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The influence of microbial factors on the susceptibility of bacteria to photocatalytic destruction

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ABSTRACT

The role that bacterial factors play in determining how bacteria respond to photocatalytic degradation is becoming increasingly recognised. Fimbriae which are thin, proteinaceous cell surface structures produced by many enterobacteria are generally considered to be important bacterial virulence determinants in the host. Recent studies, however, suggest that their expression may be increased during times of environmental stress to protect them against factors such as nutrient depletion and oxidation. In this study bacteria were grown under defined culture conditions to promote the expression of type 1 fimbriae and subjected to photocatalytic treatment.

Results showed that *Escherichia coli* grown under conditions to express type 1 fimbriae were more resistant to photocatalytic destruction than control cultures, taking 75 min longer to be destroyed. Curli fimbriae are also known to play a role in environmental protection of bacteria and they are associated with biofilm production. The ability of the *E. coli* strain to produce curli fimbriae was confirmed and biofilms were grown and subjected to photocatalytic treatment. Biofilm destruction by photocatalysis was assessed using a resazurin viability assay and a loss of cell viability was demonstrated within 30 min treatment time. This study suggests that intrinsic bacterial factors may play a role in determining an organism's response to photocatalytic treatment and highlights their importance in this disinfection process.

1. Introduction

Over the past decade there have been numerous reports on the successful use of titanium dioxide (TiO_2) photocatalysis to destroy a wide range of bacteria in both water [1–6] and on solid surfaces [7–12]. Interest in this research area has grown mainly out of a need to develop alternative disinfection strategies to those currently in use. This is partly as a result of the negative aspects of some current methods, but also because of the development of multi-drug resistant bacteria particularly in the healthcare environment, where many common disinfectants and antibiotics are no longer effective [13–16]. While photocatalytic disinfection has been shown to be a very successful disinfection technique under laboratory conditions, the plethora of parameters that can affect experimental outcome is significant. Factors such as pH, aeration, UV intensity, temperature, microbial starting concentration, growth phase and organism type have all been highlighted as important variables during experimental design [6,17–23].

Furthermore, significant differences in the response of micro-organisms to photocatalytic destruction in natural and laboratory water have been highlighted [24–28]. In some cases these differences have been attributed to the presence of suspended solids, dissolved inorganic ions and organic compounds and dissolved oxygen [26,27]. To date, however, little consideration has been given as to how bacteria behave under these, often less than optimal, conditions. Bacteria grown under laboratory conditions are provided with the right amount of nutrients, light, oxygen and temperature to promote maximum growth. In the external environment, however, these optimal growth conditions are often not available and as a result bacteria may switch on the expression of virulence determinants to protect them against environmental stresses and to aid their survival. Expression of such virulence factors could influence bacterial susceptibility to reactive oxygen species (ROS) attack and affect how bacteria respond to photo-catalytic disinfection.

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In this study, the role of several bacterial factors on bacterial response to photocatalytic destruction, were examined. Bacteria were grown under conditions to enhance expression of type 1 fimbriae and to promote biofilm formation. Type 1 fimbriae are filamentous, proteinaceous cell surface structures expressed by many enterobacteria [29–31]. They are considered to be important virulence determinants as they provide adhesive, haemagglutinating (mannose sensitive) and pellicle forming properties on the organism [29,32–34]. While many studies have undoubtedly established a role for these fimbriae during the infection process fewer have looked at their role in environmental survival of the organism and how their expression protects the organism from environmental stress. Curli fimbriae are associated with an organism's ability to form biofilms and strong evidence exists for the role of these cell surface structures in environmental survival of bacteria [34,35]. Due to their complex structure, however, biofilms are very difficult to destroy and present a serious health hazard in many environmental, health and industrial settings. Photocatalysis presents a novel way of destroying biofilms in the environment although to date only a small number of studies have investigated this possibility [36,37].

This paper reports a preliminary study examining the role that intrinsic bacterial factors may play in protecting bacteria from photocatalytic attack. Using *E. coli* as a model organism, the bacteria were grown under defined culture conditions to promote the expression of type 1 fimbriae and curli fimbriae; biofilm growth was also assessed and photocatalytic disinfection experiments were performed.

2. Materials and methods

2.1. Bacterial strains and culture conditions

E. coli strain NCTC 12241(clinical isolate) was sub-cultured from a stock culture stored at −80 °C and maintained on nutrient agar at 4 °C. To prepare bacterial control cultures (stationary phase culture) for experiments, two to three well isolated colonies from the nutrient agar (Oxoid,UK) reference plate were inoculated into 10 ml of nutrient broth (Oxoid, UK) and incubated overnight at 37 °C in an orbital incubator (Thermo Scientific MaxQ 4000, USA) set at 100 rpm. Following overnight incubation cells were harvested by centrifugation at 3500 rpm for 10 min, these cells were then washed twice and re-suspended in sterile 0.9% NaCl.

2.2. Assessment of expression of type 1 fimbriae

To promote the expression of type 1 fimbriae, statically grown bacteria were serially passaged (times three) in Brain Heart Infusion (BHI) broth (Oxoid, UK), using a method described by Humphries et al. [38]. A statically grown, non-passaged culture was also prepared along with a control culture (as described in Section 2.1). Expression of type 1 fimbriae, in all three cultures, was assessed using a mannose sensitive haemagglutination assay (MSHA) as described by Sojka et al. [39].

2.3. Assessment of susceptibility of serially passaged culture to photocatalytic degradation

Sterile 150 ml glass beakers containing 99 ml of sterile 0.9% NaCl with 1 g/l TiO_2 (P25 Evonik, Frankfurt, Germany) were inoculated with 1 ml of the appropriate washed bacterial culture ($\sim 1 \times 10^8$ CFU ml^{-1}). UV illumination was provided by a 6 × 8 W UV-A lamp (spectral output 311–415 nm peaking at 368 nm; Philips TL 8W/08 F8 T5/BLB) which was housed within a light protective box. The photonic output of the lamp was determined to be 6.8×10^{-5} Einstein's s^{-1} using ferrioxalate actinometry. The light intensity at the position where the photocatalysis was being undertaken was determined to be an average of 2.86 mW cm^{-2} using a UVP, Model UVX digital Radiometer (Cole-Parmer, UK). Reaction vessels were placed at a distance of 10 cm from the UV lamp and magnetically stirred throughout the experimental period. Reaction mixtures were sampled at 15 min intervals, with serial dilutions performed in sterile 0.9% NaCl. Then 20 μl drops of each dilution were placed, in duplicate, onto well dried nutrient agar plates. Plates were incubated for 24 h at 37 °C and viable counts determined. Controls consisting of bacterial suspensions exposed to UV light in the absence of TiO_2 and bacterial suspensions containing TiO_2 that were kept in the dark, were run in parallel.

2.4. Curli expression and biofilm formation

Curli expression by *E. coli* NCTC 12241 was assessed by morphotype determination [40]. Cells were cultivated on M17 agar (Oxoid Ltd., UK) and incubated statically at 28 °C for 5 days and plates were examined daily for the production of rough/lacy colonies. A known, non-biofilm producing strain (*E. coli* NCIMB 8110, a K12 strain from the National Collection of Industrial, Marine and Food Bacteria) was included as a control. Biofilms were grown in 24-well plates (Nunclon™ surface plate) using 1/20 TSB broth growth media, according to the method of Solomon et al. [40] and growth was assessed using a crystal violet binding assay. Control samples with media only were included in each 24 well plate.

2.5. Photocatalytic destruction of E. coli biofilms

To prepare biofilms for photocatalytic destruction experiments, growth medium was carefully removed from the surface of each biofilm and replaced with 400 μl of either TiO_2 (1 g/l) in 0.9% NaCl or with 0.9% NaCl only (UV only control). Plates were placed under a 6 × 8 W UV-A lamp, as described in Section 2.3, at a distance of 10 cm and illuminated for 3 h. Dark controls were achieved by covering wells containing TiO_2 (1 g/l) in 0.9% NaCl with aluminium foil to prevent exposure to light. Biofilm viability was then assessed using the resazurin dye reduction test. Briefly, at the end of the illumination period, medium was removed and 400 μl of fresh growth medium was added to each well along with 40 μl of 0.001 mg/l resazurin dye (Sigma–Aldrich, UK). Plates were incubated overnight at 30 °C and any colour change was visually assessed. Control samples with medium only were also included in each plate.

3. Results and discussion

3.1. Expression of type 1 fimbriae

The expression of type 1 fimbriae correlates with an organisms ability to agglutinate red blood cells, this agglutination is mannose sensitive and can thus be inhibited/reversed by the addition of

Table 1
Assessment of strength of mannose sensitive haemagglutination of *E. coli* NCTC 12241 using 4% horse erythrocytes.

Dilution factor	1:2	1:4	1:6	1:8	1:10	1:12	Mannose sensitivity
Control culture[a]	/	/	/	/	/	/	/
Non-passaged culture[b]	x	x	x	/	/	/	√
×1 passaged culture[b]	x	x	x	/	/	/	√
×2 passaged culture[b]	xxx	xxx	xx	x	x	/	√

/, no haemagglutination; x, weak haemagglutination; xx, medium haemagglutination; xxx, strong agglutination; √, haemagglutination is mannose sensitive.
[a] Control culture grown in an orbital incubator at 37 °C with shaking at 100 rpm.
[b] Non-passaged and passaged cultures grown statically at 37 °C.