

## Articles of Significant Interest Selected from This Issue by the Editors

### Protein Selection via Soil Metagenomes

Soil metagenomes are a rich source of protein-encoding genes, many of which are variants with possible functional differences. By directly cloning gene variants for a single enzyme (1-aminocyclopropane-1-carboxylate [ACC] deaminase) from a soil sample and subjecting the variants to an *Escherichia coli* growth-based competition assay, Jin et al. (p. 1050–1059) selected for optimized enzyme variants and revealed structural details of the enzyme. This work shows that soil metagenomes can provide a diverse pool of naturally occurring functional variants as a starting point for protein optimization.

### Deterministic Assembly of Bacterial Communities in Germ-Free Cockroach Guts

Although termites and cockroaches are closely related, their complex intestinal microbiotas differ distinctly. When Mikaelyan and colleagues (p. 1256–1263) inoculated germ-free cockroaches with a termite or mouse gut microbiota, they found that regardless of its origin, the community structure of the resulting xenomicrobiota was always more similar to that of conventional cockroaches than to that of the foreign donor. The study provides experimental proof that the assembly of the cockroach gut microbiota is both predictable and deterministic, with the gut microenvironment shaping community structure by preferentially selecting from the inoculum the lineages best adapted to the available niches.

### Intracellular and Extracellular Expression of *Bacillus thuringiensis* Crystal Protein Cry5B in *Lactococcus lactis* for Use as an Anthelmintic

Some *Bacillus thuringiensis* crystal proteins are lethal to nematodes. Genes encoding full-length or truncated Bt Cry5B proteins were cloned into *Lactococcus lactis* to determine if the proteins could be expressed and exported. The objective was to investigate whether *L. lactis*, a microbe used worldwide for cheese manufacture, could produce Bt proteins and possibly serve as a vehicle for oral delivery against parasitic nematode infections in the gastrointestinal tract. These infections, which occur in more than one billion people worldwide, lead to malnutrition and developmental problems, especially in children. Durmaz et al. (p. 1286–1294) showed that the two Cry5B proteins could be expressed at high levels with nisin induction. Also, using a “leaky *Lactococcus*” expression system, CryB was externalized from *L. lactis* cells without affecting culture growth. In biological activity assays against the nematode *Caenorhabditis elegans*, *L. lactis* expressing Cry5B inhibited worm development.

### Nitrogen Phosphotransferase System Essential in *Sinorhizobium fredii* Symbioses with Legume Hosts

Two general types of phosphotransferase system (PTS) have been identified in bacteria: the sugar PTS, dedicated to carbohydrate transport, and the nitrogen PTS, which exerts regulatory functions. The role of the nitrogen PTS in the nitrogen-fixing legume–rhizobium symbiosis remains elusive. Li et al. (p. 1305–1315) have shed light on this topic by using genetic analysis of nitrogen PTS components (enzyme I [EI<sup>Ntr</sup>], histidine protein [NPr], and enzyme IIA [EIIA<sup>Ntr</sup>]) in *Sinorhizobium fredii* bacteria nodulating soybean and pigeonpea plants. This work suggests a model in which the unphosphorylated EIIA<sup>Ntr</sup> component may negatively regulate nitrogen fixation while the putative glutamine-sensing domain GAF of EI<sup>Ntr</sup> is dispensable in symbiotic interactions.

### *pyrF* as a Counterselectable Marker in *Treponema denticola*

The oral bacterium *Treponema denticola*, one of the keystone pathogens of periodontitis, is recalcitrant to genetic manipulations. Using *pyrF* as a counterselectable marker, Kurniyati et al. (p. 1346–1352) developed a marker-free targeted mutagenesis system in *T. denticola*. This method provides us with a new genetic tool for studying the pathophysiology of *T. denticola*. Importantly, it will also open a new avenue for the development of similar gene knockout systems in other spirochetes and oral bacterial pathogens that have *pyrF* genes.