Cholesterol Protects *Acholeplasma laidlawii* Against Oxidative Damage Caused by Hydrogen Peroxide

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**ABSTRACT**

The aim of this study was to determine whether cholesterol, added to the cell growth medium or to cell suspension buffer, could protect *Acholeplasma laidlawii* cells against the toxic effects of hydrogen peroxide (H₂O₂). Variable concentrations of cholesterol (0.05–1.0 mg/ml) were added to the *A. laidlawii* suspension buffer and to the growth medium. Cells were then washed carefully and incubated with 0.001% (v/v) H₂O₂ at 37°C for 30 min and the viability was determined. The results indicated that cells were more viable in the presence of cholesterol than were cells grown in the absence of cholesterol. In addition, the oxygen uptake rate resulting from the oxidation of 5.5 mmol/L glucose was 2-fold and 4-fold higher for cells grown in medium supplemented with 0.05 and 0.50 mg/ml cholesterol, respectively, compared to cells grown in a medium with no added cholesterol. These findings indicate that cholesterol might play a role in protecting Mollicutes against the oxidative damage caused by H₂O₂.

**Keywords**: Acholeplasma, cholesterol, hydrogen peroxide, Mollicutes

**Abbreviations**: CFU/ml, colony-forming units per ml; DOT, dissolved oxygen tension; H₂O₂, hydrogen peroxide; NADH, nicotinamide–adenine dinucleotide (reduced form); OD, optical density; RH, Ringer-Hepes

**INTRODUCTION**

Mollicutes are a class of bacteria lacking cell wall, being bound by a plasma membrane only. *Acholeplasma*, non-cholesterol growth-dependent organisms, and *Mycoplasma*, cholesterol growth-dependent organisms, are two major genera of this class. Many species of Mollicutes, including *A. laidlawii* have been shown to produce H₂O₂ as a result of oxidation of different metabolic substrates such as glucose, NADH, glycerol and ethanol (Miles et al., 1991; Miles, 1992; Abu-Amero et al., 2000). Hydrogen peroxide is generally toxic to living cells as it causes peroxidation of polyunsaturated fatty acids, liberation of DNA bases and inactivation of enzymes (Halliwell and Gutteridge, 1985). Since Mollicutes have no cell wall and many of the species produce quantities of H₂O₂ that are sufficient to lyse red blood cells, how do these organisms...
protect themselves against the oxidative damage caused by \( \text{H}_2\text{O}_2 \)? In contrast to all other prokaryotes, Mollicutes incorporate cholesterol into their cell membranes and this might give the cells protection against their own \( \text{H}_2\text{O}_2 \) production (De Kruyff et al., 1972). Different mollicute species incorporate variable concentrations of cholesterol into their membranes if it is provided in the medium. In \( M. \text{mycoides} \), cholesterol is approximately 20% of the total membrane lipid (Rodwell, 1963), and values as high as 40% have been reported for \( M. \text{hominis} \) (Rottem and Razin, 1973). In addition, achloroplasts also incorporate smaller amounts of cholesterol into their membrane if it is available in the medium. Cholesterol accounted for 8.0% of the total cell membrane lipid of \( A. \text{laidlawii} \) cells grown in the presence of 25 mg/L of cholesterol (De Kruyff et al., 1972), and the highest recorded amounts for this species were up to 12% (Razin, 1974; Razin, 1975). The incorporation of cholesterol into mollicute membranes may help the organisms to resist the toxic effects of \( \text{H}_2\text{O}_2 \). It was previously reported that as \( A. \text{laidlawii} \) cells aged, accumulation of cholesterol in the cell membrane increased (Kapitanov et al., 1990). The objective of the present study was to determine whether cholesterol added to the cell growth medium or cell suspension buffer might aid \( A. \text{laidlawii} \) in resisting the oxidative damage caused by \( \text{H}_2\text{O}_2 \).

**MATERIALS AND METHODS**

**Organisms and media**

\( A. \text{laidlawii} \) strain PG8 (NCTC 10116), \( A. \text{oculi} \) (NCTC 10150), \( M. \text{gallisepticum} \) (NCTC 10115) and \( M. \text{pneumoniae} \) (NCTC 10119) were obtained from the National Collection of Type Cultures (Colindale Avenue, London, UK). The growth medium used was a modified SP4 broth (Tully and Whitcombe, 1983) consisting of the following: mycoplasma broth base (Oxoid, Unipath Ltd, Basingstoke, UK) 3.3 g/L; proteose peptone (Oxoid) 5.3 g/L; tryptose (Oxoid) 10.5 g/L; yeast extract (Oxoid) 2.0 g/L; glucose 5.0 g/L; glutamine 0.6 g/L; Hepes (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) 18 g/L; CMRL-1066 (synthetic culture medium without glutamine or NaHCO\(_3\); Gibco Life Technologies Inc., Paisley, UK) 50 ml/L; fresh yeast extract 20 ml/L; and heat-inactivated pig serum (Gibco Life Technologies) 200 ml/L. Fresh yeast extract was obtained by boiling baker’s yeast (25 g/100 ml) for 15 min at 100°C and removing cell debris by centrifugation. SP4 broth medium was then dispensed in 7 ml quantities in sterile plastic screw-capped test tubes and inoculated with 0.2 ml of \( A. \text{choleplasma} \) and \( M. \text{ycoplasma} \) cultures stored at –70°C. The tubes were then incubated statically at 37°C for 24 h.

**Cholesterol and hydrogen peroxide**

Cholesterol (Sigma, Poole, UK) from porcine liver was dissolved in methanol at the required concentration and stored at –20°C until required. Methanol (10 mmol/L),