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Short communication

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Cultivation in different growth media affects the expression of the cell surface hydrophobicity of bacteria

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ABSTRACT
Environmental factors may greatly influence the expression of cell surface components of bacterial pathogens. Few studies have described the effect of growth conditions on the cell surface hydrophobicity of bacterial isolates of certain Gram-negative and Gram-positive bacteria. The present study describes the effects of cultivation in four common liquid growth media on the cell surface hydrophobicity of non-clinical Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* using the microbial adhesion to hydrocarbon (MATH) test which assesses the partition of bacterial cells into a hydrocarbon phase. It was observed that growth in all the test media yielded cells with varying surface hydrophobicity increasing in the order: nutrient broth<tryptic soy broth<brain heart infusion broth<MacConkey broth, irrespective of the test strain. The changes were however, more pronounced in the Gram-positive strain. It was also observed that the surface of *Staphylococcus aureus* was more hydrophobic than that of *Escherichia coli*, irrespective of the cultivation media. The outcome of this work further point to the fact that environmental changes can influence cell surface hydrophobicity of bacteria which in turn can affect their adhesion to certain kinds of host targets through hydrophobic interactions.

Key words: Hydrophobicity; *Escherichia coli*; *Staphylococcus aureus*; Growth media

INTRODUCTION

The surfaces of bacterial cells are fundamental to their ability to interact with their environment; and characterization of these surfaces encompasses both the macromolecular constitution and physicochemical properties, such as hydrophobicity and surface charge. Cell surface hydrophobicity (CSH) plays important role in the interaction of bacteria with living cell and inanimate surfaces [1, 2]. The importance of hydrophobicity however, appears to vary with different species of bacteria [3].

Certain environmental factors may greatly influence the expression of cell surface components of bacterial pathogens [4, 5], the optimum expression of which relates to growth in suitable environment. Few studies have described the effect of growth conditions on the cell surface hydrophobicity of clinical and environmental isolates of certain Gram-negative and Gram-positive bacteria [6, 7, 8, 9]. However, the impact of cultivation in different growth media on the cell surface hydrophobicity (CSH) of non-clinical laboratory strains of bacteria, which have not been exposed to antibiotic and other environmental pressures, is not documented. The aim of the present study was to investigate the effects of cultivation in different growth media on the CSH of non-clinical laboratory Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus*.

MATERIALS AND METHODS

Bacterial strains and culture conditions

Escherichia coli (ATCC 11775) and *Staphylococcus aureus* (ATCC 12600) were used in this study. These strains were maintained on nutrient agar (NA; LAB M Ltd., UK) slants at 4°C and sub-cultured overnight on same agar prior to use. Growth media used were: nutrient broth 'E' (NB: LAB M Ltd., U.K.), tryptic soy broth (TSB: Merck Ltd., Germany), brain heart infusion broth (BHIB: LAB M Ltd., UK) and MacConkey broth (MCB: Oxoid Ltd., UK).

Measurement of cell surface hydrophobicity

Cell surface hydrophobicity was measured by the microbial adhesion to hydrocarbon (MATH), method originally described by Rosenberg et al.

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[10], with modifications as cited by Flint et al. [3] using xylene as hydrocarbon. Briefly, the test strains were grown statically for 24 h at 37°C in 15 ml each of the different media. Cells were then harvested by centrifugation (4500 rpm for 10 min) and re-suspended in sterile distilled water to an absorbance at 600 nm (A_{600}) of 1.2-1.6. The resulting cell suspensions (3.0 ml each) were added to 3.0 ml of n-xylene (Daychem Ltd., UK) in separate universal bottles and mixed briefly on a vortex mixer. These bacteria-xylene mixtures were then left for 15 minutes at ambient temperature to allow equilibration to occur. The bottles were subsequently mixed vigorously by vortexing for 2 minutes at ambient temperature and afterwards allowed to stand for 20 minutes to allow phase separation. The A_{600} of the aqueous phase after phase separation was henceforth measured. The per cent (%) hydrophobicity of the cell surfaces of the test strains cultivated in the different media were then determined from the initial absorbance of the bacterial suspension (A_I) and the absorbance of the aqueous phase after separation (A_F) using the formula:

$$Hydrophobicity(\%) = (1 - \frac{A_F}{A_I}) x100.$$

The experiment was repeated twice in all cases.

Experimental design and statistical analysis

The test strains were grown in four separate 15-ml samples of each of the four different culture media. Hydrophobicities were analyzed by the one-way analysis of variance (ANOVA) using Smith Statistical Package, version 2.5 and significance of results determined at the 5 % probability level (that is, at $P = 0.05$).

RESULTS

As can be seen in Figure 1, the surface of the Gram-positive *Staphylococcus aureus* is more hydrophobic than that of the Gram-negative *Escherichia coli* under all cultivation conditions. Also, the growth of bacteria in different media yielded cells with varying surface hydrophobicities increasing in the order: nutrient broth<tryptic soy broth<brain heart infusion broth<MacConkey broth, irrespective of the test strain. For, the Gram-negative *E. coli*, NB, TSB and BHIB promoted the expression of cell surface hydrophobicity to similar extent ($P>0.05$); but did so significantly ($P<0.05$) less than MCB. However, for the Gram-positive *S. aureus*, difference in cell surface hydrophobicity determined in TSB, BHIB and MCB were insignificant ($P>0.05$); but were significantly ($P<0.05$) more compared with NB.

DISCUSSION AND CONCLUSIONS

The observation that the surface of the Gram-positive *Staphylococcus aureus* was more hydrophobic than that of the Gram-negative *Escherichia coli* under all cultivation conditions may be accounted for by the fact of existing differences in the chemical composition of the cell envelopes of the test strains. Chemical analysis of walls of Gram-positive bacteria have revealed them to differ from the Gram-negative ones in possessing more peptidoglycan, covalently linked 'accessory polymers' (teichoic acids, polysaccharides and proteins), and lacking lipopolysaccharides, outer membrane proteins and lipoproteins [11, 12].

Also, the variation in surface hydrophobicities following growth of bacteria in different media could be explained by a possible change in the surface compositions of the test strains when grown in the different media. This is because MATH usually assesses the extent of interaction between hydrophobic cell surface components and/or adhesins with liquid hydrocarbon [10, 13]. Medium composition have, before now, been shown to influence peptidoglycan chemistry [14], synthesis of accessory anionic polymers in the Gram-positive bacteria [4, 15], the production of capsular and extracellular polysaccharides [16], and the synthesis of outer membrane proteins and lipopolysaccharides in Gram-negative bacteria [17, 18, 19]. The amino acid and peptide composition of the medium can have a profound effect on the peptide composition and degree of cross-linking of peptidoglycan [12]. This explains probably, the higher values of hydrophobicity obtained in cells grown in media containing high content of peptones and amino acids such as MacConkey, Brain-Heart Infusion and Tryptic Soy broths. Nutrient broth contains lower levels of nitrogen sources in its composition compared to the other test media. This appeared to have encouraged the preferential production of extracellular polysaccharides [20] which, to a large degree, are acidic and often impart a hydrophilic nature to the cell surface [21].

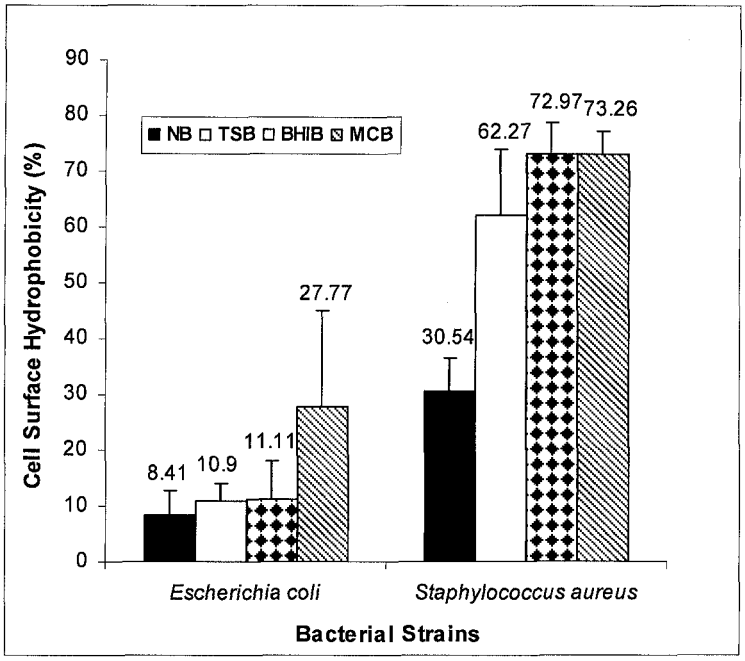

Figure 1: Cell surface hydrophobicity of test strains measured by the MATH test. Bacteria were grown in the different growth media, washed and re-suspended in PBS, then mixed with xylene for stated time period and the partitioning of cells into aqueous and xylene phase was determined by difference of absorbance (at 600 nm) of aqueous phase before and after mixing bacteria with xylene as described in *Materials and Methods*. NB: nutrient broth; TSB: tryptic soy broth; BHIB: brain heart infusion broth; and MCB: MacConkey broth.

The more pronounced effect of medium composition on the Gram-positive test strain could be explained on the basis of a perhaps greater influence of medium composition on peptidoglycan chemistry since peptidoglycan is the major component of the Gram-positive cell wall [11].

The overall results show that cultivation of non-clinical bacterial isolates in different growth media also influences the relative hydrophobicity of their surfaces, the effect being more pronounced in Gram-positive bacteria. This explains probably, why bacteria colonize certain host targets preferentially or proliferate under certain favorable environmental changes. Further work is being done to ascertain the precise effect of the media on the composition of the bacterial cell surface.

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