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Host Vector Systems of Deep-sea Piezophilic Bacteria, and the Constructions of High Pressure Glow Cells

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Summary

Deep-sea bacteria are adapted to extreme environments, such as high pressures and cold temperatures. We have isolated many piezophiles which grow well even under high pressures from deep-sea sediment. *Shewanella violacea* DSS12 and *Moritella japonica* DSK1 have the ability to grow at up to 70 MPa, and those bacteria have unique mechanisms of gene expression in response to high pressure conditions. The combination of gene expression systems in piezophiles, like the high pressure-dependent promoters and GFP reporter gene, may reveal highly fluorescent cells when exposed to high hydrostatic pressure conditions. It is predicted that a novel bio-sensing system can be made to probe high pressure environments using living bacteria. First, gene transformation into our piezophiles, strains DSS12 and DSK1, were examined. *Eschericha coli* S17-1 was used for bacterial conjugation with those piezophiles. As a result, the broad host range vector, pKT231, and the shuttle vector, pTH10, were successfully introduced to DSS12 and DSK1, respectively. Next, The pressure regulated promoters from DSS12 and DSK1 were cloned into proper vectors and combined with GFP as a reporter gene downstream of each promoter. The transformants of DSK1 and DSS12 with the recombinant pTH10 and pKT231 plasmid, which has *cadA* and *glnA* promoters (each of them is a pressure regulated promoter from DSK1 and DSS12, respectively) and GFP, were grown under high pressure and gene expression of GFP promoted by 50 MPa pressure was confirmed. This is a critical point to create a pressure-sensing bacteria, as the "High Pressure Glow Cells", which will indicate the level of environmental pressure using fluorescence of GFP as a reporter gene.

Host vector systems of *S. violacea* and *M. japonica*

Several gene clusters from *S. violacea* and *M. japonica* have been studied with respect to their expression at high pressure. In addition, the control mechanisms for gene expression *in vivo* and in *E. coli* cells have also been examined. However, how piezophile genes are actually regulated under

high-pressure in those piezophilic bacteria is not yet understood in detail. Therefore, we developed a gene transfer system in rifampicin-resistant *S. violacea* strain DSS12R (DSS12R) and *M. japonica* DSK1 to further expand genetic studies of the piezophiles. This system utilizes a biparental method for bacterial mating resulting in transformation. In this biparental system, the donor is *E. coli* S17-1 with a *tra* gene on its chromosome. The plasmid to be transferred into the recipient cells was transformed into the donor, S17-1, by a heat shock method. Early-log cells of donor (S17-1) harboring the plasmid cultivated in LB medium and late-log cells of the recipient (DSS12R or DSK1) grown in MB2216 (Difco) medium were mixed and cultivated on non-selective MB2216 agar plates so that plasmids were introduced from the donor cells into the recipient cells by mating. After mating, the cells were cultivated on the selective MB2216 agar plates to identify transconjugants. DSS12R was selected for rifampicin resistance, and DSK1 was selected by cultivated at 8°C that *E. coli* can not grow. The colonies obtained by this treatment were grown on two types of MB2216 plates, with or without antibiotics to isolate transconjugants containing the donor plasmid. After comparison of growth on both plates, those colonies showing good growth on both plates were likely to be the correct transconjugants. Plasmids were then isolated from each of the transconjugants using the alkaline-SDS method for large-scale plasmid preparation as well as the Plasmid Prep Kit (MO BIO). The results of this approach are summarized in Table 1. Fig 1. also shows one of the results of electrophoretic characterization of the plasmids in the transconjugants of DSS12R. DSS12R was successfully transconjugated with broad host-range vector pKT231, plasmid pTS4 as well as plasmid pACYC184, which is generally used for *E. coli* transformation. DSK1 was also transformed with broad host-range vector pRK415, as well as plasmid pTH10, which is shuttle vector between *E. coli* and gram positive bacteria. The efficiency of

Table 1. The efficiency of transconjugation of piezophilic bacteria, *S. violacea* DSS12R and *M. japonica* DSK1, using biparental mating with *E. coli* S17-1.

plasmid	size Kb	Replication origin	Antibiotics resistance gene	Transformation efficiency (transconjugants / recipient) DSS12R	DSK1
road host range vector					
pKT231	13.0	RSF1010	Km Sm	4.2 X10^{-7}	-
pRK415	10.5	RK2	Tc	-	2.4X 10^{-8}
Shuttle vector					
pTH10	5.0	pC194 pUC	Km Em	-	1.6X 10^{-7}
Vector for E.coli					
pTS4	2.7	pUC	Amp Cm	1 X10^{-8}	-
pACYC184	4.2	p15A	Tc Cm	2 X10^{-8}	-

transconjugation (transconjugants / recipient cell) generally appears to be much lower than for other mesophilic bacterial conjugations. As both *S. violacea* and *M. japonica* are psychrophilic bacteria, although matings were usually carried out at 37°C which is ideal for *E. coli*, it was demonstrated that optimal mating conditions for DSS12R and DSK1 was at 20°C which is near the limitting temperature for psychrophiles for over-night growth or at 10°C which is the minimal growth temperature for *E. coli* for one week. This study was the first demonstration of gene transfer in the piezophilic bacteria, DSS12 and DSK1, those are able to grow well at high pressure. This technique is essential base for the constructions of High Pressure Glow Cells.

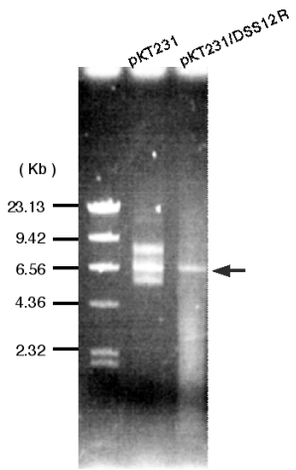

Fig. 1. Detection of the plasmid from transconjugants of *S. violacea* DSS12R.