AIMS Microbiology, 5 (2): 176–185.
DOI: 10.3934/microbiol.2019.2.176
Received: 04 April 2019
Accepted: 24 June 2019
Published: 26 June 2019

http://www.aimspress.com/journal/microbiology

Research article

Sensitivity of *Deinococcus grandis rodZ* deletion mutant to calcium ions results in enhanced spheroplast size

Yusuke Morita[1], Mai Okumura[1], Issay Narumi[2], Hiromi Nishida[1*]

[1] Biotechnology Research Center and Department of Biotechnology, Toyama Prefectural University, 5180 Kurokawa, Imizu, Toyama 939-0398, Japan
[2] Radiation Microbiology Laboratory, Department of Life Sciences, Faculty of Life Sciences, Toyo University, 1-1-1 Izumino, Itakura, Gunma 374-0193, Japan

* **Correspondence:** Email: hnishida@pu-toyama.ac.jp

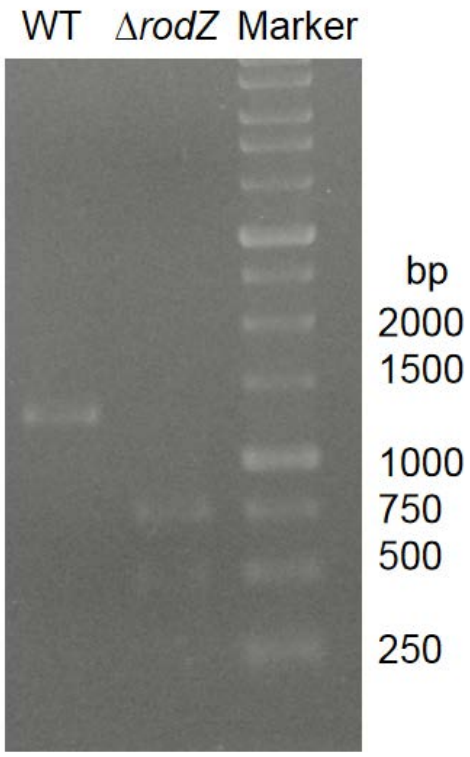

Figure S1. Confirmation of Δ*rodZ*. Disruption was confirmed by amplifying the target allele via genomic PCR using the oligonucleotide primer set HpH-FP and HpH-RP (Table 1). The size of PCR products from wild type and Δ*rodZ* are expected to be 1,295 and 1,247 bps, respectively. The *hph* gene has a *Pst*I site. Thus, the size of the PCR product from Δ*rodZ* is expected to be 492-bp and 755-bp fragments following *Pst*I treatment.

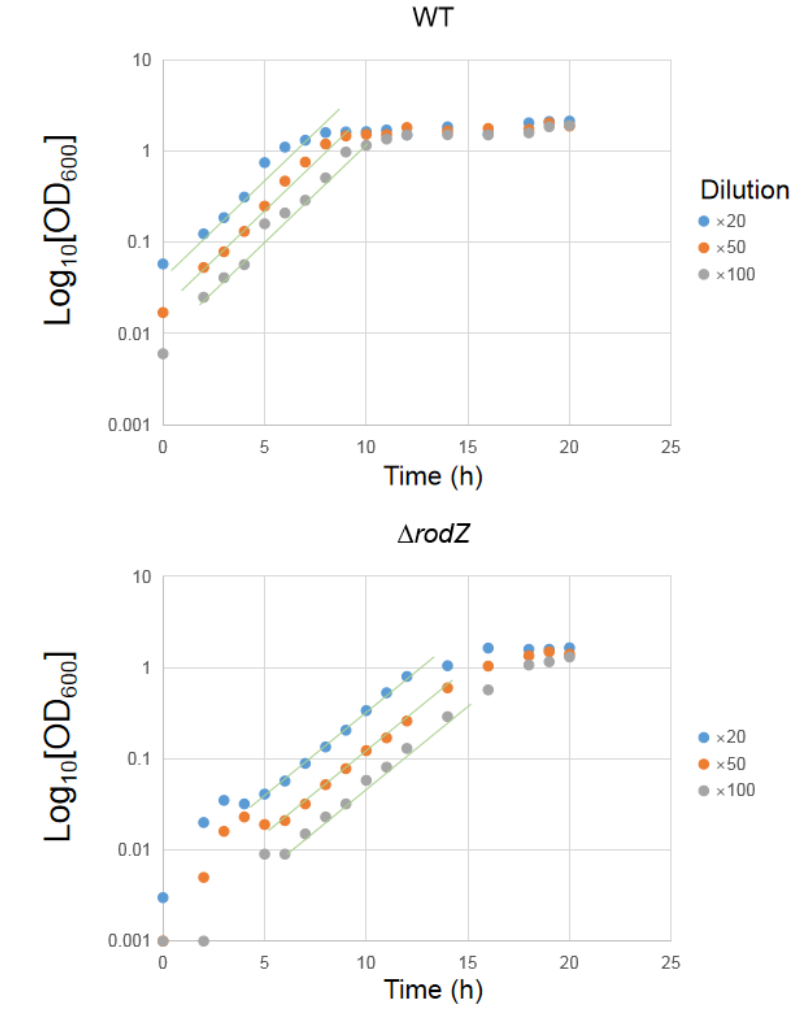

Figure S2. Growth of Δ*rodZ* and wild type incubated at 30°C in TGY broth. OD_{600} of 20, 50, and 100 times diluted overnight cultures was measured using a BioPhotometer (Eppendorf, Hamburg, Germany).

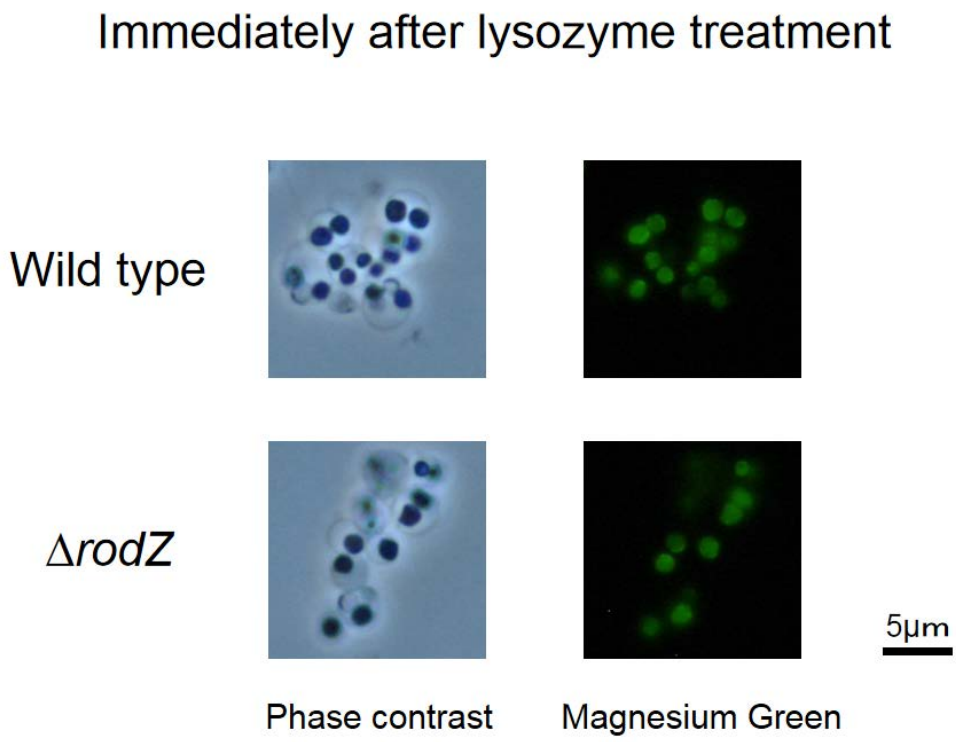

Figure S3. Microscopy images of *D. grandis* spheroplasts immediately after lysozyme treatment. *D. grandis* spheroplasts were incubated with magnesium ion indicator Magnesium Green, AM cell permeant (Thermo Fisher Scientific). Phase contrast and fluorescent microscopy images were captured using an Olympus BX51.

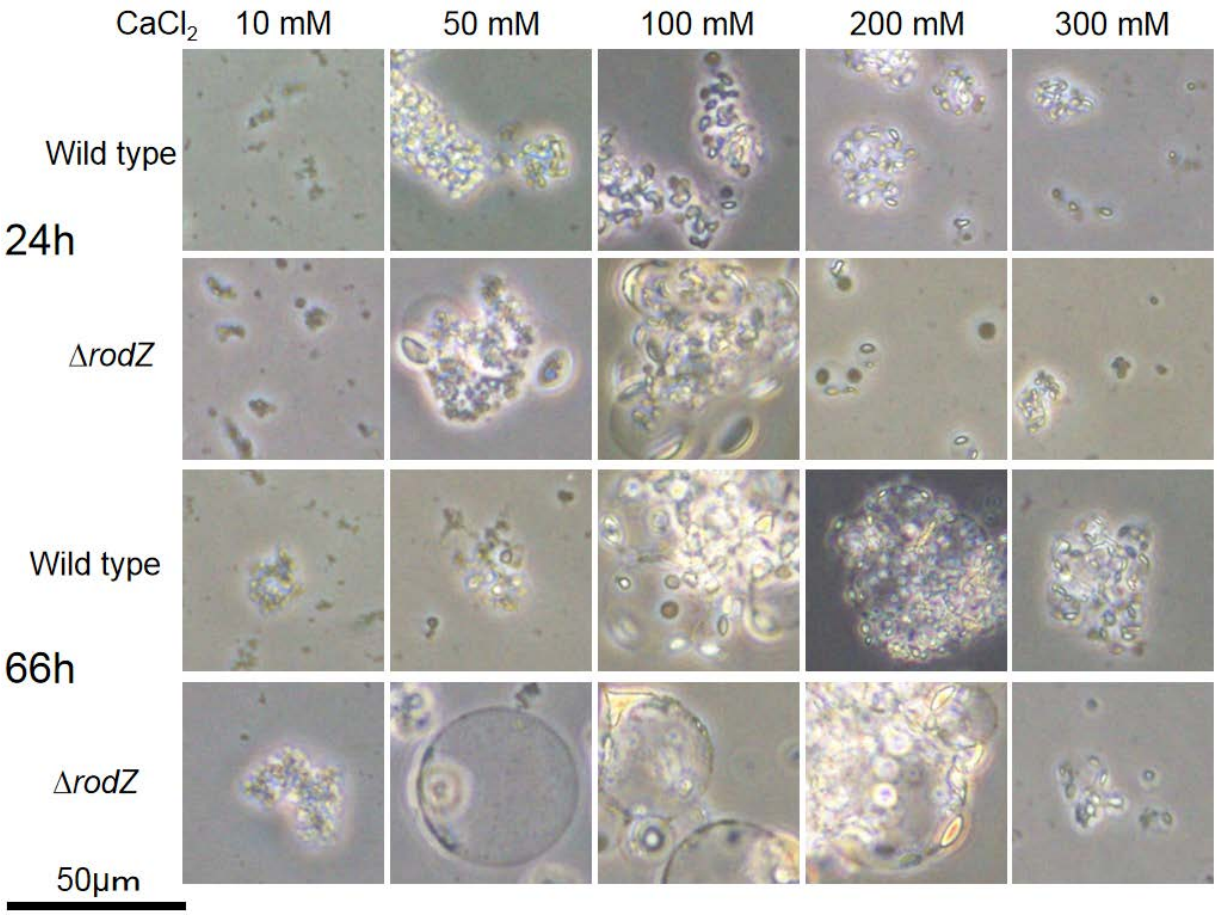

Figure S4. Micrographs of Δ*rodZ* and wild type incubated in MMB0 containing penicillin G at different concentrations of $CaCl_2$. The spheroplasts were incubated for 24 h and 66 h. Differential interference contrast microscopy images were captured using an Olympus IX73.

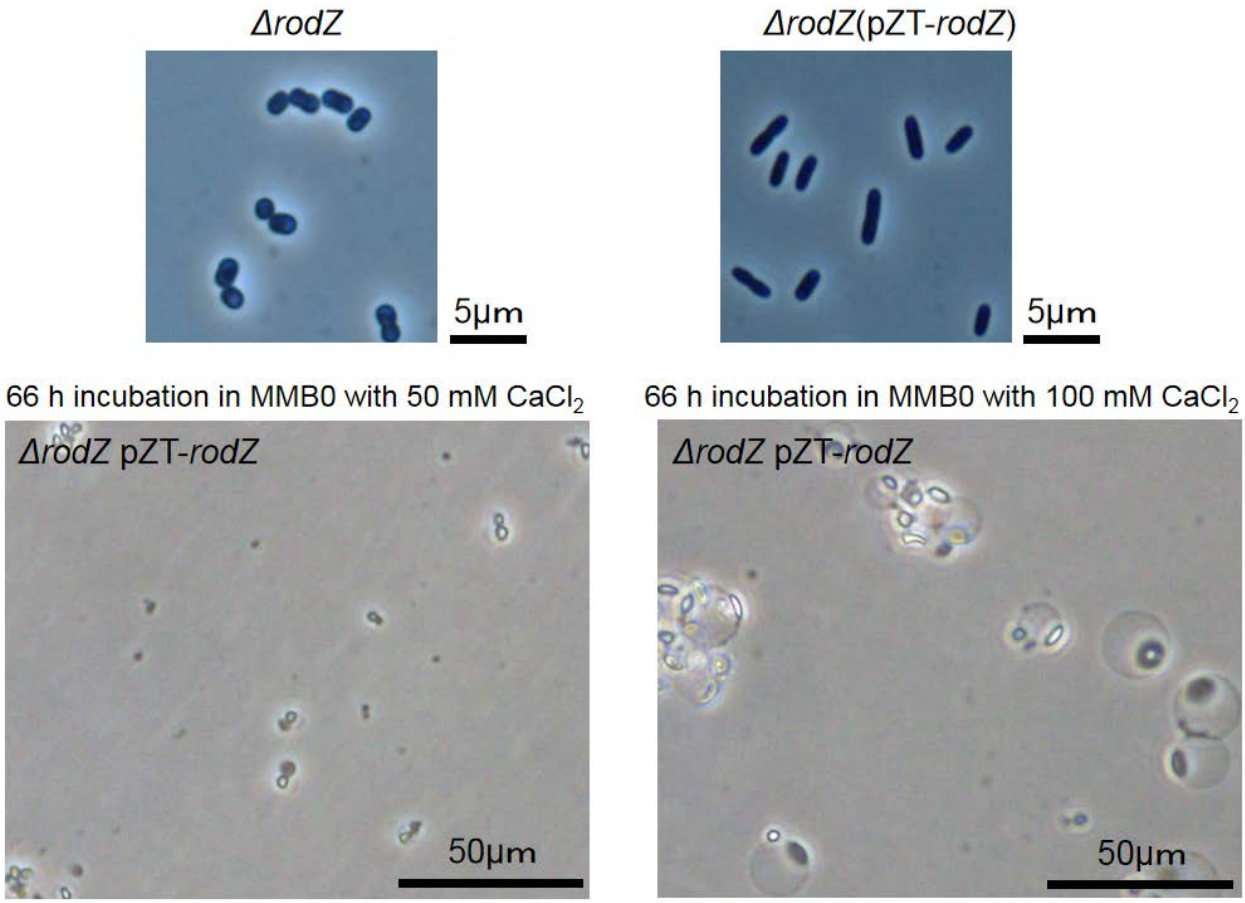

Figure S5. Δ*rodZ* and Δ*rodZ* pZT-*rodZ* micrographs were captured using an Olympus BX51 microscope micrographs of Δ*rodZ* pZT-*rodZ* spheroplasts incubated at 66 h in MMB0 containing penicillin G with 50 and 100 mM $CaCl_2$. Phase contrast microscopy images were captured using an Olympus CK X41.

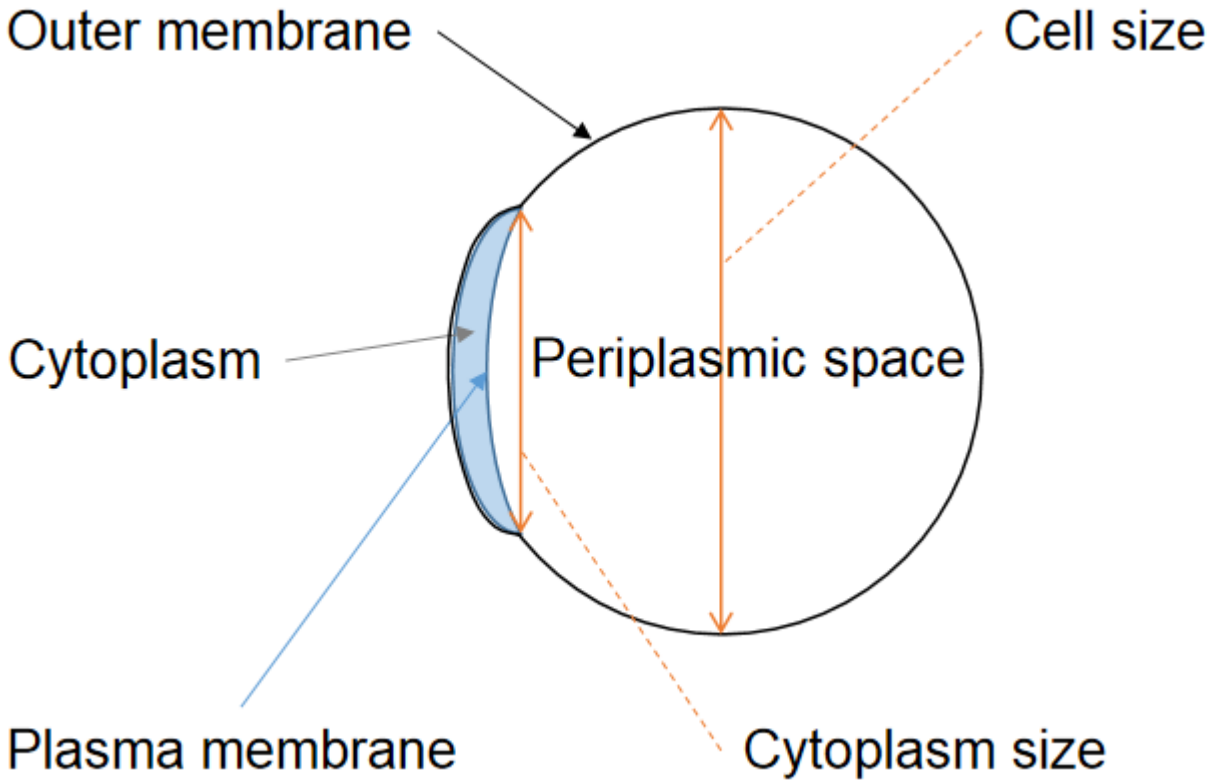

Figure S6. Cell and cytoplasm sizes measured in this study. We used phase contrast microscopy images to measure cell and cytoplasm sizes.