Testing directed evolution strategies for space exploration: genetic modification of photosystem II to increase stress tolerance under space conditions

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Plants and many microorganisms are able to convert and store solar energy in chemical bonds by a process called photosynthesis. They remove CO_2 from the atmosphere, fix it as carbohydrate and simultaneously evolve oxygen. Oxygen evolution is of supreme relevance for all higher life forms and results from the splitting of water molecules. This process is catalyzed by the so called photosystem II (PSII) complex and represents the very beginning of biomass production. PS II is also a central point of regulation, being responsive to various physical and physiological parameters. Complex space radiation is damaging PS II and reduces photosynthetic efficiency. Thus, bioregenerative life-support systems are severely disturbed at this point.

Genetic manipulation of photosynthesis checkpoints offer the possibility to adjust biomass and oxygen production to changing environmental conditions. As the photosynthetic apparatus has adapted to terrestrial and not to space conditions, we are trying to adapt a central and particularly stress-susceptible element of the photosynthesis apparatus - the D1 subunit of PS II - to space radiation by a strategy of directed evolution. The D1 subunit together with its sister subunit D2 form the reaction centre of PS II. D1 presents a central weak point for radiation energy that hits the chloroplast.

We have constructed a mutant of the green alga *Chlamydomonas reinhardtii* with a defect D1 protein. This mutant is easily transformable with D1-encoding PCR fragments without purification and cloning steps (1). When generating a pool of randomly mutated DNA fragments by error prone PCR and transforming the mutant strain with this pool (2), it is possible to expose the resulting transformants to selective conditions like space radiation. Mutants adapted best to such conditions can then be identified and analyzed without further knowledge of protein structure and function.

(1) Dauvillee, D., Hilbig, L., Preiss, S., and Johanningmeier, U. (2004) Photosynth. Res. 79, 219-224.

(2) Johanningmeier, U., Bertalan, I., Hilbig, L., Schulze, J., Wilski, S., Zeidler, E. and Oettmeier,W., http://eurekah.com/abstract.php?chapid=2851&bookid=199&catid=2