Note

Isolation and Classification of *Pediococcus halophilus* Plasmids

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*Pediococcus halophilus* is a salt-tolerant lactic acid bacterium\(^1\) which is widely used in the traditional Japanese food (*miso* and *soy sauce*) industry.\(^1\)\(^2\) But no evidence for the existence of gene transfer systems, such as transduction, transformation, conjugation, protoplast fusion or electroporation, has been reported. Although genetic engineering is a powerful means of breeding species and *P. halophilus* is a safe host, a suitable host-vector system has not been constructed and little is known about plasmids in this species. Therefore we isolated plasmids from *P. halophilus*, constructed physical maps of the smaller ones and classified them by means of Southern blot hybridization.\(^4\)

One-hundred sixty strains isolated from *miso* and *soy sauce* factories in Nagano Prefecture were screened for plasmids. Cells were cultivated in 500 ml of PAT-5 medium (yeast extract, 3 g; polypeptone, 10 g; glucose, 10 g; K\(_2\)HPO\(_4\), 5 g; CH\(_3\)COONa, 20 g; SHCH\(_2\)COONa, 1 g; NaCl, 50 g per liter, pH 7.2) for 4–6 days at 30°C. Plasmids were prepared basically by the rapid lysis extraction method described by Birnboim and Doly.\(^3\) A higher concentration of lysozyme (15 mg/ml) was used for cell lysis. Plasmids were observed in ninety-two strains, of which eighteen harbored small plasmids (less than 11 kilobases (kb)). According to the number of restriction enzyme sites for *EcoRI* and *HindIII*, and the molecular size, we divided the small plasmids into ten types (pPHA1 to pPHA10). pPHA1 and pPHA7 were widely distributed in strains isolated from 6 different samples from 4 factories.

![Fig. 1. Restriction Maps of *P. halophilus* Plasmids.](image)

The numbers in parentheses indicate the distance in kb from the basal point. B, *BamH*; E, *EcoRI*; H, *HindIII*; P, *PvuII*; S, *SalI*; X, *XhoI*; Bg, *BglII*; B, *BaiI*; EV, *EcoRV*; Ha, *HaeIII*; St, *StyI*; X, *XbaI*. Small plasmids not shown here are pPHA8 (7.4 kb), pPHA9 (~8.3 kb) and pPHA10 (~11 kb), which had 4 and 2, 3 and 2, and 4 *EcoRI* and 4 *HindIII* sites, respectively.

\(^{1}\) Offprint requests to J. Sekiguchi.
Fig. 2. Southern Transfer of Plasmid DNAs from *P. halophilus* Strains and Hybridization with pPHA2 as a Probe.

(A) Agarose gel electrophoresis of DNAs: lanes 1 to 7, strains 501, 3015, 1809, 1701, 2106, 1216 and 812, respectively. Bands, from fast (bottom) to slow (top) migrating: lane 1, pPHA1 (closed circular (cc)), pPHA1 (open circular (oc)) and pPHA101 (cc, faint band); lane 2, pPHA2 (cc), pPHA2 (oc), pPHA9 (cc) and pPHA9 (oc); lane 3, pPHA3 (cc) and pPHA3 (oc); lane 4, pPHA4 (cc) and pPHA4 (oc); lane 5, pPHA501 (cc), pPHA5 (cc) and pPHA5 (oc); lane 6, pPHA6 (cc) and pPHA6 (oc); and lane 7, pPHA7 (cc) and pPHA7 (oc). pPHA2 (cc) as a probe DNA was separated by agarose gel electrophoresis, followed by purification with low-melting agarose. Weak bands in Fig. 2A were not further examined. (B) Southern blot hybridization of A: lanes 1 to 7 correspond to the same lanes in A. 32P-Labeled pPHA2 DNA was used as a probe.

and from 7 to 2, respectively. Strains 807 and 2307, and strain 3015 harbored both pPHA1 and pPHA7, and both pPHA2 and pPHA9, respectively. Among pediococci, larger plasmids (>4.5 megadaltons (Md)) have been reported in *P. pentosaceus*, *P. acidilactici* and *P. cerevisiae*, but the functions (bacteriocin production, sucrose-fermenting ability and raffinose utilization) are known for only a few plasmids. On the other hand, reports of the isolation of smaller plasmids have been very rare. All of the small plasmids reported here, as well as the 1.2-Md and 2.3-Md plasmids in *P. pentosaceus*, were cryptic plasmids.

The physical maps of pPHA1 to pPHA7 are shown in Fig. 1. pPHA3 and pPHA4 resemble each other quite well, there being a 0.12-kb difference in the *PvuII*-EcoR1 fragments. The smallest plasmid, pPHA1, was similar to pPS-1 (1.55 kb) isolated previously from *shoyu-moromi*. Figure 2 shows the results of Southern blot hybridization of pPHA1 to pPHA7 DNAs with 32P-labeled pPHA2 DNA as a probe. pPHA2 hybridized to pPHA2 to pPHA6, but to neither pPHA1 nor pPHA7. The probe DNA also hybridized to pPHA9 (lane 2) and low-copy number plasmids (pPHA501 and a plasmid not clearly visible in Fig. 2A, lane 6). When pPHA1 DNA was used as a probe, it hybridized to a 3-kb plasmid (pPHA101, weak band in Fig. 2A, lane 1) which migrates more slowly than the oc form of pPHA1, but not hybridize to the other plasmids (figure not shown). Similar hybridization experiments were carried out with the other small plasmids as probes, and finally they were classified into three groups (group I, pPHA1; group II, pPHA7; and group III, pPHA2 to pPHA6). It is interesting that pPHA1 coexisted with pPHA7 in two strains, but group III plasmids did not coexist with pPHA1 or pPHA7 in any of the isolates. Plasmids in group III were detected in 6 samples from 5 factories. Since the small plasmids were very stable, even after many transfers to various media, they are strong candidates for *P. halophilus* vectors. Our research is now directed toward constructing shuttle plasmids between *Escherichia coli* and *P. halophilus*, and toward the development of a plasmid transfer system in *P. halophilus*.

References

Plasmids in *Pediococcus halophilus*

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