Biology of the Bacillus plasmids pUB110 and pE194

Plasmids pUB110 and pE194 are widely used in industry, both by DSM (formerly Gist-brocades) and other companies, as vectors for the production of industrially important enzymes by Bacilli. Both were originally isolated from clinical samples of Staphylococcus and have been thoroughly investigated. In fact, they are among the best characterized plasmids from gram-positive microorganisms, where they belong to class I (small, with high copy number).
Both plasmids pUB110 and pE194 are accepted as a vector-component of certified *B.subtilis* HV1 systems.
Although originally isolated from Staphylococcus both plasmids can be considered to be endogenous to Bacilli as the following evidence shows.

pUB110

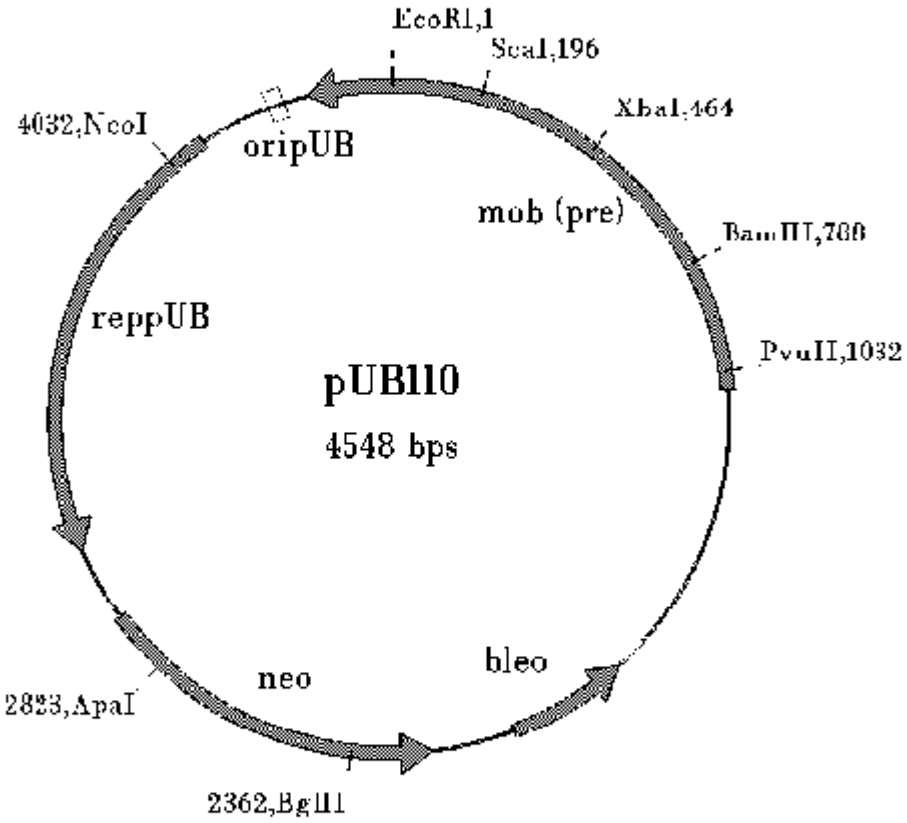
Although isolated from Staphylococcus [1], plasmid pUB110 is to be considered a Bacillus plasmid.
Plasmid pUB110 is 3.0 Mdal (4.5 kb) in size [2] and confers resistance to neomycin [1] and bleomycin [3]. Its complete nucleotide sequence [4,5], its replication gene [6] and origins of replication [7] are known (see plasmid map pUB110). The plasmid is non-conjugative but can be mobilized by some plasmids [8]. The mobilization gene (*mob* or *pre*) and origin of transfer (RS_A) are known [9], which allows the inactivation of the mobilization phenotype.
Plasmid pUB110 is capable of replicating in Bacilli [2], as some *Staphylococcus aureus* plasmids are [10]. Furthermore, plasmid pUB110 has a much higher copynumber and is much more stable in *B.subtilis* than in *S.aureus*, in contrast to other class I plasmids [1,2]. This is probably because pUB110 is the only class I plasmid isolated from *S.aureus* whose lagging-strand conversion signal (the minus-origin) functions in *B.subtilis* [23].
The physical maps of pUB110 [2] and plasmid pBC16 from *B.cereus* [16] are identical but for the resistance region [12], encoding tetracyclin resistance in pBC16 and neomycin plus bleomycin resistance in pUB110 respectively. The physical map of pBC16 is identical to that of pNS1981 [13] which is present integrated into the chromosome of some Bacilli [14]. Plasmids indistinguishable from pBC16 have been isolated from *B.subtilis* and *B.sphaericus* and very related plasmids from *B.stearothermophilus* and *B.licheniformis* [12].
Several tetracyclin resistance plasmids have been isolated from Bacilli. The tetracyclin resistance genes from pTHT9 and pTHT15 are indistinguishable from the one from pBC16 [15]. The regions involved in replication of pTHT9, pTHT15 and pTHN1 show homology to the one from pUB110 [16].
Imanaka et al [17] have isolated a neomycin and tetracyclin resistance plasmid pTB19 (27 kb) from thermophilic Bacilli. During selection on neomycin a specific deletion derivative, pBT913 (4.5 kb), was obtained [18], which is the same size as pUB110. The neomycin resistance gene was found to be identical to the one from pUB110 but for one nucleotide [19]. Also the *rep* gene [20], the RS_A-site, the bleomycin resistance gene and the *pal*U minus-origin [21] were found to be more than 99% homologous to the sequence of pUB110. The N-terminal part of the Pre protein showed more than 85% homology to the Pre protein of pUB110 [21]. The only region of the sequences of pBT913 and pUB110 that did not show significant homology to each other was the region encoding the C-terminal part of the Pre protein [21].
Hoshino et al [15] also isolated a neomycin resistance plasmid pTHN1 from a thermophilic Bacillus. The gene encoding neomycin resistance was shown to be completely identical to the one from pUB110 [16].
These data show that plasmids such as pUB110 are common throughout Bacilli, whereas pUB110 is uncommon among Staphylococci [22].

Gene and plasmid transfer is widespread between species and genera [24-27]. For instance, several plasmids almost identical to pBC16 have also been isolated from Streptococci [28]. Taken together these facts suggest that pUB110 is native to Bacilli and has only recently arrived in Staphylococci [22].

pE194

Plasmid pE194 was also originally isolated from a clinical Staphylococcus isolate [29]. It is fully characterized [29] and like pUB110 belongs to class I, but is placed in another group on the basis of its plus-origin and Rep protein. It contains a RS_A/*pre* mobilization complex that is closely related to pUB110. Its functional organisation is similar to that of pUB110 except that the Em^R determinant is inverted with respect to the other transcription units and the direction of replication.
Members of different replication groups of Class I plasmids originally isolated from Staphylococcus have several sequences in common [22]. This observation is consistent with horizontal exchange of specific segments (which are not transposons), the so-called cassettes or modules. Examples of such cassettes are the antibiotic determinants, the RS_A/*pre* complex, the replication functions or the minus-origin. Cassette exchange presumably occurs by recombination involving short intervening regions, including the known targets for site-specific plasmid cointegrate formation, RS_A and RS_B [22]. The host range of plasmids can be broadened by cointegrate formation. The cointegrate formed may either be stable (in the case the plasmid is unable to replicate on its own in the new host) or transient [30].
Plasmids having pE194-like origins and showing homology in their plus origins and Rep proteins are found in hosts as distant as *S.aureus* and *Mycoplasma mycoides* [31]. Plasmid pE194 can replicate and maintain itself without selective pressure in Bacilli, although its minus origin does not seem to be functional.
Despite the very homologous RS_A/*pre* complex of plasmid pE194 compared to pUB110, attempts to mobilize plasmid pE194 have failed [8,9]. However by providing Pre protein *in trans* from plasmid pBC16 we have been able to demonstrate mobilization, indicating there is a mutation in the *pre* gene of pE194. Besides the conjugational pathway horizontal transfer of plasmids is also possible by transduction [22,30].
Therefore its seems obvious that plasmid pE194 or derivatives are present throughout the micobial world, and can be regarded as naturally present in Bacillus.

Restriction and functional map of plasmid pUB110



REFERENCES

1. R.W. Lacey & Chopra, J. Med. Microbiol. 7 (1974) 285-297
2. T.J. Gryczan et al., J. Bacteriol. 134 (1978) 318-329
3. D. Semon et al., Plasmid 17 (1987) 46-53
4. T. McKenzie et al., Plasmid 15 (1986) 93-103
5. T. McKenzie et al., Plasmid 17 (1987) 83-85
6. M-F. Gros et al., EMBO J. 6 (1987) 3863-3869
7. L. Boe et al., J. Bacteriol. 171 (1989) 3366-3372
8. T.M. Koehler & C.B. Thorne, J. Bacteriol. 169 (1987) 5271-5278
9. L.B. Selinger et al., J. Bacteriol. 172 (1990) 3290-3297
10. S.D. Ehrlich, Proc. Natl. Acad. Sci. USA 74 (1977) 1680-1682
11. K. Bernard et al., J. Bacteriol. 133 (1978) 897-903
12. J.B. Polak & R.P. Novick, Plasmid 7 (1982) 152-162
13. K. Shishido & Y. Tanaka, Plasmid 12 (1984) 65-66
14. K. Shishido et al., Plasmid 10 (1983) 224-234
15. T. Hoshino et al., Can. J. Microbiol. 31 (1985) 339-345
16. T. Hoshino et al., Can. J. Microbiol. 31 (1985) 614-619
17. T. Imanaka et al., J. Bacteriol. 146 (1981) 1091-1097
18. T. Imanaka et al., J. Gen. Microbiol. 130 (1984) 1399-1408
19. M. Matsumura et al., J. Bacteriol. 160 (1984) 413-420
20. R.E. Muller et al., Mol. Gen. Genet. 202 (1986) 169-171
21. D. van der Lelie et al., Nucl. Acid Res. 17 (1989) 7283-7294
22. R.P. Novick, Annu. Rev. Microbiol. 43 (1989) 537-565
23. A. Gruss et al., Proc. Natl. Acad. Sci. USA 84 (1987)2165-2169
24. D. Clewell, in: Molecular Biology, Pathology and Ecology of Bacterial Plasmids (S.B. Levy et al., eds.), Plenum Publ. Corp., New York, 1981, 191-205
25. O.E. Landman et al., in: Transformation (M. Polsinelli & G. Mazza, eds.), Cotswold Press, Oxford, 1981, 219-228
26. M.F. DeFaun & S.B. Levy, in: Gene Transfer in the Environment (S.B. Levy & R.V. Miller, eds.), McGraw-Hill, New York, 1989, 1-32
27. S.B. Levy & B.M. Marshall, in: The Release of Genetically Engineered Microorganisms (M. Sussman et al., eds.), Academic Press, London, 1988, 61-76
28. J.B. Perkins & P. Youngman, J. Bacteriol. 155 (1983) 607-615
29. S. Horinouchi & B. Weisblum, J. Bacteriol. 150 (1982) 804-814
30. A. Gruss & S.D. Ehrlich, Microbiol. Rev. 53 (1989) 231-241
31. A. Bergman, J. Whitley and L. Finch, J. Bacteriol. 171 (1989) 593-595