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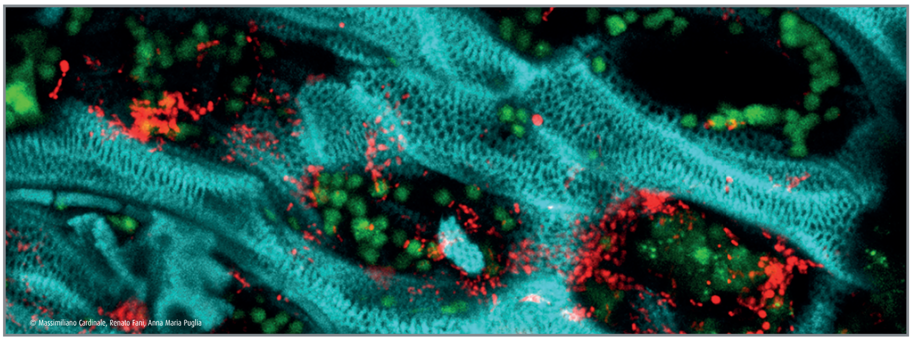
BAGECO 15

Bacterial Genetics and Ecology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Ecosystem drivers in a changing planet

26–30 May 2019
Lisbon, Portugal

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RopB protein of the root nodule bacterium *Rhizobium leguminosarum* possesses amyloid-forming properties

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Scientific question and context: Amyloids are a group of protein aggregates possessing a set of unusual features including high resistance to ionic detergent or protease treatment and ability to induce transition of some proteins from soluble to aggregated form. They play important pathogenic and functional roles and have been found in different taxonomic groups, from prokaryotes to humans and animals. Nevertheless, the observations of the last decades demonstrated the ability of amyloids to perform diverse physiological functions. The majority of functional amyloids of bacteria were identified within *Gammaproteobacteria* species including *Escherichia coli* and *Salmonella enteritidis*. Functional amyloids of *Gammaproteobacteria* play important roles in the biofilm formation, act as a storage of toxins and cause hypersensitive response in plants.

Methods: We performed screening for novel amyloid-forming proteins in the proteome of *Rhizobium leguminosarum*, an agriculturally important species of *Alphaproteobacteria*. Next, we analyzed the obtained data by various molecular genetic methods: PSIA, SDS-PAGE and C-DAG.

Results: Using previously developed Proteomic Screening and Identification of Amyloids (PSIA) approach, we identified 54 potentially amyloidogenic detergent-resistant proteins of *Rhizobium leguminosarum*. For further analysis, we selected the RopB protein that belongs to the Omp25/RopB family of the outer membrane proteins containing the trans-membrane beta-barrel domain. We produced recombinant RopB protein in the *E. coli* bacteria, and its ability to aggregate was shown. We determined the detergent-resistant properties of the obtained RopB aggregates using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). Moreover, RopB fibrils produced *in vivo* on the surface of bacterial cells in the Curli-Dependent Amyloid Generator system (C-DAG) were shown to demonstrate properties typical for amyloids including unbranched morphology and apple-green birefringence upon binding of amyloid-specific dye Congo Red.

Conclusions: Taking together, our results provide an opportunity to conclude that RopB is an amyloid-forming protein of *Rhizobium leguminosarum*. This work was supported by the Russian Science Foundation (Grant No 17-16-01100).

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Interactions between the wasp *Asobara japonica,* its endosymbiont *Wolbachia,* and the remainder of the microbiome

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Animals commonly host specific microbes, so-called symbionts. The relationship between hosts and symbionts is not a one-to-one interaction but needs to be considered in the context of the whole microbiome, i.e. the collection of all host-associated microbes. We set out to disentangle the interactions within the complex of host-symbiont-microbiome in the parasitic wasp *Asobara japonica* infected with the endosymbiont *Wolbachia.* The *A. japonica* infection with *Wolbachia* varies geographically and infected wasps show asexual reproduction, with an all-female offspring. However, *Wolbachia*-induced reproductive manipulation is imperfect, with infected females occasionally producing sons. We propose that this is due to microbe-microbe and host-microbe interactions. To investigate this hypothesis, we collected 3 uninfected (sexual) lines from 3 islands in the south of Japan and 7 infected (asexual) lines on the main island of Japan. As a first necessary step, we analyzed the microbiome of the collected field lines and their population structure. This is currently followed by the artificial removal of *Wolbachia* with heat shock and antibiotic treatment as the first manipulation of an interacting partner. In the future, we plan to manipulate each interaction partner singularly and measure the effect on the other partners, which will provide new insights into interactions within the complex of host-symbiont-microbiome.