Vectors Derived from Native *Lactobacillus casei* Plasmids

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Abstract

Lactic acid bacteria are frequently used in the preparation of fermented foods and as probiotics. They also are employed by the biotechnology industry to make valuable biomaterials, such as enzymes and lactic acid. The goal of this project was to produce shuttle vectors from the native plasmids found in different strains of *Lactobacillus casei*. Genetic regions from three native plasmids that contained the plasmid replication origin and genes encoding proteins involved with replication initiation and with partitioning of the plasmids into daughter cells were cloned into an *Escherichia coli* vector backbone. Maintenance of the resulting shuttle vectors in *L. casei* strain 12A was followed over 200 generations of growth in the absence of selection. The pDW8 vector, which was derived from a plasmid found in *L. casei* strain 12A, showed the best retention in these experiments. These vectors may prove useful for expression of foreign proteins in *L. casei*.

Methods

Using the proofreading Phusion DNA polymerase, the *rep*R to *par*AB regions of native *Lactobacillus casei* plasmids were amplified and the resulting PCR products cloned into the *E. coli* vector pGEM^{T}. Using sites incorporated in the PCR primers, inserts were recovered as *Bam*HI to *Kpn*I or *Bgl*II to *Kpn*I fragments and re-cloned into an *E. coli* vector backbone that contained an erythromycin resistance gene and a multiple cloning site. The resulting shuttle vectors were shown to contain the correct inserts by a combination of restriction digestion, sequencing, or the ability to yield the correctly sized product in PCR tests. *L. casei* strain 12A was then transformed with the shuttle vectors and transformants (four per vector) passaged under non-selective growth for up to 200 generations. Colony Forming Ability was determined on MRS plates that contained selective levels of erythromycin (2.5 µg/mL) and on nonselective MRS plates without erythromycin.

Results

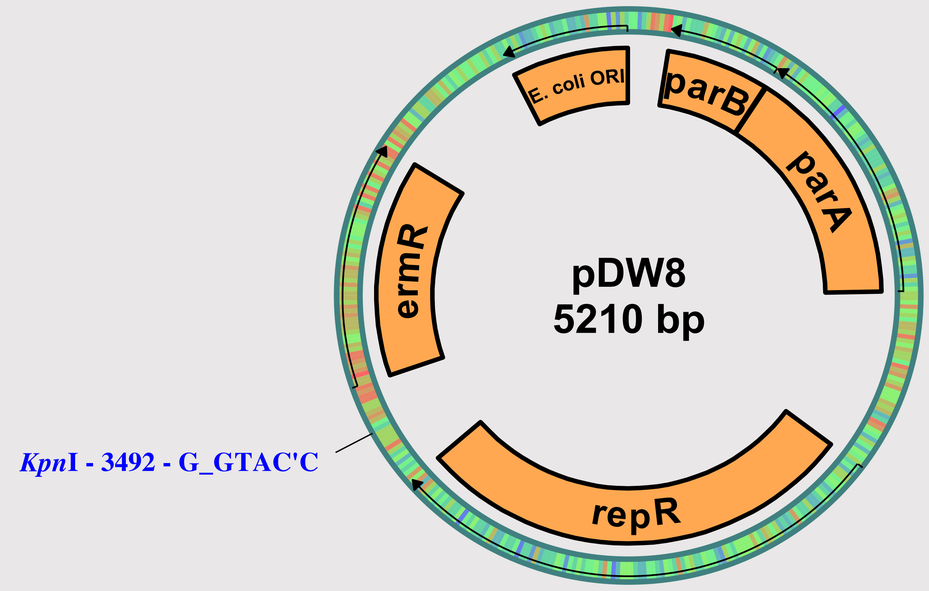
Lactobacillus casei Shuttle Vectors

Vector	Native Plasmid	Restriction Sites	Insert Size (bp)	Total Size (bp)	Vector Backbone	Rep R	Par A	Par B
pDW3	32G	*Bam*HI *Kpn*I	3180	4909	pAE1	+	+	-
pDW4	32G	*Bam*HI *Kpn*I	3480	5209	pAE1	+	+	+
pDW5	32G	*Bam*HI *Kpn*I	3631	5360	pAE1	+	+	+
pDW6	32G	*Bam*HI *Kpn*I	3786	5539	pAE10	+	+	+
pDW7	32G	*Bam*HI *Kpn*I	3988	5741	pAE10	+	+	+
pDW8	12A	*Bgl*II *Kpn*I	3481	5210	pAE1	+	+	+
pDW12	A2-362	*Bam*HI *Kpn*I	3960	5713	pAE10	+	+	+

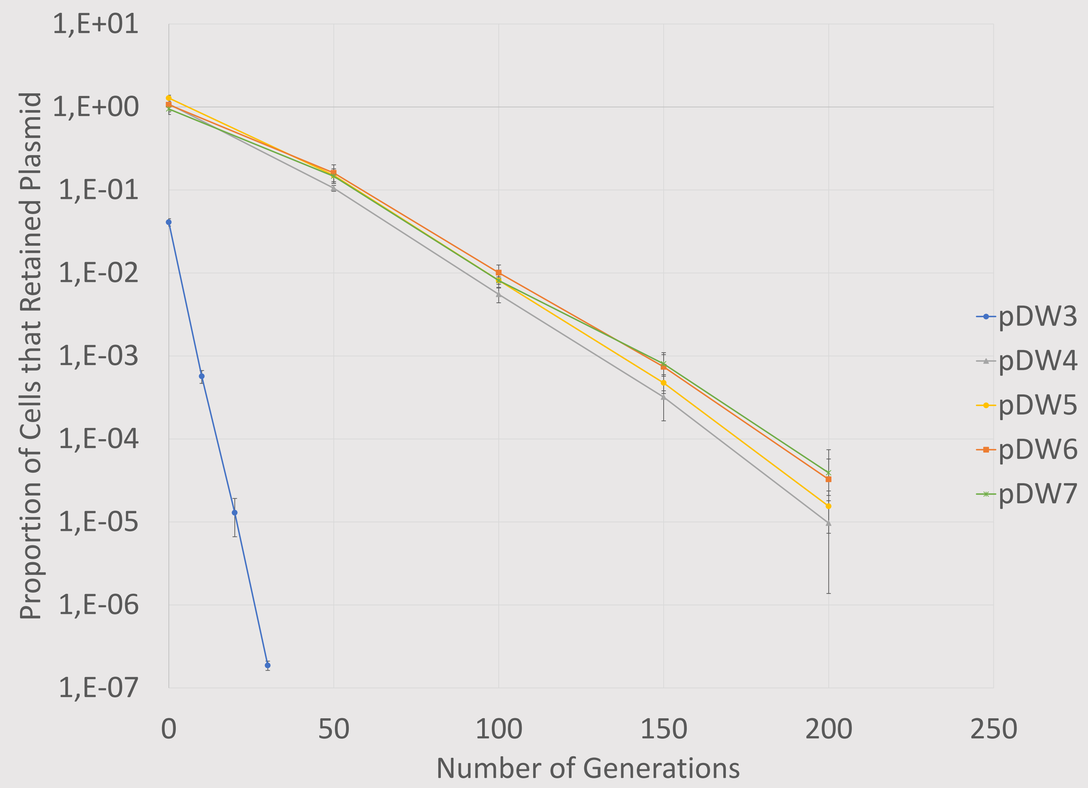
Conclusions

- Overall the pDW8 vector was best retained. This may be due to pDW8 being derived from a plasmid native to strain 12 A.
- The pDW3 vector had the worst retention. This result is likely due to its lack of *par*B.
- With the exception of pDW3, the vectors derived from the plasmid in strain 32G showed about 90 % loss every 50 generations.
- Vector pDW12, which was derived from a native plasmid found in strain A2-362, showed more variable loss than the others, but overall its loss was similar most of the vectors derived from the native plasmid in 32G.

Representative Shuttle Vector



Plasmid Maintenance for *repR* Vectors Derived from the Native Plasmid Present in Strain 32G



Plasmid Maintenance for Additional *repR* Vectors Derived from the Native Plasmids Present in Strains 12A and A2-362