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# DISTINCT BEHAVIOR BETWEEN MULTI-ANTIBIOTIC RESISTANT *ESCHERICHIA COLI* STRAINS TOWARDS REACTIVE OXYGEN SPECIES

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Antibiotic resistant bacteria have been implicated in a large number of nosocomial infections. This worldwide problem drew the attention to the development of new disinfection techniques, such as photoinactivation. During photocatalysis, the generation of reactive oxygen species (ROS) such as hydrogen peroxide ($H_2O_2$), hydroxyl radicals (•OH) and superoxide radical ($O_2^{\bullet-}$) are supposed to occur. Hence, photocatalysis has been used to inactivate organisms, through ROS attack. However, several studies reported that in *Escherichia coli* an increased tolerance to oxidative stress induced by ROS may occur, and that it may be related to antibiotic resistance. In this study, the susceptibility to photoinactivation of two antibiotic resistant *E. coli* environmental strains (A5EL5 and E5EL20) belonging to the same phylogenetic group and with similar antibiotic resistance phenotype was assessed.

After 40 min of UV/$TiO_2$ exposure, viability losses of 44.2 % and above 99.0 % were recorded for strains A5EL5 and E5EL20, respectively. Based on the hypothesis that these two strains have distinct tolerance to different ROS generators, the ROS formed after contact with $H_2O_2$, $KO_2$ or $H_2O_2$ + $Fe^{2+}$ (Fenton reaction) were quantified using a fluorescence method. The probe 2', 7'–dichlorohydrofluorescein diacetate (DCFH-DA) is widely used to measure the ROS content in cells. After hydrolysis by cellular esterases, DCFH is subsequently oxidized by ROS to highly fluorescent DCF. After 60 min of contact with DCFH-DA, cells were incubated in the pesence of $H_2O_2$ or $KO_2$ for $O_2^{\bullet-}$ and $H_2O_2$ + $Fe^{2+}$ (Fenton reaction) for •OH. Fluorescence units were measured in the crude cell extracts and converted into ROS concentration and the data was normalized by total protein content. Significant differences ($p<0.05$) between ROS content in A5EL5 and E5EL20 crude cell extracts were found for $H_2O_2$ ($1.4x10^{-5}$ ± $2.9x10^{-6}$ and $1.1x10^{-4}$ ± $1.1x10^{-6}$ µmol ROS.µg$^{-1}$ total protein, respectively) and Fenton reaction ($9.6x10^{-5}$ ± $5.6x10^{-6}$ and $1.5x10^{-4}$ ± $5.2x10^{-6}$ µmol ROS.µg$^{-1}$ total protein, respectively). No significant difference was found for $KO_2$. These preliminary tests, suggest that, in fact, the different tolerance to photocatalysis of the environmental *E. coli* may be related to oxidative stress response, but not to antibiotic resistance.

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