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

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

Jeanette M.C. Robertson [a*], Carina Sieberg[b] and Peter K.J. Robertson [a]

[a] Centre for the Theory and Application of Catalysis (CenTACat), School of Chemistry and Chemical Engineering, Queen's University Belfast, David Keir Building, Stranmillis Road, Belfast, BT9 5AG, UK

[b]School of Pharmacy and Life Sciences, Robert Gordon University, Garthdee Road, Aberdeen, AB10 7GJ, UK

* Corresponding author (J.M.C. Robertson)
Centre for the Theory and Application of Catalysis (CenTACat), School of Chemistry and Chemical Engineering, Queen's University Belfast, David Keir Building, Stranmillis Road, Belfast, BT9 5AG, UK
Tel: +44 2890 974627
Fax: +44 1224 2890 976524
E-mail address: j.robertson@qub.ac.uk

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 minutes 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 minutes 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.

Keywords: photocatalysis, bacteria, type 1 fimbriae, curli fimbriae, biofilm.

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

51 solid surfaces [7-12]. Interest in this research area has grown mainly out of a need to develop
52 alternative disinfection strategies to those currently in use. This is partly as a result of the
53 negative aspects of some current methods, but also because of the development of multi-drug
54 resistant bacteria particularly in the healthcare environment, where many common
55 disinfectants and antibiotics are no longer effective [13-16]. While photocatalytic disinfection
56 has been shown to be a very successful disinfection technique under laboratory conditions,
57 the plethora of parameters that can affect experimental outcome is significant. Factors such as
58 pH, aeration, UV intensity, temperature, microbial starting concentration, growth phase and
59 organism type have all been highlighted as important variables during experimental design [6,
60 17 – 23].
61
62 Furthermore, significant differences in the response of microorganisms to photocatalytic
63 destruction in natural and laboratory water have been highlighted [24-28]. In some cases these
64 differences have been attributed to the presence of suspended solids, dissolved inorganic ions
65 and organic compounds and dissolved oxygen [26, 27]. To date, however, little consideration
66 has been given as to how bacteria behave under these, often less than optimal, conditions.
67 Bacteria grown under laboratory conditions are provided with the right amount of nutrients,
68 light, oxygen and temperature to promote maximum growth. In the external environment,
69 however, these optimal growth conditions are often not available and as a result bacteria may
70 switch on the expression of virulence determinants to protect them against environmental
71 stresses and to aid their survival. Expression of such virulence factors could influence
72 bacterial susceptibility to reactive oxygen species (ROS) attack and affect how bacteria
73 respond to photocatalytic disinfection.
74
75 In this study, the role of several bacterial factors on bacterial response to photocatalytic
76 destruction, were examined. Bacteria were grown under conditions to enhance expression of
77 type 1 fimbriae and to promote biofilm formation. Type 1 fimbriae are filamentous,
78 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^{0}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 3,500 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 1g/L TiO_2 (P25 Evonik, Frankfurt, Germany) were inoculated with 1 ml of the appropriate washed bacterial culture (~ $1x10^8$ CFU mL^{-1}). UV illumination was provided by a 6 x 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 $x10^{-5}$ 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 minute 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 hrs 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* ATCC 8110) was included as a control. Biofilms were grown in 24-well plates (Nunclon ^{TM} 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.

2.4 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 (1g/L) in 0.9 % NaCl or with 0.9 % NaCl only (UV only control). Plates were placed under a 6 x 8 W UV-A lamp, as described in section 2.3, at a distance of 10 cm and illuminated for 3 hrs. Dark controls were achieved by covering wells containing TiO_2 (1g/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 mannose [29,39]. Results from the MSHA (table 1) show that both the, statically grown, serially passaged and non-passaged cultures expressed type 1 fimbriae, however expression was clearly stronger in the serially passaged culture and therefore this culture was chosen for further study. The control culture, which was not grown under conditions to

promote the expression of type 1 fimbriae i.e. non-static culture conditions, did not show any haemagglutination. In addition, on visual examination of all broth cultures, a white pellicle was present on the surface of both the statically grown serially passaged and non-passaged cultures however it was more prominent in the former. There was no pellicle evident in the control culture. This white pellicle is a cell mesh indicative of the presence of fimbriated cells growing at the surface of the broth. The importance of this was first highlighted by Old and Duguid [29] who proposed that fimbriated bacteria had a growth advantage over non-fimbriated strains as by establishing themselves rapidly in a pellicle at the surface of a broth culture their growth could be promoted by the supply of atmospheric oxygen. These findings agree with published results which show that growth in static broth results in a culture containing a large fraction of type 1 fimbriated cells and which increase in number after serial passage in static culture [29, 38].

3.2 Effect of serial passaging on susceptibility of E. coli to photocatalytic destruction

Results from Figure 1 show that the serially passaged culture, expressing type 1 fimbriae, took 75 minutes longer to be completely destroyed than the non-passaged control culture, which did not express any type 1 fimbriae, according to the MSHA. **Whilst the majority of bacteria from both test groups were destroyed within 15 minutes, the surviving bacteria in the serially passaged culture group persisted for up to 90 minutes and this effect was repeatedly seen in replicate experiments. These findings do therefore indicate a trend, towards persistence, in the fimbrial producing group of bacteria and therefore this phenomena warrants further study.** Data from the UV-A only and dark control groups showed that no bacterial destruction took place. **The optimal growth conditions afforded to bacteria grown under laboratory conditions may not available in the external environment therefore bacteria may have to switch on and off the expression of many virulence determinants (a phenomena known as phase variation), according to physiological conditions, to aid their survival [41,42].** In terms of fimbrial expression this means that bacteria can switch between fimbriated and non-fimbriated states [42] and as our

data suggest, this may influence how bacteria respond to photocatalytic disinfection. Further studies are required to ascertain the specific reasons for this difference in susceptibility; however one reason could be prevention of interaction between catalyst and bacterial cell surface by the presence of large numbers of fimbriae. It has been established that the cause of bacterial cell death by photocatalysis is loss of membrane structure [2,43-48]. This process is dependent on physical contact between the catalyst and bacteria taking place and anything that interferes with this is likely to greatly reduce the efficacy of the process. The reduced photocatalytic disinfection efficiency observed here, in cultures expressing greater numbers of type 1 fimbriae, may be due to the increased presence of these surface appendages which could be physically preventing the photocatalyst from coming into contact with the bacterial cell surface. Krishna *et al* [49] suggested that the decreased efficiency of TiO_2 coated multi-wall carbon nanotubes compared to a commercial TiO_2 nanopowder (Degussa P25) against *E. coli* vegetative cells may be due to the presence of surface appendages, such as fimbriae which could stearically hinder the carbon nanotubes from coming into contact with the bacterial cell wall and therefore reduce photocatalytic efficiency. **However consideration should also be given as to what, if any, effects fimbrial production may have on cell wall architecture and how this might influence photocatalytic response.**

Sub-successive sub-culturing of a bacterial culture is known to induce mutations within that culture. Such changes may affect how bacteria respond to photocatalytic treatment. Rincon and Pulgarin [23] showed that bacteria harvested at the third generation of culture were less sensitive to irradiation than those taken from the seventh one and suggested that this was due to mutations that arose from successive sub-culturing of bacteria. **In our study however a serially passaged culture, which was grown under static culture conditions, was more resistant to photocatalytic treatment and we have shown that one such change induced by these growth conditions is increased expression of type 1 fimbriae (as evidenced by results from the MSHA assay). However, other potentially influencing factors which**

could be induced by this culture technique should not be overlooked. These include the induction of other mutations or the presence of increased protein (fimbriae) in the culture media which could compete with bacteria for ROS.

3.3 Expression of curli fimbriae and biofilm formation

Results of growth on M17 agar showed that *E. coli* NCTC 12241 expressed curli fimbriae, as indicated by the presence of rough, dry, lacy colonies (figure 2) and a crystal violet binding assay for biofilm formation showed that this strain was a strong biofilm producer (data not shown). The non-biofilm producing strain (*E. coli* NCTC 8110) formed smooth, moist and regular shaped colonies, indicating no curli fimbrial expression (figure 2). The association of curli fimbrial expression with biofilm production has been shown by several authors [34,35,50,51]. White *et al* [35] demonstrated that their expression enhanced long term survival of an organism and increased organism resistance to desiccation and chlorination. These studies show that expression of curli fimbriae and biofilm formation could aid long term survival of the organism in the environment and promote passage to further hosts and thus highlight the need for an effective disinfection technique to control their survival.

3.4 *Effect of expression of curli fimbriae on susceptibility of E. coli biofilms to photocatalytic destruction*

Results from photocatalytic degradation studies showed that *E. coli* biofilms were destroyed after 30 minutes treatment time. This was assessed using the viability stain resazurin, in which microbial respiration is indicated by a colour change from blue to pink. No biofilm destruction was observed within either the dark control group or the UV-A only treatment group (figure 3a and 3b). Biofilms are complex surface associated communities of bacterial cells enclosed within a polymeric matrix and are the main mode of bacterial growth in water rich, nutrient limited environments [52]. They present a serious health hazard in many environments in particular in drinking water distribution systems where problems can arise

when biofilms attached to water pipes are sloughed off and cell clusters are released into drinking water [53]. Their complex structure means that they are also more resistant to environmental stresses such as dehydration and oxidative stress [54-56] and are hence more difficult to destroy by conventional water treatment methods. Photocatalysis represents an effective technology to destroy biofilms however to date there are a limited number of reported studies [36,37]. Our study demonstrates the successful destruction of *E. coli* biofilms using photocatalysis and suggests a possible role for this technology in water treatment systems.

4. Conclusion

Using defined culture conditions this study highlights the importance of bacterial factors in the photocatalytic disinfection process. Cultures grown under conditions to promote the expression of type 1 fimbriae (serial passaging in static culture) showed strong mannose sensitive haemagglutinating properties and took 75 minutes longer to be completely destroyed by photocatalysis than control stationary phase cultures, which were grown under conditions that do not promote type 1 fimbrial expression. **While the influence of other bacterial factors, on photocatalytic susceptibility, that may be expressed under these growth conditions cannot be ruled out, our observations support those of previous studies which have shown that serial passaging in static culture increases the numbers of type 1 fimbriae present in a culture [29,38]. Future studies to confirm the exact role of bacterial surface structures in the susceptibility of bacteria to photocatalytic attack, however, should involve the use of defined genetic mutants.**

The expression of curli fimbriae has been associated with an organism's ability to produce biofilms and with increased survival and persistence of these bacteria in the environment [34,35,50,51]. In this study the expression of curli fimbriae correlated well with the ability of an *E. coli* strain to produce biofilms, as assessed by the expression of a lacy colony type on M17 agar and a crystal violet binding assay respectively. Due to their complex structure, biofilms are extremely difficult to destroy and present a serious health hazard in

environmental, medical and industrial settings [52,57]. In this study, however, *E. coli* biofilms were readily destroyed by photocatalytic treatment. Using a cell viability assay, biofilm destruction was observed after 30 minutes treatment time. To date there are only a few published studies which examine the role microbial factors play in bacterial susceptibility to photocatalysis ; however their importance is becoming increasingly recognised [21,23,58]. Our findings highlight the important role that bacterial cell surface structures play in this process and add to the ever growing list of microbial variables that can impact on the successful outcome of photocatalytic disinfection studies.

References

[1] T. Matsunaga, R. Tomoda, T. Nakajima, H. Wake, Photoelectrochemical sterilization of microbial cells by semiconductor powders. FEMS Microbiol. Lett. 29 (1985) 211–214.

[2] T. Saito, T. Iwase, J. Horie, T. Morioka, Mode of photocatalytic bactericidal action of powdered semiconductor TiO_2 on mutans streptococci, J. Photochem. Photobiol. B, 14 (1992) 369-379.

[3] J.C. Ireland, P. Klostermann, E.W. Rice and R.M. Clarke, Inactivation of *Escherichia coli* by Titanium Dioxide Photocatalytic Oxidation, Appl. Environ. Microbiol., 59 (1993) 1668-1670.

[4] J.A. Ibáñez, M.I. Litter, R.A. Pizarro, Photocatalytic bactericidal effect of TiO_2 on Enterobacter cloacae: comparative study with other Gram (-) bacteria. J.Photochem. **Photobiol**. A 157 (2003) 81–85.

[5] J.M.C. Robertson, L.A. Lawton, P.K.J. Robertson, The production of small colony variants of pathogenic bacteria following treatment of contaminated potable water with UV light, J. Photochem. Photobiol. A: Chemistry, 175 (2005) 51-56

[6] R. van Grieken, J. Marugán, C. Pablos, L. Furones, Ainhoa López, Comparison between the photocatalytic inactivation of Gram-positive *E. faecalis* and Gram-negative *E. coli* faecal contamination indicator microorganisms, Appl. Catal. B, Environ. 100 (2010) 212-220.

[7] N. Huang, Z. Xiao, D. Huang, C. Yuan, Photochemical disinfection of *Escherichia coli* with a TiO_2 colloid solution and a self-assembled TiO_2 thin film, **Supramol. Sci.,** 5 (1998) 559-564.

[8] D. Gumy, A.G. Rincon, R. Hajdu, C. Pulgarin, Solar photocatalysis for detoxification and disinfection of water: Different types of suspended and fixed TiO_2 catalysts study. Solar Energy, 80 (2006) 1376-1381.

[9] K. Sunada, T. Watanabe, K. Hashimoto, Studies on photokilling of bacteria on TiO_2 thin film. Journal of **Photochem. and Photobiol**. A: Chemistry, 156 (2003) 227-233.

[10] Y. Kikuchi, K. Sunada, T. Iyoda, K. Hashimoto, A. Fujishima, Photocatalytic bactericidal effect of TiO_2 thin films: dynamic view of the active oxygen species responsible for the effect. Journal of Photochemistry and Photobiology A: Chemistry, 106 (1997) 51-56.

[11] P. Evans, D.W. Sheel, Photoactive and antibacterial TiO_2 thin films on stainless steel. Surf. Coat. Tech. 201 (2007) 9319-9324.

[12] K.P. Kühn, I.F. Chaberny, K. Massholder, M. Stickler, V.W Benz, H-G. Sonntag, L. Erdinger, Disinfection of surfaces by photocatalytic oxidation with titanium dioxide and UVA light, Chemosphere 53 (2003) 71-77.

[13] I.M.Gould, Costs of hospital-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) and its control. Int. J. Antimicrob. Ag. 28 (2006) 379-384.

[14] N.Ben Saida, M. Marzouk, A. Ferjeni, J. Boukadida, A three-year surveillance of nosocomial infections by methicillin-resistant *Staphylococcus haemolyticus* in newborns reveals the disinfectant as a possible reservoir. Pathol. Biol. 57 (2009) 29-35.

[15] E.O. Morente, M.A. Fernández-Fuentes, M.J. Grande Burgos, H. Abriouel, R.P. Pulido, A. Gálvez, Biocide tolerance in bacteria, Int J Food Microbiol. 162 (2013) 13-25.

[16] B. González-Zorn, J.A. Escudero, Ecology of antimicrobial resistance: humans, animals, food and environment, Int Microbiol. 15 (2012) 101-9.

[17] S.Malato, P. Fernández-Ibáñez, M.I. Maldonado, J. Blanco, W. Gernjak, Decontamination and disinfection of water by solar photocatalysis: Recent overview and trends, Catal. Today 147 (2009) 1-59.

[18] A.G. Rincon, C. Pulgarin, Use of coaxial photocatalytic reactor (CAPHORE) in the TiO_2 photo-assisted treatment of mixed *E. coli* and *Bacillus* sp. and bacterial community present in wastewater, **Catal. Today**, 101 (2005) 331–344.

[19] A. Paleologou, H. Marakas, N. P. Xekoukoulotakis, A. Moya, Y. Vergara, N. Kalogerakis, P. Gikas, D. Mantzavinos, Disinfection of water and wastewater by TiO_2 photocatalysis, sonolysis and UV-C irradiation, Catal. Today 129 (2007) 136-142.

336 [20] C. Sichel, J. Tello, M. de Cara, P. Fernández-Ibáñez, Effect of UV solar intensity and dose
337 on the photocatalytic disinfection of bacteria and fungi, Catal. Today 129 (2007) 152-160.
338 [21] T.P. Cushnie, P. K.J.Robertson, S. Officer, P. M. Pollard, C. McCullagh, J. M.C. Robertson,
339 Variables to be considered when assessing the photocatalytic destruction of bacterial pathogens,
340 Chemosphere 74 (2009) 1374-1378.
341 [22] A.K. Benabbou, Z. Derriche, C. Felix, P. Lejeune, C. Guillard, Photocatalytic inactivation
342 of *Escherischia coli*: Effect of concentration of TiO_2 and microorganism, nature, and intensity
343 of UV irradiation, Appl. Catal. B, Environ. 76 (2007) 257-263.
344 [23] A.G. Rincon, C. Pulgarin, Bactericidal action of illuminated TiO_2 on pure
345 *Escherichia coli* and natural bacterial consortia:post-irradiation events in the dark and
346 assessment of the effective disinfection time, Applied Catalysis B: Environmental 49
347 (2004) 99-112.
348 [24] N. Lydakis-Simantiris, D. Riga, E. Katsivela, D. Mantzavinos, N. P. Xekoukoulotakis,
349 Disinfection of spring water and secondary treated municipal wastewater by TiO_2
350 photocatalysis, Desalination 250 (2010) 351-355.
351 [25] A.G. Rincón, C. Pulgarín, Solar Photolytic and Photocatalytic Disinfection of Water at
352 Laboratory and Field Scale. Effect of the Chemical Composition of Water and Study of the
353 Postirradiation Events, J. Sol. Energy Eng. 129 (2007) 100–110.
354 [26] A.G. Rincón, C. Pulgarin, Effect of pH, inorganic ions, organic matter and H_2O_2 on *E.*
355 *coli* K12 photocatalytic inactivation by TiO_2: Implications in solar water disinfection, Appl.
356 Catal. B. - Environ. 51 (2004) 283-302.
357 [27] D. M.A. Alrousan, P.S.M. Dunlop , T.A. McMurray, J.A. Byrne, Photocatalytic
358 inactivation of *E. coli* in surface water using immobilised nanoparticle TiO_2 films, Wat. Res.
359 43 (2009) 47-54.
360 [28] J. Marugan, R. van Grieken, C. Pablos, C. Sordo, Analogies and differences between
361 photocatalytic oxidation of chemicals and photocatalytic inactivation of microorganisms, Wat.
362 Res. 44 (2010) 789-796.
363 [29] D.C. Old, J.P. Duguid, Selective outgrowth of fimbriated bacteria in static liquid medium,

364 J. Bacteriol. 103 (1970) 447-456.
365 [30] C.J. Thorns, Salmonella fimbriae: novel antigens in the detection and control of
366 ***Salmonella*** infections, Brit. Vet. J. 151 (1995) 643-658.
367 [31] C. Le. Bouguenec, Adhesins and invasins of pathogenic *Escherichia coli*, Int. J.
368 Med.Microbiol. 295 (2005) 471-478.
369 [32]Y. Tanaka, Y. Katsube, Infectivity of *Salmonella typhimurium* for mice in relation to
370 fimbriae, Japn. J. Vet. Sci. 40 (1978) 671-681.
371 [33] M.R. Darekar, J.P. Duguid, The influence of fimbriation on the infectivity of *Salmonella*
372 *typhimurium*, Proc. Ind. Acad. Sci. 35 (1972) 283-293.
373 [34] A.L. Cookson, W.A. Cooley, M.J. Woodward, The role of type 1 and curli fimbriae of shiga
374 toxin-producing *Escherichia coli* in adherence to abiotic surfaces, Int. J. Med. Microbiol. 292
375 (2002) 195-205.
376 [35] A.P. White, D.L. Gibson, W. Kim, W.W. Kay, M.G Surette, Thin aggregrative fimbriae
377 and cellulose enhance long-term survival and persistance of ***Salmonella***, J. Bacteriol. 188(9)
378 (2006) 3219 - 3227 doi:10.1128/JB. 188.9.3219-3227.2006
379 [36] P.S.M Dunlop, C.P. Sheeran, J.A. Byrne, M.A.S. McMahon, M.A. Boyle, K.C. McGuigan,
380 Inactivation of clinically relevant pathogens by photocatalytic coatings, J. Photochem.
381 Photobiol. A: Chem. 261 (2010) 303-310.
382 [37] Y.Ohko, Y.Nagao, K. Okano, N. Sugiura, A. Fukuda, Y. Yang, N. Negishi, M. Takeuchi,
383 S. Hanada, Prevention of *Phormidium tenue* biofilm formation by TiO_2 photocatalysis,
384 Microbes Environ. 24 (2009) 241-5.
385 [38] A.D. Humphries, M. Raffatellu, S. Winter, E.H. Weeing, R.A. Kingsley, R. Droleskey, S.
386 Zhang, J. Figueiredo, S. Khare, J. Nunes, L.G. Adams, R.M. Tsolis, A.J. Baumler, The use of
387 flow cytometry to detect expression of subunits encoded by 11 *Salmonella enterica* serotype
388 Typhimurium fimbrial operons, Molecular Microbiology 48 (2003) 1357-1376.
389 [39] M.G. Sojka, M. Dibb-Fuller, C.J. Thorns, Characterisation of monoclonal antibodies
390 specific to SEF21 of *Salmonella enteritidis* and their reactivity with other *Salmonellae* and
391 Enterobacteria, Vet. Microbiol. 48 (1996) 207-221.

392 [40] E.B. Solomon, B.A. Niemira, G.M. Sapers, B.A. Annous, Biofilm formation, cellulose
393 production, and curli biosynthesis by *Salmonella* originating from produce, animal and
394 clinical sources, J. Food Protection 68 (2005) 906-912.
395 [41] S.L. Walker, M. Sojka, M.P. Dibb-Fuller, M.J. Woodward, The effect of pH, temperature
396 and surface contact on the elaboration of fimbriae and flagella by *Salmonella entertidis*, J.
397 Med. Microbiol. 48 (1999) 253-261.
398 [42] B.I. Eisenstein, Fimbriae. In: ***Escherichia coli*** and ***Salmonella typhimurium***:Cellular
399 and Molecular Biology, eds. F.C. Neidhardt et al. 1 (1987) 84-90 Washington D.C. :
400 American Society for Microbiology.
401 [43] P-C. Maness, S. Smolinski, D. M. Blake, Z. Huang, E. J. Wolfrum, W. A. Jacoby,
402 Bactericidal Activity of Photocatalytic TiO_2 Reaction: toward an Understanding of Its Killing
403 Mechanism, Appl. Environ. Microbiol., 65 (1999) 4094-4098.
404 [44] Z. Huang, P-C. Maness, D.M. Blake, E.J. Wolfrum, S.L. Smolinski, W.A. Jacoby,
405 Bactericidal mode of titanium dioxide photocatalysis, J. Photochem. Photobiol A, 130 (2000)
406 163-170.
407 [45] K. Sunada, T. Watanabe, K. Hashimoto, Studies on photokilling of bacteria on TiO_2 thin
408 film, J. Photochem. Photobiol. A, 156 (2003) 227-233.
409 [46] Y. Kikuchi, K. Sunada, T. Iyoda, K. Hashimoto, A. Fujishima, Photocatalytic
410 bactericidal effect of TiO_2 thin films: dynamic view of the active oxygen species responsible
411 for the effect, J. Photochem. Photobiol. A, 106 (1997) 51-56.
412 [47] M. Suwalsky, C. Schneider, H.D. Mansilla J. Kiwi, Evidence for the hydration effect at
413 the semiconductor phospholipid-bilayer interface by TiO_2 photocatalysis, J. Photochem.
414 Photobiol. B, 78 (2005) 253-258
415
416 [48] P. Amézaga-Madrid, R. Silveyra-Morales, L. Córdoba-Fierro, G. V. Nevárez-Moorillón,
417 M. Miki-Yoshida, E. Orrantia-Borunda, F. J. Solís, TEM evidence of ultrastructural alteration
418 on *Pseudomonas aeruginosa* by photocatalytic TiO_2 thin films, J. Photochem. Photobiol. B,
419 70 (2003) 45-50.

420 [49] V. Krishna, S. Pumprueg, S.-H. Lee, J. Zhao, W. Sigmund, B. Koopman, B.M. Moudgil,
421 Photocatalytic disinfection with titanium dioxide coated multi-well carbon nanotubules,
422 Process Safety and Environmental Protection 83 (2005) 393-397.
423 [50] M. Dibb-Fuller, E. Allen-Vercoe, M.J. Woodward, C.J. Thorns, Expression of SEF17
424 fimbriae by *Salmonella enteritidis*, Letters in Applied Microbiol. 25 (1997) 447-452.
425 [51]U. Römling, W.D. Sierralta, K. Eriksson, S. Normark, Multicellular and aggregrative
426 behaviour of *Salmonella typhimurium* strains is controlled by mutations in the agfD promoter,
427 Mol. Microbiol. 28 (1998) 249-264.
428 [52] J.W. Costertson, P.S. Stewart, Bacterial biofilms: a common cause of persistent infections,
429 Science 284 (1999) 1318-1322.
430 [53] M-H. ne Castonguay, S. van der Schaaf, W. Koester, J. Krooneman, W. van der Meer, H.
431 Harmsen, P. Landini, Biofilm formation by ***Escherichia coli*** is stimulated by synergistic
432 interactions and co-adhesion mechanisms with adherence-proficient bacteria, **Res. Microbiol.**
433 157 (2006) 471-478.
434 [54] I. Albesa, M.C. Becerra, P.C. Battan, P.L. Paez, Oxidative stress involved in the
435 antibacterial action of different antibiotics, Biochem. Biophys. Res. Commun. 317 (2004) 605-
436 609.
437 [55] W.S Chang, L.J. Halverson, Reduced water availability influences the dynamics,
438 development and ultrastructural properties of *Pseudomonas putida* biofilms, J. Bacteriol. 185
439 (2003) 2847-2857.
440 [56] W.M. Dunne Jr, Bacterial adhesion:Seen any good biofilms lately?, Clin. Microbiol. Rev.
441 15 (2002) 1550166.
442
443 [57] U. Römling, C. Balsalobre, Biofilm infections, their resilience to therapy and innovative
444 treatment strategies, J. Intern. Med. 272 (2012) 541-61.
445 [58] T.Y. Leung, C.Y. Chan, C. Hu, J.C. Yu, P.K. Wong, Photocatalytic disinfection of marine
446 bacteria using fluorescent light, Water Research 42 (2008) 4827-4837.
447

List of captions for figures

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

Figure 1 Effect of fimbrial expression on photocatalytic destruction of *E. coli* **NCTC 12241** (a) control (stationary phase) culture and (b) serially passaged culture ◇: TiO_2 and UV; □: UV only; Δ: dark control (TiO_2 only).

Figure 2 *E. coli* cultures showing (a) normal colony phenotype (non-biofilm producing *E. coli* NCTC 8110) and (b) lacy colony phenotype indicating expression of curli fimbriae (biofilm producing *E. coli* NCTC 12241).

Figure 3 Viability assessment of *E. coli* biofilms following photocatalysis using E. coli NCTC 12241 (a) section a: *E. coli* with media control, section b: UV only control, section c: TiO_2 and UV; (b) section a: *E. coli* with media control, section b: dark control with TiO_2 only, section c:TiO_2 and UV; column labels 1 and 4, 2 and 5, 3 and 6 correspond to 30, 60 and 90 minutes treatment time.

Table 1

Dilution factor	**1:2**	**1:4**	**1:6**	**1:8**	**1:10**	**1:12**	**Mannose sensitivity**
Control culture*	/	/	/	/	/	/	/
Non-passaged culture§	x	x	x	/	/	/	√
x1 passaged culture§	x	x	x	/	/	/	√
x2 passaged culture§	xxx	xxx	xx	x	x	/	√

*Control culture grown in an orbital incubator at 37^0C with shaking at 100 rpm

§ Non-passaged and passaged cultures grown statically at 37^0C

/ = no haemagglutination

x = weak haemagglutination

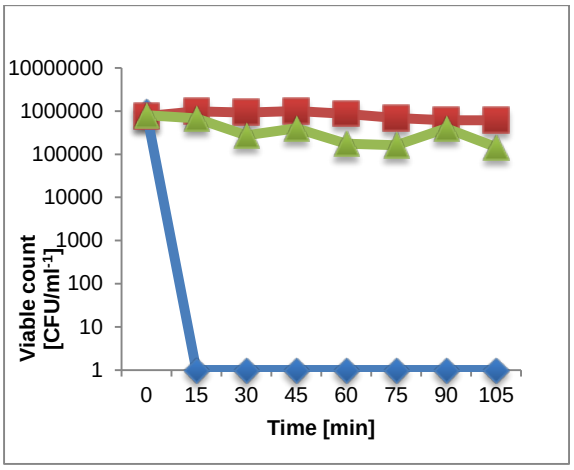
xx = medium haemagglutination

xxx = strong agglutination

√ = haemagglutination is mannose sensitive

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

(a)



(b)

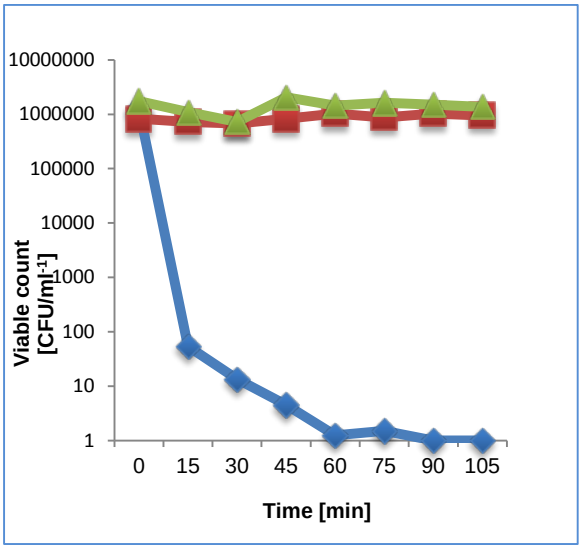
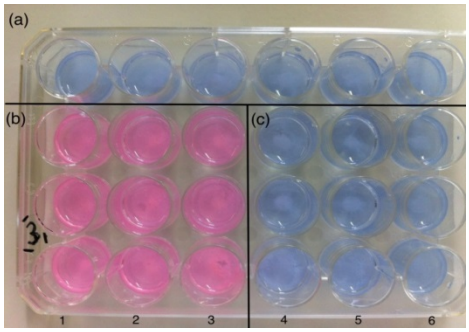


Figure 1 Effect of fimbrial expression on photocatalytic destruction of *E. coli* NCTC 12241 (a) control (stationary phase) culture and (b) serially passaged culture ◇: TiO_2 and UV; □: UV only; Δ: dark control (TiO_2 only).

(a) (b)

Figure 2 *E. coli* cultures showing (a) normal colony phenotype (non-biofilm producing *E. coli* NCTC 8110) and (b) lacy colony phenotype indicating expression of curli fimbriae (biofilm producing *E. coli* NCTC 12241).

(a)



(b)

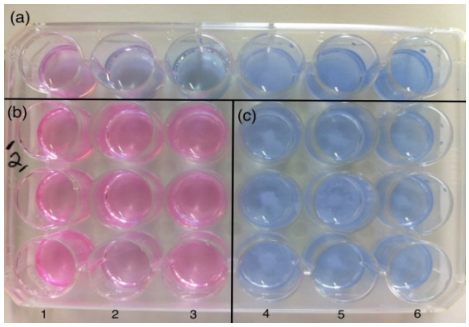


Figure 3 Viability assessment of *E. coli* biofilms following photocatalysis using E. coli NCTC 12241 (a) section a: *E. coli* with media control, section b: UV only control, section c: TiO_2 and UV; (b) section a: *E. coli* with media control, section b: dark control with TiO_2 only, section c:TiO_2 and UV; column labels 1 and 4, 2 and 5, 3 and 6 correspond to 30, 60 and 90 minutes treatment time.