Excimer laser radiation to mutate industrial microorganisms

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Bacteria are excellent producers of several potent bioactive compounds. Many of the products currently used for human or animal therapy, in pharmaceutical and food industry and in agriculture are produced by microbial secondary metabolism or by chemical modification of a microbial products. However, it is rare for these microorganisms to produce biological molecules at concentration so high as to initiate the production on an industrial scale. Therefore, an important challenge in industrial microbiology is to improve the secondary metabolites production by microorganisms.

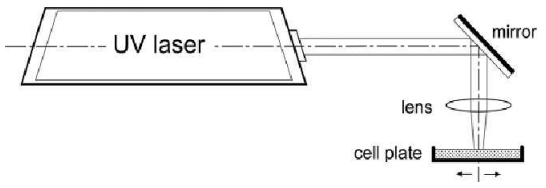

Figure 1. Experimental apparatus.

Mutation-selection procedures are widely used in biotechnology to improve the performance of producer microorganisms. The mutagenic effects of ultraviolet (UV) light of microorganisms are fairly well known from studies with UV lamps. The interaction of UV light with cellular systems is responsible for photochemical, photothermal, photodecomposition and photodinamic processes. When short-wavelength radiation strikes biological material, DNA is damaged. It can be repaired or can cause cell killing, mutagenesis or, in mammalian cells, carcinogenesis [1,2].

Mutagenic and lethal mechanisms of $UV_{254}nm$ radiation have been elucidated in several microorganisms. RecA, a multi-functional protein, plays a fundamental role in bacterial response to $UV_{254}nm$ nm radiation [3,4]. Escherichia coli strains harboring loss-of-function mutations in recA gene are extremely sensitive to far-UV by germicidal lamps [5]. DNA photoproducts are maximally represented by pyrimidine dimers, and it is believed they are responsible of the far-UV lethal effects [1]. The extreme sensitivity of recA-deficient strains to far-UV radiation is dependent on the inability to both repair DNA by means of the post-replicative recombination pathway, and to activate additional error-free and error-prone (mutagenic) DNA repair pathways (the so-called SOS response) [6,7]. Owing to the failure to activate the SOS response, recA-deficient strains are also inefficiency to UV254 nm-induced mutagenesis. This is a frequent condition that limits diffusion of the $UV_{254}nm$ nm mutagenesis for research or industrial purposes because many strains are naturally SOS response-defective [8].

Since the eighties, due to the development of excimer lasers, new frontiers in the study of UV applications have been opened. In this context we, analyze the effects of the $UV_{308}nm$ nm laser radiation generated by an homemade XeCl 308 nm excimer laser [9], using E. coli, as a model organism, and Nonomuraea ATCC 39727, as a microorganism of industrial interest.

The E. coli strains were grown to late logarithmic phase (optical density = 1.0 at a wavelength of 550 nm) in LB broth at $37°C$ with vigorous shaking. We used two strains: the strain JC1553 harbours the recA mutation and it is congenic to the the recA-proficient strain JC411.

The results confrmed previous data on the extreme far-UV-radiation sensitivity of strains carrying the recA mutation as a result of the inability to repair UV-radiation-induced damage to DNA [10,11].

The Nonomuraea sp.ATCC 39727 spores (about $510^8 ml^{-1}$) were collected by centrifugation and re-suspended in medium 707 (for rehydration).

The UV sources were a $UV_{308}nm$nm XeCl excimer laser and a $UV_{254}nm$ germicidal lamp. The laser fluences were fixed at 25 or 50 mJ/cm^2 per laser shot, while the $UV_{254}nm$ nm germicidal lamp intensity was $0.3mJcm^{-2}s^{-1}$. Irradiation was performed by opening the LB agar miniplates. Figure 1 shows the experimental apparatus.

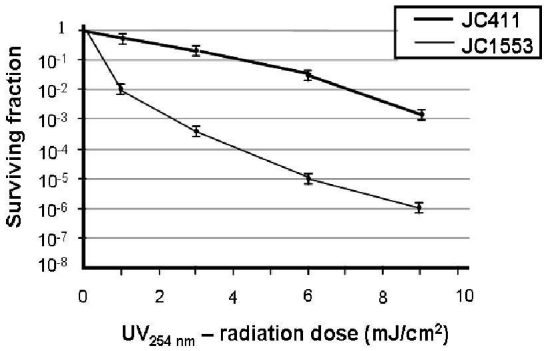

Figure 2. Survival of recA-proficient and recA-defective E. coli strains exposed to lamp-generated $UV_{254}nm$

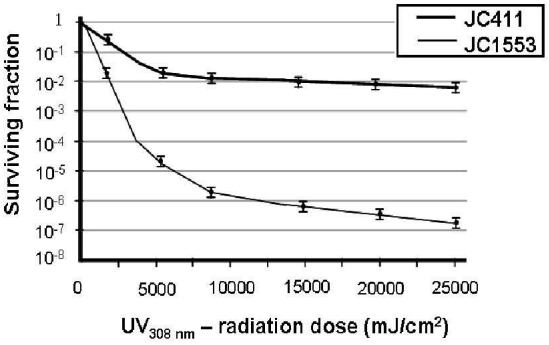

Figure 3. Survival of recA-proficient and recA-defective E. coli strains exposed to excimer laser-generated $UV_{308}nm$ radiation

We started our study by determining the effect of $UV_{254}nm$ radiation of E. coli strains. The results are represented in Fig. 2 and Fig. 3.

In the recA-proficient strain, the decrease in survival as a function of UV254 nm-radiation dose was smaller at lower doses ($< 3mJcm^{-2}$) than at higher doses ($> 3mJcm^{-2}$). An opposite behavior was observed in the recA-defective strains, which showed less effect at higher doses ($> 1mJcm^{-2}$) than at lower doses ($< 1mJcm^{-2}$) (Fig.2). In parallel experiments E. coli strains were subjected to $UV_{308}nm$ coherent radiation. In the survival plot, a net change in the slope was apparent at doses higher than $500mJcm^{-2}$; cell killing increased only slightly with further increasing in $UV_{308}nm$-radiation dose. The same effect was observed with the recA-defective strain at about the same doses (Fig.3).

Next mutation frequencies to rifampicin resistance were determined. Unexpectedly, the $UV_{308}nm$ laser treatment was much more mutagenic for JC1553 (recA) than for JC411, resulting in an impressive increase in mutation frequencies as a function of dose. At 2400, 3600 and $6000mJcm^{-2}$ the increment was 720-, 1200- and 1900-fold, respectively.

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