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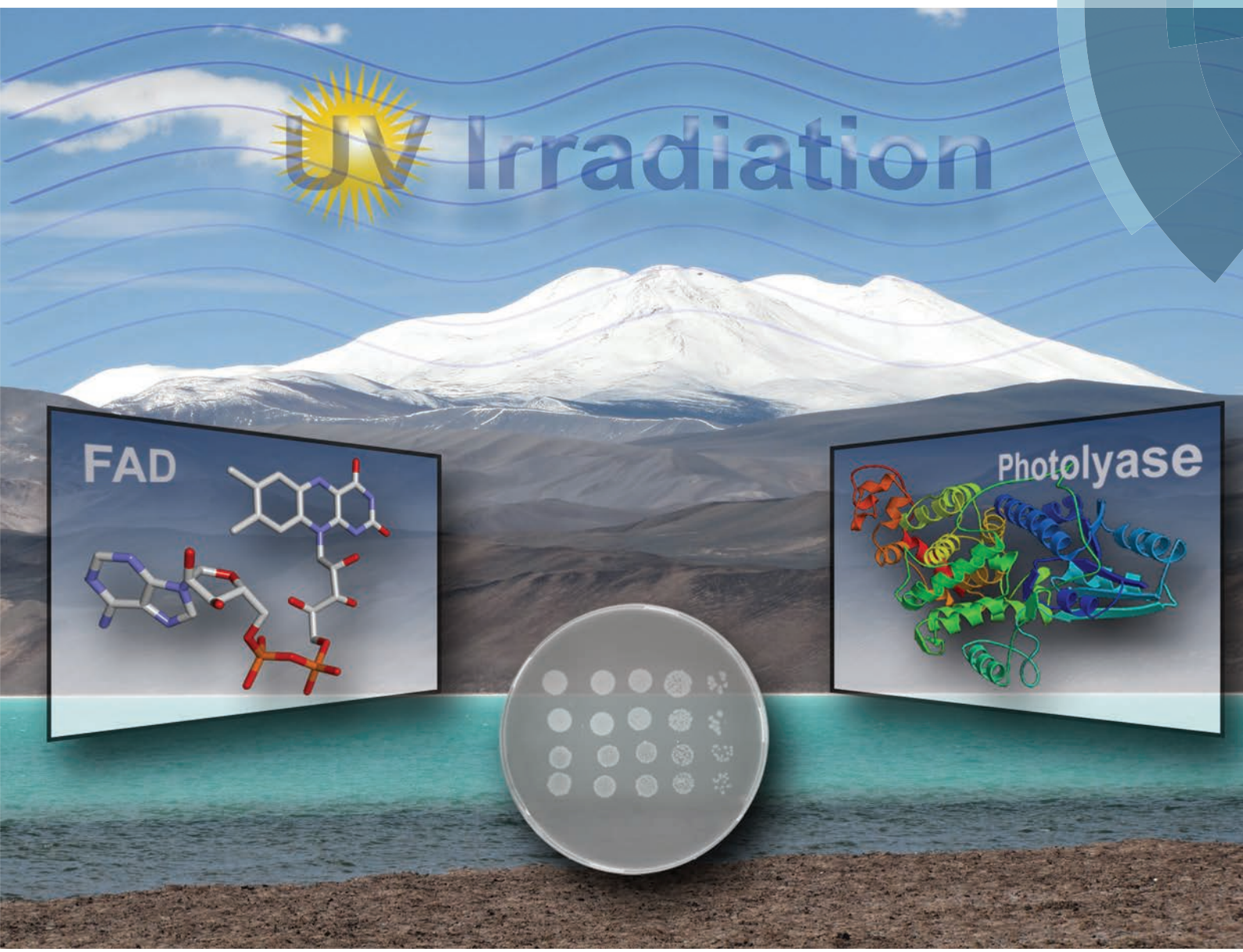


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PAPER

# First characterisation of a CPD-class I photolyase from a UV-resistant extremophile isolated from High-Altitude Andean Lakes†

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UV-resistant *Acinetobacter* sp. Ver3 isolated from High-Altitude Andean Lakes (HAAL) in Argentinean Puna, one of the highest UV exposed ecosystems on Earth, showed efficient DNA photorepairing ability, coupled to highly efficient antioxidant enzyme activities in response to UV-B stress. We herein present the cloning, expression, and functional characterization of a cyclobutane pyrimidine dimer (CPD)-class I photolyase (Ver3Phr) from this extremophile to prove its involvement in the previously noted survival capability. Spectroscopy of the overexpressed and purified protein identified flavin adenine dinucleotide (FAD) and 5,10-methenyltetrahydrofolate (MTHF) as chromophore and antenna molecules, respectively. All functional analyses were performed in parallel with the ortholog *E. coli* photolyase. Whereas the *E. coli* enzyme showed the FAD chromophore as a mixture of oxidised and reduced states, the Ver3 chromophore always remained partly (including the semiquinone state) or fully reduced under all experimental conditions tested. Functional complementation of Ver3Phr in Phr$^-$-RecA *E. coli* strains was assessed by traditional UFC counting and measurement of DNA bipyrimidine photoproducts by HPLC coupled with electrospray ionisation-tandem mass spectrometry (ESI-MS/MS) detection. The results identified strong photoreactivation ability *in vivo* of Ver3Phr while its nonphotoreactivation function, probably related with the stimulation of nucleotide excision repair (NER), was not as manifest as for EcPhr. Whether this is a question of the approach using an exogenous photolyase incorporated in a non-genuine host or a fundamental different behaviour of a novel enzyme from an exotic environment will need further studies.

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## 1. Introduction

Extreme environments are considered to enclose a large variety of exceptional and yet unexplored microbial life.[1] Such environments are represented by the High-Altitude Andean Lakes (HAAL), pristine ecosystems located at the Dry Central Andes region between 2000 and 6000 m above sea level, asl.[2,3] Predominant harsh conditions at the HAAL are strong UV irradiation, desertic weather and large temperature fluctuations during the day, alkalinity, hypersalinity (up to 30%), and volcanic settings together with a high concentration of arsenic on soils and in water (up to 200 ppm) as a consequence of the geological foundation.[2,3] Yet, an outstanding microbial diversity has developed there, and almost 500 strains of prokaryotes (archaea, cyanobacteria and eubacteria) and lower eukaryotes (fungi and yeast) were isolated from bacterioplankton, benthos, microbial mats and soils surrounding the lakes.[4–7] Even microbial mats ordered in multi-layered flat mats and stubby pillars called stromatolites were found to be widespread at the HAAL; these stromatolites are the first ones described at such extreme environments and high altitudes.[8]

The extremophilic *Acinetobacter* sp. Ver3 from HAAL has been previously described as an exceptionally UV-resistant strain.[4,7] This strain was isolated from Lake Verde (4400 m asl), a hypersaline lake in the HAAL. Recently, we could provide evidence that this interesting UV-resistance phenotype is due in part to efficient photorepair capability,[9] coupled to highly efficient antioxidant enzyme activities in response to UV-B stress.[6]

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