

GENOME WATCH

A bacterial toolkit for plants

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This month's Genome Watch highlights efforts to engineer microorganisms and their plant hosts to address the challenges of sustainable agriculture.

Close your eyes and imagine stalks of barley and corn waving gently against a butterscotch-coloured sky under the light of Phobos and Deimos — on the ruddy plains of a terraformed Mars! Although this science fiction fantasy has been re-imagined many times in cinema and print, the following recent studies bring this make-believe scenario infinitesimally closer to the realm of credibility. But more urgently, these studies begin to address potential solutions for alleviating pressures arising from agricultural burdens and climate change on our own planet.

Geddes, Paramasivan, Joffrin et al.¹ successfully engineered *Medicago trunculata* (barrelclover) and *Hordeum vulgare* (barley, a cereal crop) with a synthetic pathway for bacterial-derived rhizopine, which was exuded into the plant root milieu and functioned as a signal to recruit a rhizopine biosensor-carrying *Rhizobium leguminosarum*. Engineering crops, cereals in particular, to gain important nutrients such as N₂ without costly and environmentally detrimental fertilizer input is the 'Holy Grail' of agronomists, and the study by Geddes et al.¹ proves this goal is achievable. N₂ fixing bacteria or other growth-promoting bacteria could be specifically recruited and induced by engineering plant signalling and control. Also evidenced in this study, a lack of comprehensive functional knowledge of genes that participate in rhizopine metabolism proved an impediment, and considerable effort was expended in elucidating the native bacterial

pathway, with partial success. Ultimately, success was achieved with an alternate rationally designed pathway (comprising genes from two different bacterial sources) that was transferred to the plant host with the desired effects.

Taking a complementary approach, Ryu et al.² re-designed the bacterial partner by engineering and improving the performance of N₂ fixation of naturally occurring epiphytic or endophytic denizens of cereal crops. They used bioinformatics, protein engineering, complete genetic reconstruction or refactoring approaches to identify the best-performing native and engineered *Nif* gene clusters (involved in N₂ fixation) from, and in, multiple sources and host strains. The team performed further engineering to implement inducible control of nitrogenase expression (responsive to various agriculturally relevant signals, including opines related to rhizopine), reduce effects of ammonium repression (so N₂ fixation is unimpeded by added nitrogenous fertilizer) or reduce oxygen sensitivity of nitrogenase (to enable functionality of the enzyme in micro-aerobic environments). The most promising candidates were *Azorhizobium caulinodans* (native cluster) and *Pseudomonas protegens* Pf-5 (engineered with an *Azotobacter vinelandii* cluster), which achieved high levels of inducible nitrogenase activity with reduced oxygen sensitivity.

Beyond N₂ fixation, a few other plant growth-promoting factors are known (for example, ACC deaminase, phytohormones and inorganic phosphate solubilization); however, many more have not yet been identified owing to inadequate genomic and functional characterization of plant-associated microorganisms, their genes and plant-microorganism interactions, posing a bottleneck for future engineering efforts. To this end,

Levy, Gonzalez et al.³ established a compendium of 3,837 new and previously sequenced plant-associated genomes (PAG) and performed comparative analysis with a non-plant-associated (NPA) control set to predict an expanded arsenal of genes and pathways involved in plant-bacterial interaction. Briefly, proteins from PAG and NPA genomes were clustered, and statistically significantly PAG-enriched protein clusters were delineated, which included known plant-associated functions such as nodulation, N₂ fixation and gibberellin (phytohormone) synthesis, among others. Notably, numerous plant-associated functions were detected across phylogenetically diverse taxa, including 'plant mimic' proteins possessing sequence similarity to eukaryotic plant proteins and possibly modulating host interactions by either hijacking host functions or outcompeting host proteins. In all, a new knowledgebase with a compelling set of new targets for further assessment was presented, with potential to modify crops for improved agronomic traits, including resistance to diseases or pests, enhanced nutritional quality and increased productivity. Certainly many other challenges need to be overcome, but piecing together all these recent efforts brings us ever nearer to realizing the dream of sustainable agriculture here on Earth and to possibly someday "boldly go where no man has gone before".

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Competing interests

The author declares no competing interests.

