility leading to disruption during cell harvesting and washing, as indicated by the NADH oxidase activity of whole cells grown in these conditions. Indeed, little increase in NADH oxidase activity was observed in lysed protoplasts of cells grown under these conditions.

![Graph 1](https://academic.oup.com/femsle/article-abstract/78/1/95/587120)

*Fig. 1. Inhibition of NADH oxidase by HQNO. Protoplast (0.1 ml) were incubated in 50 mM potassium phosphate buffer, pH 7, with HQNO for 2 min prior to the addition of NADH (1.6 mM).*

4.2. Respiratory inhibitors

NADH oxidase activity of lysed protoplasts was inhibited by low concentration of 2-heptyl-4-hydroxyquinoline-N-oxide (HQNO), with 50% inhibition occurring at 3.5 μM (Fig. 1). Rotenone also inhibited NADH oxidase activity, with 50% inhibition at 6 μM (Fig. 2), however, concentrations of rotenone up to 60 μM failed to bring about more than 40% inhibition.

Addition of potassium cyanide caused inhibition of glucose oxidase activity in whole cells and
Table 2

Localisation of enzyme activities in *L. monocytogenes*

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Enzyme activity</th>
<th>Isocitrate dehydrogenase ($\mu$mol NADP min$^{-1}$ mg dry wt. cells$^{-1}$)</th>
<th>NADH oxidase (ng-atom 0 min$^{-1}$ mg dry wt. cells$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protoplast preparation</td>
<td>47.6</td>
<td>591.8</td>
<td></td>
</tr>
<tr>
<td>'Cytoplasmic fraction'</td>
<td>33.4</td>
<td>75.8</td>
<td></td>
</tr>
<tr>
<td>'Cell membrane fraction'</td>
<td>16.9</td>
<td>475.5</td>
<td></td>
</tr>
</tbody>
</table>

NADH oxidase activity in lysed protoplasts. The inhibition of NADH oxidase activity in lysed protoplasts was significantly affected by the initial concentration of NADH employed in the assay. Dixon plots of 1/v (NADH oxidase activity) against cyanide concentration at different concentrations of NADH yielded parallel lines indicative of an uncompetitive type of inhibition (Fig. 3).

4.3. **Localisation of NADH oxidase activity**

Lysis of protoplasts in hypotonic buffer followed by centrifugation resulted in 70% of isocitrate dehydrogenase activity in the supernatant indicating substantial damage to the cells with release of cell contents (Table 2). NADH oxidase activity largely sedimented with the cell wall and membranes suggesting this activity is localised on the cell membrane.

4.4. **Conclusion**

*L. monocytogenes* possesses a NADH oxidase activity associated with the cell membrane. The oxidase activity is inhibited by the classical respiratory inhibitors rotenone, HQNO and cyanide and therefore appears to be a membrane associated with respiratory activity. Interestingly however, the concentration of cyanide required to inhibit oxidase activity was relatively high. Cytochromes were not detected by reduced minus oxidised difference spectra at room temperature of whole cells and lysed protoplasts of *L. monocytogenes* (data not shown). Earlier reports note the presence of cytochromes *a*, *b*, *d*, *o* in *Listeria* sp. [7] although cytochromes were not detected by Trivett and Meyer [6]. The action of respiratory inhibitors suggests the presence of a respiratory chain, thus the failure to detect cytochromes in this work may be indicative of a low concentration of respiratory components. This would suggest a high specific activity of the oxidase system. The role of this respiratory activity is as yet unclear. During anaerobic conditions lactate is produced [3]. Use of oxygen as an electron acceptor for the reoxidation of NADH may enable the formation of ATP and acetate via acetyl phosphate as suggested for *Leuconostoc* spp. [10]. In the absence of oxygen NADH must be reoxidised by the formation of lactate to allow metabolism to proceed. The possible conservation of energy in the form of proton translocation to generate a transmembrane pH gradient and membrane potential during NADH oxidation by *L. monocytogenes* is unknown. These points require further investigation.

REFERENCES