Control of bacterial lipopolysaccharide modifications and rare metal nanoparticle production

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Research aims

The PmrA/PmrB two-component regulatory system confers cationic peptide polymyxin B and ferric iron resistance to *Salmonella enterica* by modifying phosphoryl groups in lipopolysaccharide (LPS). LPS is the major component of the outer membrane of Gram-negative bacteria (Fig. 1) [1, 2]. Recently, we have shown that reciprocal control of the PmrA/PmrB system and LPS modifications, in combination with Ugd/PbgPE- and PmrC-mediated modifications of phosphoryl groups affecting the lipid A moiety of LPS and the PmrR dependent inhibition of pyrophosphorylated lipid A formation, (catalyzed by LpxT), all act to decrease the net negative charge of the LPS. This in turn hinders the accessibility of Fe^{3+} to the cell surface, as well as to the Fe^{3+} sensor PmrB (Fig. 1) [3, 4].

Shewanella oneidensis is a free-living Gram-negative bacterium that can produce nanoparticles (NPs) from several metal ions, including iron, gold, platinum, silver, and palladium. *S. oneidensis* can therefore be used for the recycling of precious and rare metals, although it is important to understand and improve the physiology and function of the organism to fully realize its potential. In this study, the LPS-modifying gene clusters of *S. enterica* were utilized to alter the binding affinity and specificity of *S. oneidensi*, in turn limiting the types of metal NPs produced from a mixture of several metal ion species, improving the metal recovery technology.

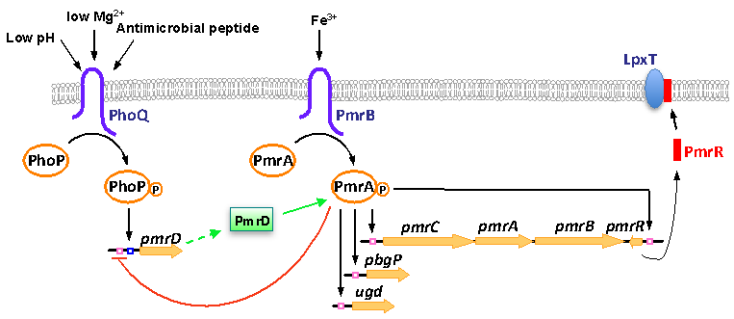

Fig.1.Regulatory network of the LPS modification genes that confers resistance to iron and polymyxin in *Salmonella enterica*

Methods

(1) Prior to the introduction of the *S. enterica* LPS modification gene clusters into *S. oneidensis*, several constructs were created. FRT sites were used to define a chromosomal target DNA fragment that encoded the LPS modification enzymes. These would then be used for *in vivo* specific cloning (Fig. 2).

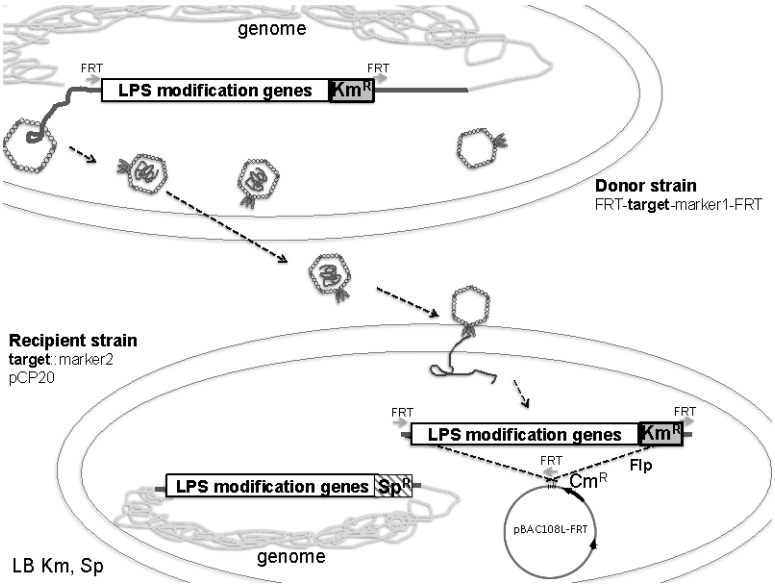

Fig. 2. Model illustrating processes of the P22 phage- and Flp-mediated *in vivo* cloning.

Two consecutive lambda Red-mediated recombination events were induced using two copies of the same antibiotic marker. Following this, P22 transduction was utilized to prepare and transfer insert DNA from the donor strain harboring the target segment to a recipient strain containing the vector plasmid (Fig. 2). Some phage particles injected into *Salmonella* chromosomal DNAs would include the LPS modification gene cluster, Km^R, and the FRT sites. This provided the targets for chance recombination and integration into FRT sites in the pBAC108L-FRT plasmid in the recipient strain (Fig. 2).

(2) In order to use the periplasmic space of *S. oneidensis* as a platform for specific metal NP production, genes encoding the major c-type cytochrome were disrupted using the allelic exchange vector pDMS197. Upstream and downstream DNA fragments (500 bp) of the target gene cluster site were cloned into pDMS197, creating the pDMS197-up-FRT-down construct. An FRT site, which could also be used as a site of integration for the LPS modifying gene cluster segmented by the two copies of FRT, was inserted between the upstream and downstream DNA fragments.

(3) To construct defective mutants of the two-component regulatory system in *S. oneidensis*, a generalized procedure was developed. The pDMS197 plasmid was

employed as a vector to clone a portion of each target site in the response regulator (RR)/histidine kinase (HK) operons (except for the *sacB* gene).

(4) The types of metal species in NPs produced by the *S. oneidensis* mutant strain containing the LPS modification gene clusters were analyzed by a scanning electron microscope using Energy Dispersive X-ray (SEM/EDX).
The LPS modification gene clusters on the chromosome were dissected by FRT site and antibiotic marker-FRT sequence (i.e., Km-FRT) in a *Salmonella* strain. P22 phage lysate was prepared from this donor strain. The recipient strain possessed another antibiotic marker (e.g. Sp) at a site corresponding to the original antibiotic marker-FRT insertion site in the donor strain. This was used to counter-select and prevent RecA-dependent homologous recombination, and harbored the pCP20 and pBAC108L-FRT plasmids respectively. A portion of the P22 phage particles package and transfer the target DNA, together with the marker (i.e. Km) and FRT sites, from the donor chromosome into the recipient strain. This results in the site-specific integration of the target segment into the pBAC108L-FRT plasmid.

Results

Accurate *in vivo* cloning[5)] employing the P22 phage and FLP recombinase was used to introduce the relatively large DNA segments containing LPS modification gene clusters from *S. enterica* into *S. oneidensis*. Approximately 70% of clones contained a correct insert of up to at least 20 kb in length. The LPS gene cluster that had been fragmented by FRTs was cloned into the FRT site of the pBAC108L-FRT vector. Importantly, the insert from the clone can be transferred into other plasmids possessing an FRT via FLP-mediated exchange. The plasmid pDMS197-up-FRT-down was designed with a targeted FRT site to delete the major outer membrane c-type cytochrome in *S. oneidensis*. After the exchange reaction between the two plasmids, the LPS gene cluster was introduced into the *S. oneidensis* chromosome using conjugation transfer and homologous recombination. To construct defective mutants of the two-component regulatory system of *S. oneidensis*, part of the coding region for the first gene in each RR/HK operon was cloned into a pDMS197 vector. After conjugative transfer into *S. oneidensis*, and a single crossover event, target genes were disrupted by integration of the pDMS197 plasmid. This will likely influence downstream HK and RR genes. Analysis of the metal NPs produced by the *S. oneidensis* mutant strains

constructed in this study is ongoing.

Conclusions

In *S. oneidensis*, genetic tools that allow wide-scale gene disruptions and introduction of large gene clusters are relatively limited. Therefore, it is important to develop improved strategies for the cloning and transfer target DNA fragments, even in a donor bacterium. We have developed a cloning method that enables the insertion of large DNA fragments *in vivo*, such as the LPS gene clusters. Moreover, the FRT sites derived from the construction steps of the *in vivo* cloning method were successfully reused for targeting DNA delivery into the *S. oneidensis* chromosome. To fully realize the potential of *S. oneidensis* mutant strains containing the LPS modifying genes, it is necessary to optimize expression of genes derived from *Salmonella*, a distinct genus of *Shewanella*, to increase the metal selectivity. Continuous breeding of the strains constructed in this study and repeat analyses of metal NPs profiles by SEM/EDX will be the next challenge.

References

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