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# Bacterial quorum sensing and cell surface electrokinetic properties

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**Abstract** The hypothesis tested in this paper is that quorum sensing influences the microbial surface electrokinetic properties. *Escherichia coli* MG1655 and MG1655 LuxS- mutant (lacking quorum-sensing gene for Autoinducer synthase AI-2) were used for this study. AI-2 production (or lack of) in both strains was analyzed using the *Vibrio harveyi* bioassay. The levels of extracellular AI-2 with and without glucose in the growth medium were consistent with previously published work. The surface electrokinetic properties were determined for each strain of *E. coli* MG1655 by measuring the electrophoretic mobility using a phase amplitude light-scattering (PALS) Zeta potential analyser. The findings show that the surface charge of the cells is dependent upon the stage in the growth phase as well as the ability to participate in quorum sensing. In addition, significant differences in the electrophoretic mobility were observed between both strains of *E. coli*. These findings suggest that quorum sensing plays a significant role in the surface chemistry of bacteria during their growth.

**Keywords** Cell surface charge · Electrokinetic potential · Quorum sensing · *Escherichia coli* · Aggregation

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## Introduction

Bacterial cell electrokinetic surface properties have been shown to play a crucial role in bacterial aggregation (Eboigbodin et al. 2005; Marshall 1984). This is due to the presence of charged macromolecules on the bacterial outer membrane, lipopolysaccharides (i.e., the carboxyl group carries a net negative charge), sialic acids and/or membrane proteins (Torimura et al. 1999). Surface electric potentials for non-biological colloids are generally obtained by the measurement of the movement of the charged particles in an external electric field, i.e., the electrophoretic mobility (EPM). The magnitude of the EPM gives an indication of the overall net charge on the surface of a particle. A negative EPM value indicates that a particle has a negative charge, while a positive EPM value indicates that the particle has a net positive charge. This measurement of movement is related to the Zeta potential (Hayashi et al. 2003; Sonohara et al. 1995; Tsuneda et al. 2004) usually via the Smoluchowski's mobility formula (Jucker et al. 1996) and gives an indication of the energy barrier between colloids, which needs to be lowered to promote aggregation (Russell et al. 1989).

However, the assumptions in the Smoluchowski calculation are not suitable for use with bacteria due to their porous surface and non-spherical shape (Sonohara et al. 1995). Ohshima's soft-particle electrophoresis theory, developed by Ohshima and Kondo (Ohshima 1995; Ohshima and Kondo 1989), also assumes a spherical shape, but is suitable for colloids with a soft outer layer. This model assumes the presence of an ion-penetrable layer of finite thickness around a core spherical particle, and this approach has been found to be useful in estimating the surface charge of several biological systems from the electrophoretic mobility (Hayashi et al. 2003; Sonohara et al. 1995;

Tsuneda et al. 2004). Hayashi et al. (2003) and Eboigbodin et al. (2005) were able to demonstrate that the growth phase of different bacteria dictates their cell surface properties as measured by electrophoretic mobility. In both cases, a maximum in electrophoretic mobility occurred during the early stationary phase. Sonohara et al. (1995) also used electrophoretic mobility to show that Gram-negative bacteria are more negatively charged and have a less soft surface than Gram-positive bacteria.

More recently, the same soft particle theory was used to investigate the differences in two *E. coli* mutants, which had differences in the length of the charged macromolecules present in their cell surface (de Kerchove and Elimelech 2005). Therefore, the measurement of the electrophoretic mobility has been successful in being able to compare and characterise the electrokinetic surface properties of a variety of different bacterial strains (van Loosdrecht et al. 1987). However, whilst it is possible to quantify the changes in cell surface electrokinetic properties using a colloidal approach, the key biological question in cell–cell aggregation and biofilm formation is what governs the change in cell surface properties to promote or restrict adhesion.

There is evidence suggesting that cell-to-cell communication via quorum sensing, may influence bacteria cell surface properties, and cell aggregation, with differences in colony surface morphology and aggregation ability observed for cells, which have lost their ability to produce quorum-sensing molecules (Park et al. 2003; Zhang and Pierson 2001). Cell-to-cell communication via quorum sensing has been well studied in several biological systems (for reviews, see Waters and Bassler (2005); Xavier and Bassler (2003)). Bacteria are able to monitor the microbial community via quorum sensing by producing, detecting and responding to low molecular weight signal molecules, called autoinducers (AI). Recent findings have shown that most bacteria produce, detect and respond to an autoinducer AI-2, suggesting that it may be used in interspecies cell-to-cell communication (Schauder et al. 2001; Waters and Bassler 2005).

AI-2 has been shown to play a crucial role in biofilm formation of *Streptococcus mutans* by the regulation of glucosyltransferase genes (Merritt et al. 2003; Yoshida et al. 2005). Park et al. (2003) also observed differences in aggregation properties between *Escherichia coli* strains lacking the ability to produce AI-2 (deleted LuxS gene) and the wild-type strains. More recently, AI-2 has been shown to control biofilm formation in *Escherichia coli* by controlling the genes involved in the motility (Gonzalez Barrios et al. 2006) and the regulation of outer membrane proteins (Winzer et al. 2000). Gonzalez Barrios et al. (2006) recently demonstrated that a dramatic change in biofilm formation was observed on the addition of AI-2 by intensifying motility. Winzer et al. (2000) showed that quorum sensing also regulates outer membrane proteins such as lectins, which in some bacteria are involved in cell-to-cell adhesion. These findings suggest that the outer surface of bacteria, and hence the electrokinetic properties, will be altered if quorum sensing regulates these outer-membrane macromolecules. DeLisa et al. (2001) used DNA microarray analysis to identify genes regulated by AI-2 in *Escherichia coli* and found that *ompG* (an outer membrane porin protein) *rfaY* (biosynthesis of core lipopolysaccahride), *flip* (Flagellar biosynthesis), *rfaj*(UDP-D-glucose: (galactosyl) lipopolysaccharides glucosyltransferase) and *b1502* (putative adhesin) were all regulated by AI-2.

Although there is strong evidence suggesting that quorum sensing may alter outer-membrane polymers, quantification of the change in cell-surface electrokinetic properties, altered by quorum sensing, still remains unclear. From a bioengineering point of view, the cell-surface electrokinetic properties are a major factor that governs bacterial adhesion and aggregation. This is because as the cells are brought into close proximity, an attractive interaction potential must be present to promote cell–cell aggregation, and it is the cell-surface structure that will dictate the strength and type of this interaction (Hayashi et al. 2003). Hence, we seek to quantify the influence of quorum sensing (AI-2) on the cell-surface electrokinetic properties.

## Materials and methods

### Bacterial strains

*E. coli* strains MG1655 (wild-type) and MG1655 LuxS- mutant as well as *Vibrio Harveyi* BB170 (luxN::Tn5, sensor 1−, sensor 2+) were used in this study (supplied by Prof. Paul William, University of Nottingham, UK). The *E. coli* MG1655 LuxS- mutant mutation is identical to the *E. coli* LuxS- mutant that has been previously described in *E. coli* BL21 (Chen et al. 2002). The mutation was transferred into *E. coli* MG1655 by P1 phage transduction. (Winzer, Tavender, and Hardie, personal communication, 2006). The *E. coli* LuxS- mutant contained a deletion of LuxS over a 500-bp range, and this was confirmed using PCR analysis. PCR was used to amplify the LuxS gene from genomic DNA extracted (GeneElute NA2100, Sigma, UK) from both the *E. coli* MG1655 wild type and LuxS- mutant (PCR D4545 kit, Sigma, UK, using the protocol one cycle at 94° C for 2 min, 30 cycles of 95°C for 1 min, 52°C for 1 min, 72°C for 2 min and, finally, 72°C for 10 min). The LuxS gene was amplified using the primers LuxS-F3 (5′-TGCCDTTRTTAGAYAGCTTCA -3′) and LuxS-R3 (5′-TCCTGCARYTTYTCTTTCGG -3′) designed from Primer3 software (Rozen and Skaletsky 2000).