

# A milestone for endophyte biotechnology

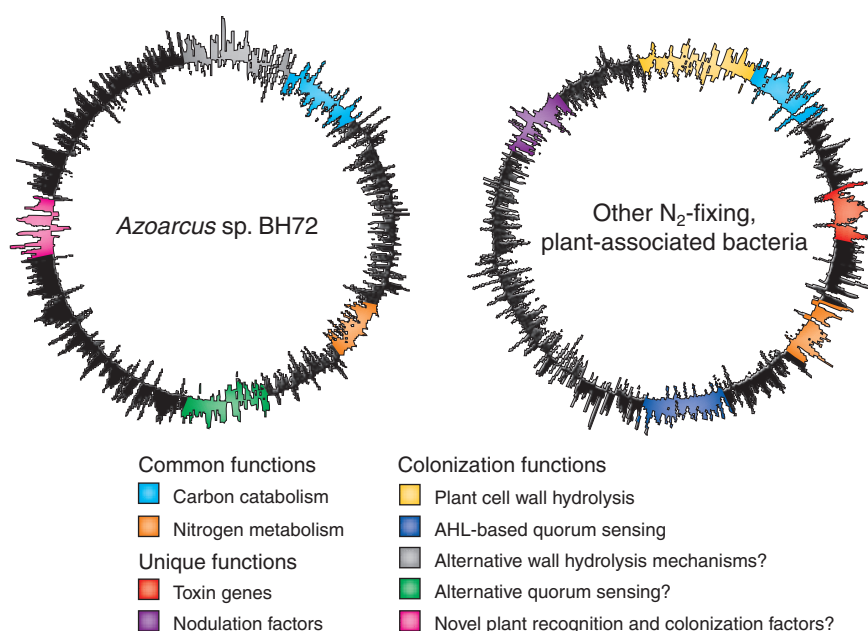
Kiwamu Minamisawa

The genome sequence of a nitrogen-fixing bacterium sheds new light on symbiosis between plants and bacteria.

Many agricultural plants depend on a symbiotic relationship with nitrogen-fixing microorganisms (diazotrophs) for proper growth. Among these microorganisms are bacterial endophytes such as *Herbaspirillum seropedicae*, *Gluconacetobacter diazotrophicus* and *Azoarcus* sp., which have been found in the tissues of some crops and grasses<sup>1</sup>. In this issue, Krause *et al.*<sup>2</sup> report the complete genome sequence of the nitrogen-fixing endophyte, *Azoarcus* sp. strain BH72, and compare it to the genomes of the related soil bacterium strain EbN1 and other plant-associated bacteria. Their results clarify the molecular mechanisms that govern the symbiotic lifestyle of *Azoarcus* and suggest strategies for applying its nitrogen-fixing abilities in agriculture (Fig. 1).

Plant-associated bacteria such as rhizobia, rhizobacteria and various pathogens share some common mechanisms that underlie their beneficial or pathogenic interactions with host plants. For example, symbiotic rhizobia possess type III and type IV secretion systems<sup>3,4</sup> and ethylene biosynthesis regulation systems<sup>5</sup> similar to those of some pathogens. Bacterial endophytes such as BH72, which colonize plant tissue, have been presumed to contain these genes. However, the BH72 genome lacks genes encoding type III and type IV secretion systems, toxins, nodulation factors, common enzymes that hydrolyze plant cell walls, and the N-acyl homoserine lactone (AHL)-based quorum-sensing system. Conversely, the sets of genes responsible for carbon catabolism and nitrogen assimilation reported for BH72 closely overlap expected metabolic capabilities<sup>1,2</sup>. These findings place BH72 in a class of what might be called 'disarmed' bacteria, as opposed to pathogens and rhizobia.

Kiwamu Minamisawa is at the Graduate School of Life Sciences, Tohoku University, 2-1-1 Katahira, Aoba-ku, Sendai 980-8577, Japan.  
e-mail: kiwamu@ige.tohoku.ac.jp



**Figure 1** Comparison of the biology of the nitrogen-fixing bacterial endophyte *Azoarcus* sp. strain BH72 to that of other nitrogen-fixing, plant-associated bacteria. The low plasticity of the BH72 genome, as inferred from sequence analysis, suggests that the bacterium has adapted to stable, low-stress microenvironments in plants. In contrast, rhizobia and other plant pathogens have developed mechanisms for rapid adaptation to the changing conditions in and outside of their host plants through frequent genomic rearrangements. Although the BH72 genome appears 'disarmed' in comparison with other plant pathogens and rhizobia, its many hypothetical proteins include potential host recognition and colonization factors.

However, Krause *et al.* do identify other factors encoded by the BH72 genome that are involved in host interaction. These include type IV pili, surface polysaccharides, the sci-genomic island of human pathogens, type I and II protein secretion systems, flagella and chemotaxis proteins, and a large number of ferric-siderophore uptake systems. Most of these are not present on the genome of the related soil bacterium EbN1.

In addition, the BH72 genome has many hypothetical proteins that are well conserved

in rhizobia or pathogens, suggesting the presence of interaction systems shared with other plant-associated bacteria. Bacterial endophytes face a strong plant defense system, which they must overcome to colonize the host. For example, although the plant defense response involving jasmonate reduces colonization by *Azoarcus* sp. strain BH72 (ref. 6), the bacterium may escape complete rejection through the activity of some of the newly discovered genes. Such mechanisms for avoiding the host defense response might be harnessed

Katie Ris

to engineer endophytic bacteria that colonize their host more efficiently.

To rapidly adapt to the very different conditions inside and outside their host plants, rhizobia and other plant pathogens use genetic mobile elements to mediate horizontal gene transfer events and genome rearrangements necessary for symbiosis and pathogenesis<sup>7</sup>. In contrast, the genome of *Azoarcus* sp. strain BH72 contains few mobile genetic elements such as phage and insertion sequences. This low-plasticity genome is probably better suited to relatively stable, low-stress microenvironments in plants.

Although the BH72 genome provides valuable insights into the biology of bacterial endophytes, many questions remain to be investigated if bacterial endophytes are to be developed for new biotechnological applications<sup>1,3</sup>. Which bacterial and plant factors affect the host range of strain BH72? What molecular switches trigger the shift of BH72 cells toward hyperinduction of nitrogenase at low oxygen concentration<sup>1</sup>? Although significant nitrogen fixation in kallar grass has been carefully verified in laboratory experiments<sup>8</sup>, it is not known whether and to what extent inoculated BH72 or naturally occurring *Azoarcus* enhance plant growth via nitrogen fixation in the field. Fortunately, strain BH72 is also able to infect rice plants and express high levels of nitrogenase in this host, which should allow the use of molecular tools to study the specifics of the interaction between *Azoarcus* BH72 and its hosts<sup>1</sup>. Finally, a question of great interest is whether engineered strains of BH72 armed

with genes borrowed from other more 'aggressive' symbionts or plant pathogens may show enhanced utility.

In addition to applications for enhanced nitrogen fixation, other potential applications of transgenic and of the vast diversity of natural bacterial endophytes include bio-control systems, gene delivery systems, plant growth promotion and phytoremediation<sup>9</sup>. Indeed, diazotrophic bacteria including *Herbaspirillum* sp., *Ideonella* sp., *Enterobacter* sp. and *Azospirillum* sp. have been isolated from rice<sup>10</sup>—and a few of these isolates have been shown to act as antagonists against pathogens or insects. The availability of the *Azoarcus* sp. strain BH72 genome will facilitate biotechnological applications by allowing comprehensive comparative genomic and metagenomic analyses of bacterial endophytes to discover novel determinants of beneficial traits.

1. Hurek, T. & Reinhold-Hurek, B. J. *Biotechnol.* **106**, 169–178 (2003).
2. Krause, A. *et al. Nat. Biotechnol.* **24**, 1385–1391 (2006).
3. Buttner, D. & Bonas, U. *Curr. Opin. Microbiol.* **9**, 193–200 (2006).
4. Thieme, F. *et al. J. Bacteriol.* **187**, 7254–7266 (2005).
5. Sugawara, M. *et al. Biotechnol. Adv.* **24**, 382–388 (2006).
6. Miche, L. *et al. Mol. Plant Microbe Interact.* **19**, 502–511 (2006).
7. Kaneko, T. *et al. DNA Res.* **9**, 189–197 (2002).
8. Hurek, T. *et al. Mol. Plant Microbe Interact.* **15**, 233–242 (2002).
9. Bacon, C.W. & Hinton, D.M. in *Plant-Associated Bacteria* (ed. Gnanamanickam, S.S.) 155–194 (Springer, Dordrecht, 2006).
10. Elbeltagy, A. *et al. Appl. Environ. Microbiol.* **67**, 5285–5293 (2001).

By the early 1990s it was recognized that a highly efficient defense against plant viruses could be achieved in plants by transforming them with virus-derived sequences expressing either translatable or nontranslatable RNA<sup>2</sup>. This defense was later shown to be mediated by homology-dependent gene silencing, a post-transcriptional RNA-degradation system that involves short interfering RNAs (siRNAs)<sup>3</sup>. Interestingly, this biotechnological approach turned out to rely on a natural antiviral defense mechanism induced in wild-type plants in response to viral infections<sup>4</sup>.

Given the functional similarities of miRNAs and siRNAs as regulatory elements, it has been postulated that miRNAs may also play a role in antiviral defense and host-range definition<sup>5</sup>. In fact, endogenous miRNAs have been shown to target engineered plant viruses<sup>6</sup>. However, as yet there is no experimental evidence for antiviral activity of miRNAs against natural plant virus infections. In contrast, cellular miRNAs targeted to animal viruses have been characterized<sup>7</sup>, although it has been suggested that such miRNAs, rather than being involved in antiviral defense of mammalian cells, could be a means by which the virus avoids excessive proliferation, which would kill the cell and threaten its survival.

Whatever the role of miRNAs in natural viral infections, Niu *et al.* have shown that these small RNAs can be engineered to protect plants against viruses. Efficient amiRNA-mediated defense was demonstrated for two very different plus-strand RNA viruses, a potyvirus and a tymovirus, suggesting that the approach should be of broad utility. It will be interesting to determine whether it will also be useful against viruses with different types of genomic nucleic acids.

Compared with homology-dependent gene silencing, amiRNA has several advantages for generating viral immunity. First, it should have fewer off-target effects, as the amiRNA sequences are shorter than those usually required for homology-dependent gene silencing. This could be especially advantageous when knowledge of the complete genome sequence of the plant to be transformed enables selection of antiviral amiRNAs that have no complementary host target sequences.

A second important advantage relates to environmental biosafety. Concerns about transforming plants with viral sequences that might complement or recombine with non-target viruses clearly do not apply to plants expressing amiRNAs.

Still, the approach of Niu *et al.* does raise several issues. In many plant viruses, sequence divergence is very high, and it may be difficult to select amiRNAs to target sequences that are

## A micropunch against plant viruses

Juan Antonio García and Carmen Simón-Mateo

**Artificial microRNAs show promise for combating viral infections in plants.**

Since their discovery a few years ago, microRNAs (miRNAs) and other small noncoding RNAs have been recognized as key components in several regulatory processes in animals and plants. These small RNAs are now being developed into tools for biological research and for diverse applications in medicine and

agriculture. The work of Niu *et al.*<sup>1</sup> in this issue demonstrates the potential value of miRNAs as antiviral agents in plant biotechnology. The authors have engineered miRNA precursors of abundant natural plant miRNAs to contain complementary sequences to particular plant viruses. Once mature, these 'artificial' miRNAs (amiRNAs) target the genomic RNAs of two plant viruses, *Turnip yellow mosaic virus* (TYMV) and *Turnip mosaic virus* (TuMV), and *Arabidopsis thaliana* plants transformed with the recombinant miRNA precursors became specifically immune to infection with these viruses (Fig. 1).

Juan Antonio García and Carmen Simón-Mateo are at the Centro Nacional de Biotecnología-CSIC, Campus Universidad Autónoma de Madrid, 28049 Madrid, Spain.  
e-mail: jagarcia@cnb.uam.es