SENSITIVITY OF *ESCHERICHIA COLI* TOWARDS CHLORHEXIDINE DIACETATE AND POLYMIXIN B ASSOCIATED WITH GROWTH-RATE-MEDIATED CHANGES IN ENVELOPE PHOSPHOLIPID AND PROTEIN COMPOSITION

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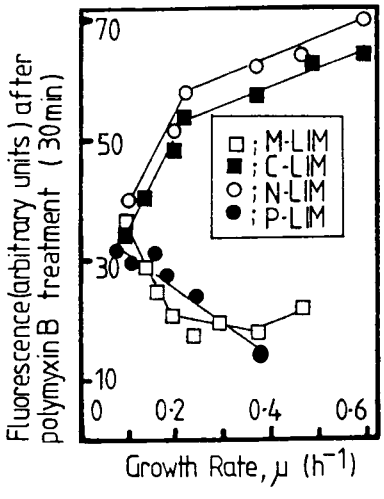
Chlorhexidine diacetate and polymyxin B are cationic surface active agents for which action is mediated through acidic phospholipids such as phosphatidylglycerol (PG) and diphosphatidylglycerol(DPG) (Elferink & Booij 1974, Conrad & Gilleland 1981). This directly influences envelope permeability and function. Resistance towards both agents has been associated with changes in acidic phospholipid content and in the case of polymyxin with a 21Kdal outer membrane protein H1 (Broxton *et al* 1984, Kenward *et al* 1979, Nicas & Hancock 1980).

We have investigated the interdependence of growth-rate, nutrient limitation and envelope composition for *E.coli* ATCC8739 and assessed the associated levels of sensitivity towards these agents. Cells were grown in the chemostat at a range of growth rate (μ=0.05-0.6h^{-1}) under carbon (C-lim), nitrogen (N-lim), phosphorus (P-lim) or magnesium limitation (M-lim). Sensitivity towards chlorhexidine was assessed by a viable counting method, whilst that towards polymyxin was determined by a fluorometric assay which measured the degree of penetration to the cytosol of the dye, 2-p-toluidinylnapthalene-6-sulphonate (Newton 1954). After preliminary experimentation, fixed concentrations of chlorhexidine (0.01mM) and polymyxin (120 i.u./ml) were used throughout.

Similar patterns of sensitivity were demonstrated towards both agents. Resistance increased with increasing growth rate for P-lim and M-lim, yet decreased for N-lim and C-lim. Gross composition of the cell envelopes were determined gravimetrically from freeze dried samples. The relative and total amounts of the different phospholipids were determined upon chloroform-methanol extracted fractions by TLC and densitometry. Envelope protein composition was determined by SDS-PAGE (Williams *et al* 1984). At all growth rates studied P-lim and M-lim cultures possessed less acidic phospholipid per unit cell mass than C-lim and N-lim. The content of these decreased in all cases with increasing growth rate and could not therefore account for the pattern of sensitivity observed. DPG content was significantly lower for M-lim and P-lim whilst phosphatidylserine content was higher but decreased markedly with growth rate under M-lim and P-lim. The outer-membrane protein H1 was also present, but in greater amounts at high growth rates for M-lim and P-lim, and low growth rates for N-lim and C-lim.



Whilst interactions between the acidic phospholipids and these drugs has been clearly demonstrated *in vitro* and is supported by work in intact cells (Broxton *et al* 1984, Conrad & Gilleland 1981), data generated in this study over a range of growth rates suggest that a far more complex interaction(s) must account for the antimicrobial action *in vivo*.

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