# Transformation efficiency of spheroplasts and normal cells of *Deinococcus radiodurans*

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We generated spheroplasts of *Deinococcus radiodurans*. Transformation experiments revealed that the transformation efficiency of spheroplasts is lower than that of normal cells, suggesting that the presence of a cell wall may play a role in the uptake of DNA by *D. radiodurans*.

*Key words*: cell wall, *Deinococcus radiodurans,* spheroplast, trasnformation

## 1. Introduction

*Deinococcus radiodurans* has an inner membrane, a peptidoglycan layer, an outer membrane, and a surface layer (S-layer) (Farci et al., 2014). Despite the complexity of the cell surface components and the presence of an extremely thick peptidoglycan layer, this bacterium is highly capable of transformation (Gerber et al., 2015). To elucidate the relationship between transformation ability and peptidoglycan structure of the bacterial cell wall, we generated spheroplasts of *D. radiodurans* and compared its transformation efficiency with that of normal cells.

## 2. Materials and Methods

In this study, we used *D. radiodurans* $R_1$ obtained from the American Type Culture Collection (ATCC 13939$^T$). *D. radiodurans* was grown at 30℃ in Tryptone Glucose Yeast Extract (TGY) broth (0.5% Bacto tryptone, 0.1% glucose, 0.3% yeast extract) or TGY agar (TGY broth supplemented with 1.5% agar). Kanamycin (50 μg/mL) was added to the medium if necessary. We generated the spheroplasts of *D. radiodurans* according to the methodology of the Ph. D. thesis of Al-Bakri (1985). Cells grown in TGY broth were inoculated in 10 mL of fresh TGY broth and incubated at 30℃ with shaking until the culture reached late exponential phase. Cells from 800-μL aliquots were harvested by centrifugation (7000 rpm, RT, 5 min), washed once with an equal volume of PS buffer (0.47% $Na_2HPO_4$, 0.46% $KH_2PO_4$, 17.1% sucrose, pH 7), and resuspended in 800 μL of the same buffer. Fresh lysozyme (in PS buffer) was added to the suspension at a final concentration of 2 mg/mL; EDTA was added to a final concentration of 2 mM. The mixture was incubated at 37℃ with gentle shaking for 6 h.

Transformation of *D. radiodurans* was performed using the direct insertional mutagenesis technique described by Funayama et al. (1999), with slight modifications. First, a 2.4-kb DNA fragment *containing DR1238* was amplified by PCR using the primers 5’-ACATGCGGGCAAGCAGGC-3’ and 5’-CGCTATTTCGAGGAATTG-3’. *DR1238* encodes homocitrate synthase (Nishida, 2001; Nishida and Nishiyama, 2012). We designed the PCR primers so that the *Srf*I site was at the middle of the PCR fragment, at which a 1.0-kb *Hinc*II fragment from pKatAPH2, a kanamycin-resistant version of pKatCAT (Funayama et al., 1999), can be inserted to split the PCR fragment. The PCR product containing the kanamycin-resistant region was used for transformation (Nishida and Narumi, 2002). *D. radiodurans* was cultured in TGY broth (800 μL) until an $OD_{600}$ value of 0.68 was reached. The cells (normal cells and spheroplasts) were collected by centrifugation (7000 rpm, 5 min), washed with 800 μL of TGY broth, resuspended in 50 μL of TGY broth, and mixed with 20 μl of 0.3 M $CaCl_2$. Aliquots (30 μL) of

the suspension were added to 14 μL (2.0 μg) of the PCR product prepared above, and the mixture was incubated at 30℃. After 90 min incubation, 1 mL of TGY broth was added to the sample and this was incubated for an additional 24 h. Finally, the cells were collected by centrifugation (7000 rpm, 5 min), and 80 μL of the suspension was spread onto TGY agar containing kanamycin. Colonies grown on the plate were picked up as candidate strains with a knockout in the *DR1238* gene. Disruption of *DR1238* was confirmed by PCR using the primers 5'-CCGTGGCACCGTCATCC-3' and 5'-AGCGGCGTAATGCCGTTG-3', the positions of which in the genomic sequence of *D. radiodurans* were just outside those of the primers used for constructing the disruption.

## 3. Results and discussion

Colonies formed on TGY agar without kanamycin (Fig. 1). Thus, the D. *radiodurans* spheroplasts turned into normal vegetative cells following incubation on TGY agar. Transformants were obtained from both the normal cells and the spheroplasts (Figs. 1 and 2). Spheroplast transformants were clearly fewer than the transformants from normal cells (Fig. 1). This suggests that the presence of a cell wall may play an important role in the uptake of DNA by *D. radiodurans*.

DNA uptake mechanisms are well-investigated in *Thermus thermophilus,* a species closely related to *D. radiodurans*. In *T. thermophilus,* DNA transformation largely depends on the type IV pilli-related competence proteins (Rumszauer et al., 2006). Although *D. radiodurans* does not possess any pilus, the type IV pilli-related competence proteins are highly conserved in *D. radiodurans;* this implies that the DNA uptake mechanisms of *T. thermophilus* and *D. radiodurans* are similar. Since the DNA uptake machinery is located throughout the S-layer, outer membrane, periplasmic space, peptidoglycan, and inner membrane, the loss of cell wall in spheroplasts may result in the destabilization of the DNA uptake machinery.

Another possibility to explain the lower transformation efficiency in spheroplasts is that, considering the time required for recovering spheroplasts over normal cells, the difference may have been due to a constant post-transformation incubation time used in the transformation procedure. Future studies would be required to elucidate the role of cell wall in the uptake of DNA by *D. radiodurans*.

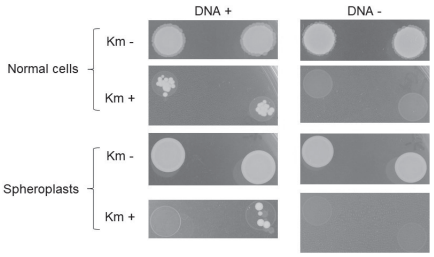


**Figure 1.** Colony formation. DNA indicates the *DR1238* fragment with a kanamycin (Km) resistant region. Km− : TGY agar without kanamycin. Km+ : TGY agar containing kanamycin.

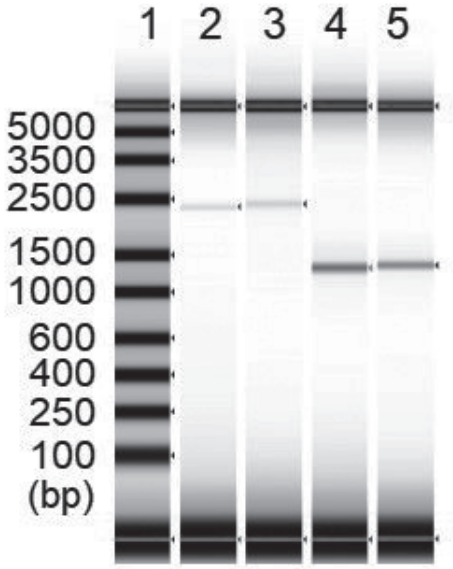


**Figure 2.** Confirmation of *DR1238* disruption by PCR. Lanes: 1, DNA ladder; 2, product from the spheroplast transformant on TGY agar containing kanamycin; 3, product from the normal cell transformant on TGY agar containing kanamycin; 4, product from the spheroplast on TGY agar without kanamycin; 5, product from the normal cell on TGY agar without kanamycin.

## Acknowledgments

We would like to thank Taku Oshima for his helpful comments. We would like to thank Editage for English language editing.

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# デイノコッカス・ラディオデュランスのスフェロプラストと通常分裂細胞の形質転換の効率

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**要旨**

我々はデイノコッカス・ラディオデュランスのスフェロプラストを作製した。通常の分裂細胞における形質転換率に比べ、スフェロプラストのそれは低いことがわかった。このことは、細胞壁が本細菌のDNA取り込みに何かしらの役割を果たしている可能性を示している。

*Key Words*：細胞壁、*Deinococcus radiodulans*、スフェロプラスト、形質転換