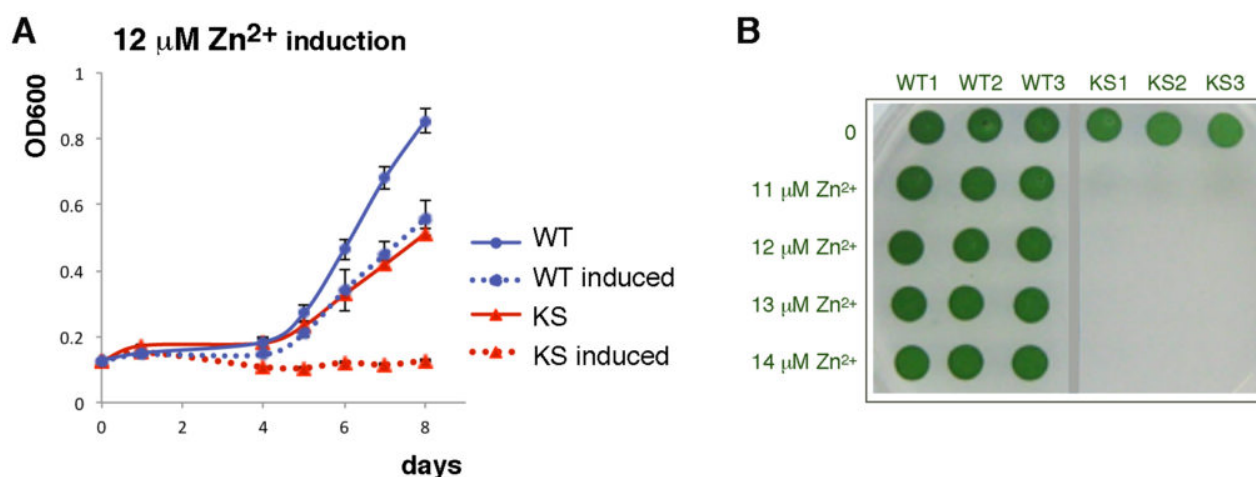


Improved biosafety of genetically modified cyanobacteria

Biosafety is a major concern in biotechnology when genetically modified (GM) organisms are used in a process. With increasing interest for cyanobacterial biotechnology and synthetic biology, it is astonishing that there has been, up to now, no published research on biosafety of GM cyanobacteria (also called blue-green algae).

A research group from University of Ljubljana, Slovenia, has addressed this important topic through development and incorporation of a biosafety mechanism in the unicellular cyanobacterium *Synechocystis* sp. PCC 6803, widely used as a model organism and production strain. Results were published in the April issue of Biology Open.



Induced autokilling of *Synechocystis* sp. PCC6803 carrying nuclease-based suicide switch. (A) Growth curves of cyanobacterial cells harboring plasmid-encoded kill switch (KS), in which the nuclease gene is driven by a metal-inducible promoter and the nuclease inhibitor gene is driven by a constitutive promoter. After induction of cyanobacteria with Zn^{2+} ions no growth is observed for cells harboring the suicide switch, while cells lacking the suicide switch WT grow normally. (B) Cultures from (A) were plated on solid media at day 13. Complete killing was observed for suicide-switch cells (KS triplicates 1-3) after zinc induction, while wild type (WT triplicates 1-3) remained viable.

Two biocontainment strategies were employed. In the first, GM cells could be triggered to start production of a nuclease that degrades the cell's own nucleic acids. The genes for a non-specific DNA/RNA nuclease and its inhibitor from a related cyanobacterium were incorporated into PCC 6803 in such a way that addition of subtoxic concentrations of metal ions triggers production of the nuclease, while nuclease inhibitor production remains constant regardless of the inducer. In absence of metal-ion trigger, the presence of the inhibitor helps neutralize any traces of nuclease

produced as a result of promoter leakage. In synthetic biology, such genetic devices are called suicide switches. After checking a broad palette of regulatory sequences, a combination consisting of a zinc-inducible promoter proved most efficient, leading to complete killing of GM cyanobacteria.

In the second approach, one of the toxin-antitoxin pairs that are already encoded in the genome of PCC 6803 was re-organized at the DNA level. Toxin-antitoxin systems only act within cyanobacterial cells and they present no harm for humans or the environment. They consist of a stable toxic protein and a less stable neutralizing antidote involved in various cellular functions. Similarly to the first approach, several variants of suicide switches were prepared and tested for efficiency. It turned out that unlike the nuclease-based safeguards, the suicide constructs based on a toxin caused reduced growth of bacteria rather than efficient cell killing, suggesting that bacteria were able to cope with the damage inflicted by the toxin.

For the success of the project, the choice of promoters used for construction of biocontainment safeguards was crucial. To achieve an optimal promoter combination for the kill switches, several metal-ion responsive promoters were tested along with two constitutive promoters. In addition, selected proteins were made less stable to achieve the desired equilibrium within the cells.

The newly developed suicide switches are the first-ever successfully implemented synthetic biology devices for improved biosafety of GM cyanobacteria. It took researchers almost three years to optimize them, but there is still space for further improvements towards higher efficiency and faster mode of action.

Marko Dolinar, Helena S. Celesnik
University of Ljubljana, Faculty of Chemistry and Chemical Technology

Publication

[Biosafety of biotechnologically important microalgae: intrinsic suicide switch implementation in cyanobacterium *Synechocystis* sp. PCC 6803.](#)

Čelešnik H, Tanšek A, Tahirović A, Vižintin A, Mustar J, Vidmar V, Dolinar M
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