

Novel biological function of polyester in insect-bacterium symbiosis

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(Left) The bean bug *Riptortus pedestris*. (Center) Dissected intestine of *R. pedestris*, whose posterior is specialized as a symbiotic organ (yellow arrow) for harboring a specific bacterial symbiont. (Right) The symbiont cells wherein accumulated PHA granules are seen (red arrowheads).

Researchers from AIST have demonstrated that an intestinal symbiotic bacterium, which has beneficial effects on the growth and reproduction of the bean bug (*Riptortus pedestris*) known as a soybean pest, accumulates polyhydroxyalkanoate (PHA), a type of polyester, as intracellular granules under symbiotic conditions, and that this intracellular polyester accumulation is required for maintenance of the normal symbiotic relationship.

Many bacteria synthesize large quantities of PHA (sometimes exceeding 90% of bacterial dry weight) from sugars and lipids, accumulating PHA

granules within the cell as [carbon storage](#) for survival during periods of starvation and environmental stresses. On the other hand, PHA has attracted attention of researchers as a bio-[plastic material](#). AIST's finding that PHA is involved in the maintenance of the insect-[bacterium symbiotic relationship](#) is not only interesting biologically but also sheds light on unexpected connections between biotechnology and symbiosis.

These results have been published online in an American academic journal, *Proceedings of the National Academy of Sciences*, on June 11, 2013.

Microorganisms are much more diverse than animals and plants, and their sophisticated biological functions have been utilized for a variety of purposes such as producing medical drugs, pesticides, foods, fuels, and detergents, thereby supporting human life and creating huge economic benefits. In recent years, [symbiotic bacteria](#), which live together with animals and plants and exhibit various elaborate biological functions, have attracted much attention as "unexplored bio-resources". Moreover, on the grounds that microbe-derived bio-plastics are recyclable and biodegradable, entails no consumption of fossil fuels in principle, and therefore potentially involves lower environmental loads, not only basic researches on bio-plastics but also application and commercialization of bio-plastics are promoted in the world.

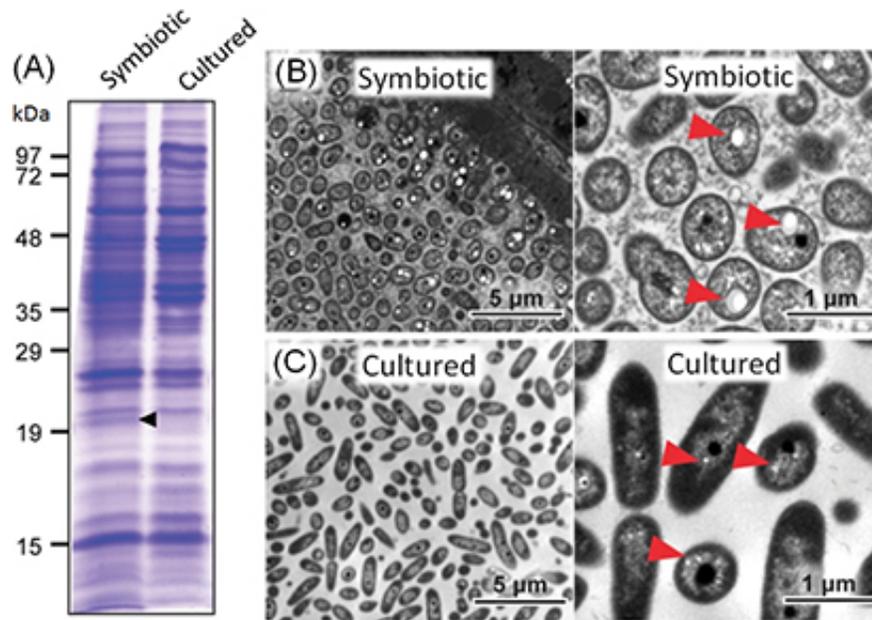


Figure 1 : (A) Comparison of proteins extracted from symbiotic Burkholderia and cultured Burkholderia. A protein band with molecular weight of approximately 19,000 is preferentially detected under the symbiotic condition (black arrowhead). (B) Electron microscopic images of the symbiotic Burkholderia cells wherein well-developed PHA granules are seen (red arrowheads). (C) Electron microscopic images of cultured Burkholderia cells wherein PHA granules are less developed (red arrowheads).

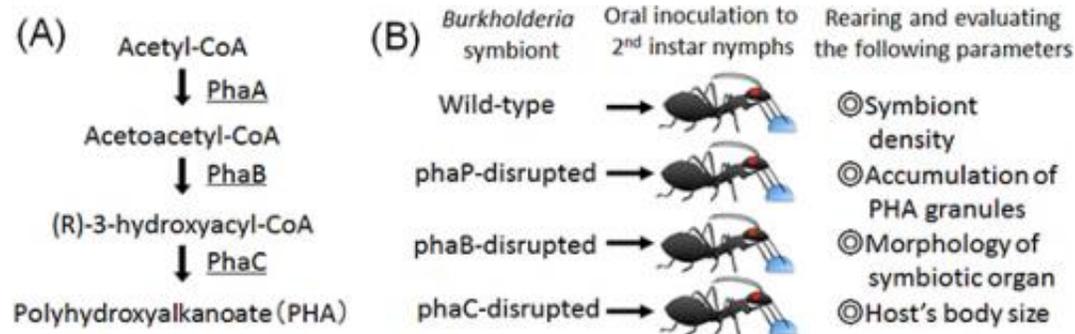


Figure 2 : (A) Synthetic pathway of PHA. (B) Experimental design of Burkholderia inoculation test.

AIST has published a number of research achievements in various [biological functions](#) of symbiotic bacteria associated with diverse insects, which include symbiont-mediated plant adaption, pest evolution, and insecticide resistance (AIST Press releases on March 25, 2004, June 13, 2007, and April 24, 2012) and in elaborate host-symbiont interactions including nutrient supply, reproductive manipulation, and vertical transmission (AIST Press release on December 22, 2009 and AIST research results announced on July 3, 2007 and May 28, 2012).

Most of symbiotic bacteria have no free-living phase in their life cycle. However, the researchers found that the bean bug (*Riptortus pedestris*), known as a soybean pest, acquires a bacterial symbiont of the genus *Burkholderia* from the soil at a nymphal stage and harbors the symbiont in a symbiotic organ located at a posterior region of the digestive tract. The symbiont has beneficial influences on the host fitness including faster growth, larger body size, and more egg production. Furthermore, reflecting its free life in the environmental soil, the *Burkholderia* symbiont is easily cultivable and genetically manipulatable. Hence the bean bug and *Burkholderia* comprise a useful model experimental system to elucidate molecular mechanisms underlying symbiosis. AIST focusing on the symbiotic system of the bean bug and *Burkholderia*, started the international joint research project with the group of Prof. Lee Bok Luel, Pusan National University in South Korea, who is an outstanding expert in the field of insect biochemistry and immunology (AIST TODAY Vol.12 No.4).

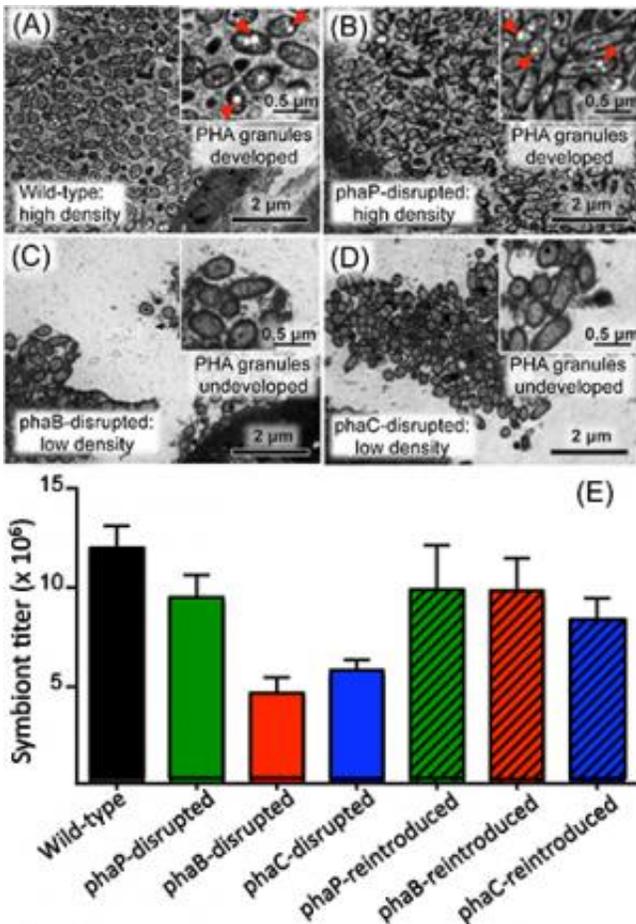


Figure 3 : (A)-(D) Comparison of electron microscopic images of wild-type Burkholderia strain and mutant Burkholderia strains in which a PHA-related gene is disrupted. Red arrowheads indicate PHA granules. (E) Comparison of infection densities of wild-type Burkholderia strain, mutant Burkholderia strains with PHA-related gene disruption, and mutant Burkholderia strains to which the disrupted PHA-related gene was reintroduced.

In an attempt to identify molecules involved in the symbiotic relationship between the bean bug and Burkholderia, the researchers prepared symbiotic Burkholderia cells from the host intestine and non-symbiotic Burkholderia cells cultured in a nutrition medium, extracted, analyzed and compared their proteins, and identified a protein over-produced under the symbiotic condition (Fig. 1A). Amino acid

sequencing identified the over-expressed protein as PhaP protein that is known to bind to PHA granules. These results suggest that PHA granules are accumulated under the symbiotic condition. Electron microscopy and fluorescent imaging confirmed that symbiotic *Burkholderia* cells under the symbiotic condition contain well-developed PHA granules (Fig. 1B) whereas cultured *Burkholderia* cells contain less-developed PHA granules (Fig. 1C).

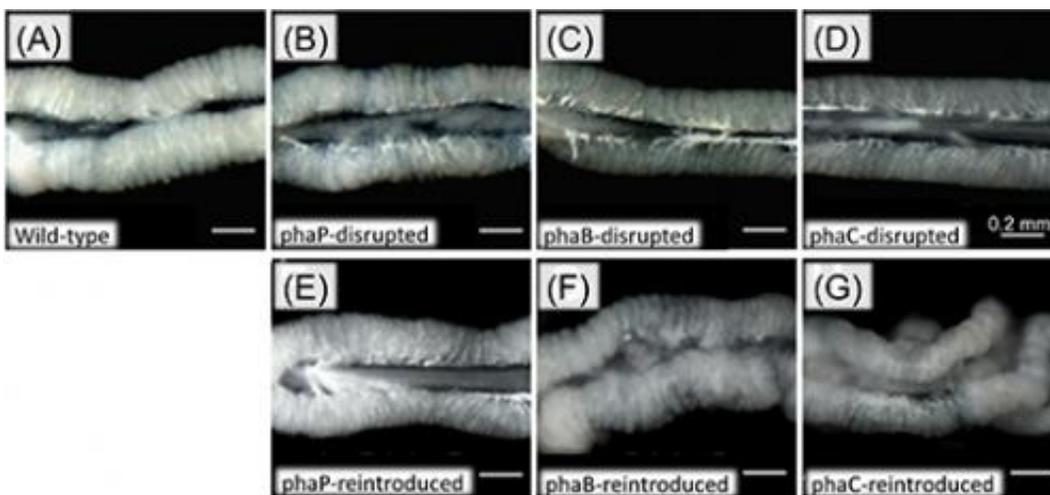


Figure 4 : External appearance of the host symbiotic organs infected with wild-type *Burkholderia* strain, *Burkholderia* strains with PHA-related gene disruption, and *Burkholderia* strains to which the disrupted PHA-related gene was reintroduced. The organ appears swollen and whitish when infection density of the symbiont is high.

Within bacterial cells, PHA is synthesized by three enzymatic reactions (the enzymes are PhaA, PhaB, and PhaC proteins, respectively) (Fig. 2A). The researchers generated *Burkholderia* strains with the disruption of *phaB* gene encoding PhaB protein or *phaC* gene encoding PhaC protein. The bean bugs were infected with the *phaB*-disrupted *Burkholderia* strain, the *phaC*-disrupted *Burkholderia* strain, the wild-

type *Burkholderia* strain, or a *Burkholderia* strain with the disruption of *phaP* gene encoding PhaP protein that is not involved in the PHA synthesis though it binds to PHA granules (Fig. 2B). In the wild-type strain and the *phaP*-disrupted strain, normal colonization to the host intestine, normal infection at a high density, and normal accumulation of intracellular PHA granules were observed (Figs. 3A, 3B, and 3E). In the *phaB*- and *phaC*-disrupted strains, by contrast, PHA granules were hardly accumulated and the infection density was very low in the host intestine (Figs. 3C-E). Furthermore, the host insects infected with the *phaB*-disrupted strain or the *phaC*-disrupted strain exhibited a less developed symbiotic organ and smaller body size than the host insects infected with the wild-type strain or the *phaP*-disrupted strain (Figs. 4A-D and Fig. 5).

Next, using plasmids encoding the normal *phaB* or *phaC* gene, the researchers genetically complemented the *phaB*- and *phaC*-disrupted *Burkholderia* strains, thereby generating the *phaB*- and *phaC*-reintroduced *Burkholderia* strains. Then the host insects were infected with one of these symbiont strains, which also included the wild-type strain containing a blank plasmid and the *phaP*-reintroduced strain. In the *phaB*- and *phaC*-reintroduced strains, accumulation of PHA granules, infection density in the host intestine, development of the host symbiotic organ, and body size of the host insects were all restored to the same levels as those observed with the wild-type strain and the *phaP*-reintroduced strain (Figs. 3E, 4F, 4G, and 5).

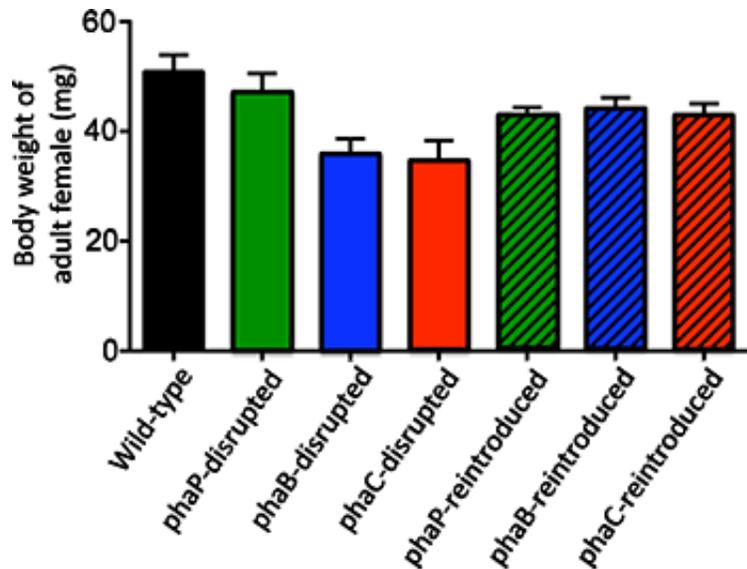


Figure 5 : Body weight of the female adult insects infected with wild-type Burkholderia strain, Burkholderia strains with a disrupted PHA-related gene, and Burkholderia strains to which the disrupted PHA-related gene was reintroduced.

Why does accumulation of PHA granules play an important role in the bean bug-Burkholderia symbiosis? A possible explanation is proposed as "symbiont-mediated stress resistance" hypothesis as follows.

Burkholderia cells are harbored in the host symbiotic organ at a very high density (Figs. 1B, 3A, and 3B), and their growth rate in the host body is very slow. Hence, during the host lifetime for about two months, symbiotic Burkholderia might be subjected to environmental stresses caused by high density symbiosis and growth control via, for example, host's immune regulation. Hence, it is expected that, although speculative, the accumulation of PHA granules may provide the symbiotic Burkholderia with stress resistance. Actually, when experimentally subjected to such [environmental stresses](#) as nutritional depletion, high temperature, and high osmotic pressure, the phaB- and phaC-disrupted Burkholderia strains were vulnerable to the stress treatments, while the phaB- and phaC-reintroduced Burkholderia strains

were resistant (Fig. 6).

The researchers are planning to further investigate the mechanisms as to how PHA is associated with the establishment and maintenance of the symbiotic relationship between the bean bug and *Burkholderia*, by narrowing down the symbiotic stress factors, comprehensive analysis of gene expression, functional analysis, and identification and analysis of symbiosis-related and PHA accumulation-related genes.

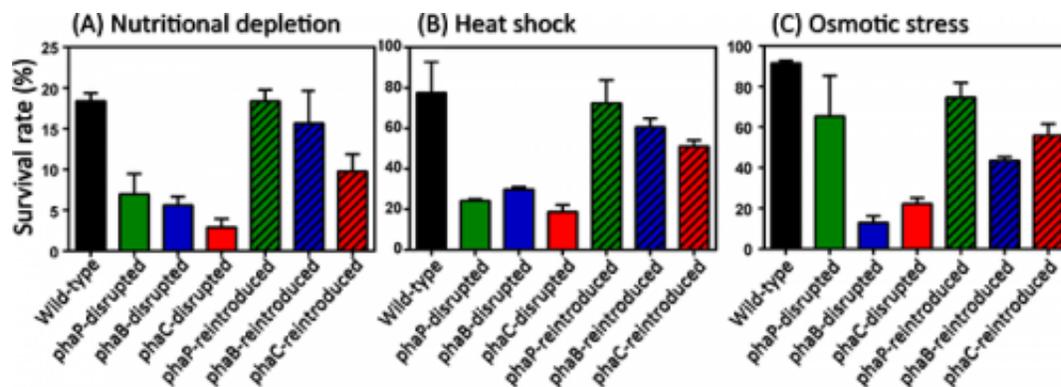


Figure 6 : Survival rate of wild-type *Burkholderia* strain, *Burkholderia* strains with a disrupted PHA-related gene, and *Burkholderia* strains to which the disrupted PHA-related gene was reintroduced, under stress conditions. (A) Two days within phosphate buffer solution (starvation). (B) 10 minutes in a 45 °C bath (heat shock). (C) 24 hours within 1 mole of glucose solution (hyper-osmotic).

This study suggests that the PHA accumulation is involved in bacterial ability of symbiosis, or enduring high density conditions within host organism, which leads to the possibility that, by genetic introduction and/or reinforcement of the PHA synthetic pathway, it may be possible to confer within-host stability and maintenance of bacteria, in particular intestinal ones utilized for probiotic purposes. The researchers are also

planning to conduct the research in such an applied perspective.

Provided by Advanced Industrial Science and Technology

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