Evaluation and Licensing Opportunities

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Patent Literature

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Biological phosphorous clean-up and recycling

Microalgae remove excess phosphorous from water - then reused as fertilizer

Novel strains of green algae have dramatically increased capacity to sequester phosphorous

Phosphorus (P) is a finite, non-renewable resource primarily mined in a limited number of geographical regions. It is a major cause of environmental pollution in industrial wastewater, municipal sewage effluent, and agricultural run-off causing algae bloom. Enhanced biological phosphorus removal (EBPR) is an environment-friendly, energy-efficient and sustainable alternative to energy-intensive and conventional water treatment processes. While algae can perform luxury P uptake (ability to take up more P than necessary for immediate growth), so far the development of algae-based EBPR systems has been limited by their low P removal rates and restricted maximum P accumulation capacity.

Now Dr Keke Yi and co-workers at the Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing have succeeded to **vastly increase the capability of microalgae to take up P** by combining two approaches: (a) increasing the expression of Phosphate Starvation-Responsive 1 (PSR1) in *Chlamydomonas reinhardtii* lines combined with (b) a mutation in the tonoplast-located Pi efflux transporter (CrPTC1).

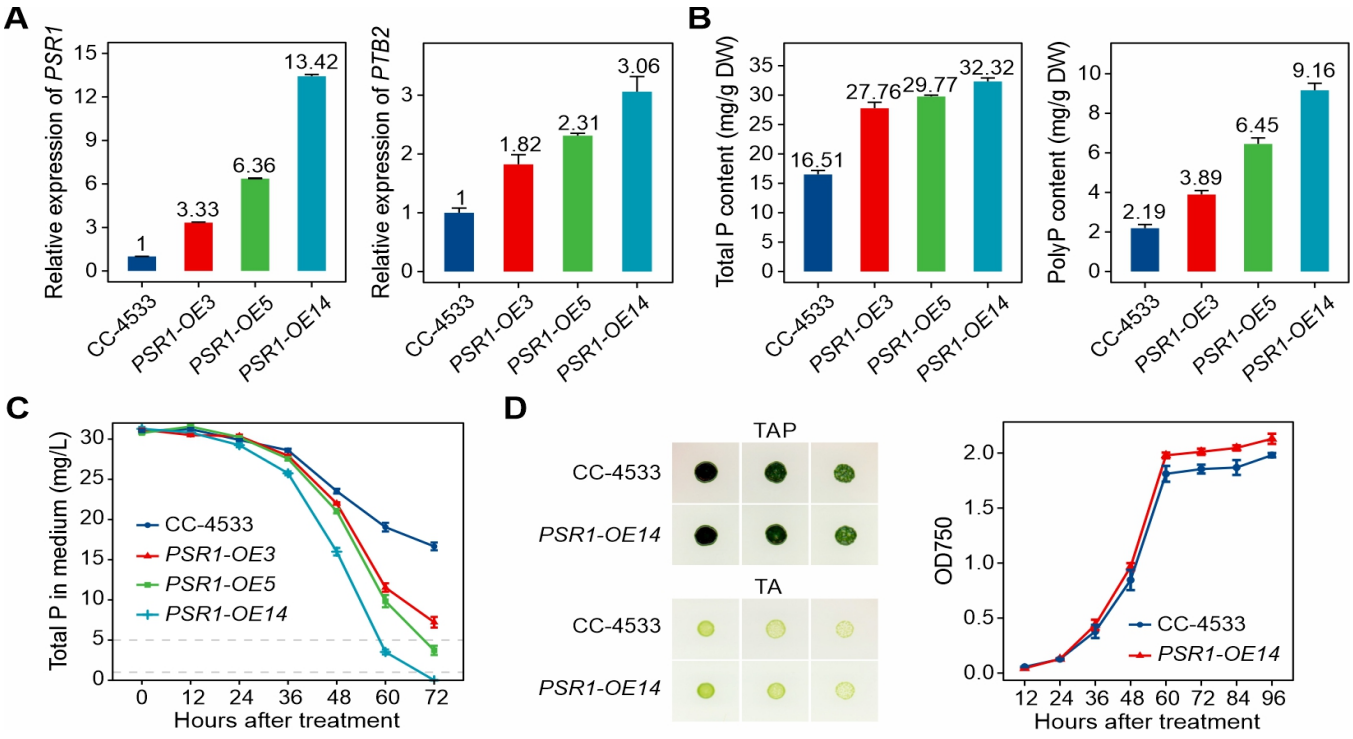

Figure 1: Over-expression of PSR1 confers high P removal capacity. (A) Relative expression levels of PSR1 and PTB2 of three representative PSR1-OE lines. (B) Total P and polyP contents in the PSR1-OE lines. (C) P removal ability of the PSR1-OE lines with 1mM Pi supply. (D) Growth of CC-4533 (wild type) and the PSR1-OE14 line in the TAP and TA mediums. Colonies from left to right are a series of dilutions. The right panel shows growth curves of CC-4533 and the PSR1-OE14 line under 1mM Pi supply conditions.

The evaluation of PSR1 overexpression lines in **Figure 1** above shows a correlation between the expression level of PSR1 and the ability to remove P. Both total P and polyP content showed significant elevation in all PSR1-OE lines (**1B**) which was also demonstrated by the **excellent P removal ability** from medium (**1C**). Meanwhile, the strain with a higher expression of PSR1 showed a higher P removal efficiency (PRE). Growth assessment found no growth defects in the highest accumulating line (PSR1-OE14) whether under conditions of Pi sufficiency or deficiency (**1D**).

In algae **excess Pi is stored as polyP in vacuoles** and *CrPTC1* is involved in cellular P homeostasis. Absence (or reduction) of the CrPTC1 transporter causes accumulation of P and polyP. The *CrPTC1* mutant shows no growth deficiencies and had significantly higher P content and polyP contents (see **Figure 2** below). Furthermore, expression profiles show differences between the *Crptc1* mutant and wild type (**2D**) with the former having upregulated ion transport **(2E**) and Pi homeostasis (**2F**) genes. In summary **CrPTC1 mutation results in high accumulation of polyP in vacuoles** and its P removal capability is very effective, with a dramatically improved rate compared to wild type, achieving full P removal after 120 hours.

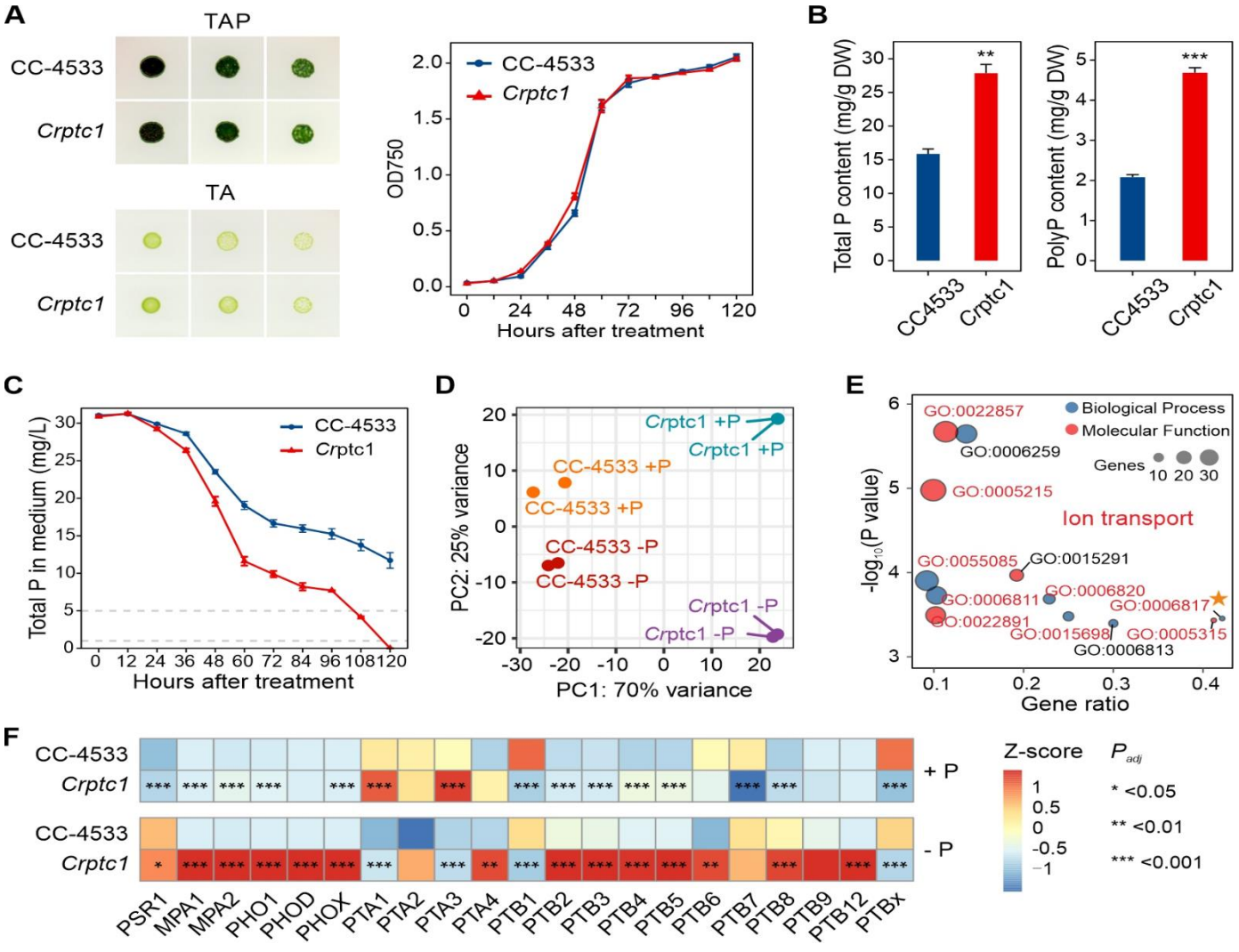

Figure 2. Knock-out of CrPTC1 confers high P removal capacity without compromising cell growth. (A) Growth of CC-4533 and the Crptc1 mutant strains in the TAP (with Pi supply) and TA (without Pi supply) mediums. Colonies from left to right are a series of dilutions. The right panel shows the growth curves of CC-4533 and the Crpsr1 mutant under Pi supply (+P) and Pi deprivation (-P) conditions. (B) Total P and polyP content of CC-4533 and the Crptc1 mutant. (C) Assessment of P removal ability of CC-4533 and the Crptc1 mutant with 1mM Pi supply. (D) Principal component analysis (PCA) shows the global similarity and divergence of transcriptome data. The first two components are shown in the plot. (E) Gene ontology (GO) enrichment analysis of significantly up-regulated genes in the Crptc1 mutant under -P condition. GO terms are highly enriched in ion transport-related terms. GO: 0006817 is P transport. (F) Heatmap of expression profiles of genes involved in P homeostasis under +P and -P conditions.

While these two approaches described above already showed impressive results, combining these two approaches yielded even more dramatic outcomes. These combined strains with high expression of PSR1 in the Crptc1 mutant background (termed SPAO lines), enable **removal of 30mg/L P from wastewater in 2 days**. In comparison wild type strains take more than 7 days and only achieve incomplete removal. In **Figure 3** below the superior accumulation of P and polyP (**B**) are shown, as well as the rapid removal of P from the medium (**C**).

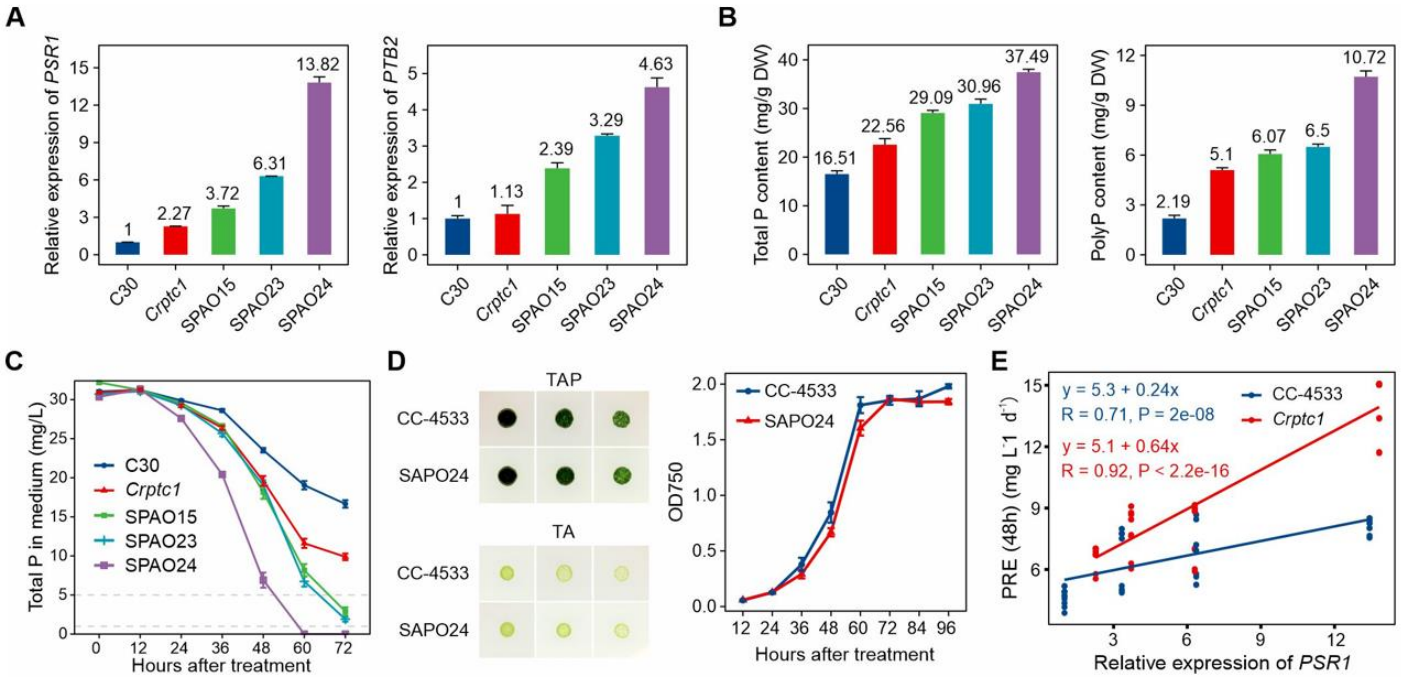

Figure 3: Over-expression of PSR1 in the Crptc1 mutant enhances P removal of the Crptc1 mutant. (A) Relative expression levels of PSR1 and PTB2 of three representative SPAO lines. (B) Total P and polyP contents in the SPAO lines. (C) Assessment of P removal capacity of the SPAO lines under 1mM Pi supply. (D) Growth of CC-4533 and the SPAO24 line in the TAP and TA mediums. (E) Correlation of P removal efficiency (PRE) and relative expression of PSR1 under backgrounds of CC-4533 (blue) and the Crptc1 mutant (red).

In summary the inventors have demonstrated that in microalgae:

- Overexpression of PSR1 leads **to 5 times higher polyP** accumulation
- The *CrPTC1* mutant has **150% more stored polyP**
- Phosphorous from medium (e.g. wastewater) removal is considerably accelerated
- Growth rates of microalgae in low or high P conditions is not impacted
- Combination of PSR1 overexpression with *CrPTC1* mutant has a **synergistic effect on P removal**:
  - **P removal** from the medium is achieved **at 15mg/L/day**

The modified microalgae can be used for ***in-situ treatment*** of aqueous environments in permeable floating photobioreactors (PBRs), in various designs with vertical tubes, flat panel or soft frame and other hybrid PBRs with suspended or immobilised strains. The resulting biomaterial, with high levels of stored polyP, can be collected and then **used as biofertilizer** on crops thereby **recycling phosphorous** leaching from agricultural land and reducing dependence on P mining and conventional P fertilizer production

References:

Not yet published in the scientific literature.