TITLE: FUNCTIONAL CHARACTERIZATION OF CC_2332 AND CC_2333 GENES IN THE SOS RESPONSE IN *CAULOBACTER CRESCENTUS*

AUTHORS: FERNÁNDEZ-SILVA, F.; MERINO, R.; RODRIGUEZ, T.R.; MENCK, C.F.; GALHARDO, R. S.

INSTITUTION: DEPARTAMENTO DE MICROBIOLOGIA, INSTITUTO DE CIÊNCIAS BIOMÉDICAS, UNIVERSIDADE DE SÃO PAULO (AV. PROF. LINEU PRESTES, 1374 – SALA 246-A CEP: 05508-000 CIDADE UNIVERSITÁRIA - SÃO PAULO, BRAZIL)

ABSTRACT:
DNA repair is crucial to the survival of all organisms. UV radiations induce harmful lesions in DNA impairing the vital process of replication and transcription. The predominant UV-induced lesions are the thymine dimers which include the cis-syn cyclobutane pyrimidine dimers (CPD) and the pyrimidine–pyrimidone (6-4) photoproducts. To maintain genome integrity, living organisms have evolved mainly two DNA repair pathways to repair these damages, the nucleotide excision repair (NER) mediated by *uvrABC* genes, which removes and replaces the damaged nucleotides and the direct reversal repair mechanism by the well-documented DNA photolyases that use blue light energy to trigger the direct reversal repair of the CPD and the (6-4) photoproducts. Interestingly, in bacterial spores, an enzyme unrelated to DNA photolyases, spore photoproduct (SP) lyase is found which carries out the direct reversion of a specific and distinct photoproduct, the so-called SP into two thymine bases, in a light-independent manner. SP lyase belongs to the radical S- adenosyl-L-methionine (SAM) superfamily of enzymes and therefore contains a [4Fe–4S] cluster and a SAM cofactor directly involved in catalysis. In *Caulobacter crescentus,* previous work has identified two genes (CC_2332 and CC_2333) that are part of the SOS regulon. The SOS response is a universal bacterial regulon involved in the cellular response to DNA damage and other forms of stress. The CC_2332 gene encodes a protein of the "Radical SAM" superfamily, and is part of an operon with CC_2333 gene that encodes a protein with two distinct domains. A domain of unknown function (DUF4130) and another domain with similarity to Uracil-DNA glycosylases. In this way, we believe that CC_2333 / CC_2332 compose a photoproduct repair system associated with a mechanism of prevention of mutagenesis induced by cytosine deamination. First, we constructed strains with knockouts for these genes, and analysed the survival and mutagenesis after exposure to UV radiation. The results showed that the mutant CC_2332 presents an 8-fold higher UV sensitivity than the wild-type strain, and the double mutant *uvrA* / CC_2332 had a 130-fold greater sensitivity than the *uvrA* mutant. UV-induced mutagenesis is decreased in the absence of CC_2332. Overall, these results show for the first time that the product of the widely conserved bacterial gene CC_2332 acts in the repair of UV damage.

keywords: SOS response; UV damage ;DNA repair; SP lyase-like; radical SAM enzymes; Uracil-DNA glycosylases; *Caulobacter crescentus.*

Development Agency: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP)

E-mail: frank_sydney@usp.br