Oral Microbiology Immunology 2004: 19: 340–342
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Oral Microbiology and Immunology

Short communication

Clonal distribution of natural competence in *Actinobacillus actinomycetemcomitans*

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Fujise O, Lakio L, Wang Y, Asikainen S, Chen C. Clonal distribution of natural competence in Actinobacillus actinomycetemcomitans.
Oral Microbiol Immunol 2004: 19: 340–342 © Blackwell Munksgaard, 2004.

The competence for natural transformation was investigated in 67 *Actinobacillus actinomycetemcomitans* strains. The transformation assays were performed with both cloned DNA fragments and chromosomal markers of *A. actinomycetemcomitans*. Competence was found in 12 of 18 serotype a strains, 0 of 21 serotype b strains, 0 of 14 serotype c strains, 3 of 6 serotype d strains, 3 of 4 serotype e strains, 0 of 3 serotype f strains, and 0 of 1 nonserotypeable strain. The transformation frequencies varied from 5×10^{-3} to 4×10^{-6} (median 1.5×10^{-4}). The distribution pattern of natural competence is concordant with the major clonal lineages of *A. actinomycetemcomitans*. Serotype a strains are predominantly competent for transformation, while serotypes b and c strains are apparently non-competent.

Key words: clonal analysis; natural competence; natural transformation; periodontal disease

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Accepted for publication May 13, 2004

Natural transformation is a genetically regulated process in which a bacterium takes up extracellular DNA and incorporates it into a genome by homologous recombination (7, 15). Natural transformation has been found in a limited number of species in the domains of *Bacteria* and *Archaea* but not in *Eukarya* (7, 15). There are two well-characterized natural transformation systems, the gram-positive *Streptococcus-Bacillus* system and the gram-negative *Haemophilus-Neisseria* system (7, 15). More recently, a third distinct natural transformation system was identified in *Helicobacter pylori* (25).

Gram-negative facultatively anaerobic *Actinobacillus actinomycetemcomitans* is a causative agent of periodontitis and non-oral infections (2, 17, 24, 28). This bacterium is naturally competent for transformation and shares a similar transformation system with that found in *Haemophilus influenzae* (29, 30). We previously reported that two of 16 *A. actinomycetemcomitans* study strains were naturally competent (29). However, neither the genetic distinctions between nor the clinical origins of competent and non-competent strains were examined.

A complex and highly regulated process, natural transformation is selected for and maintained at a cost to the host bacteria. The purpose of natural transformation is not fully understood (8, 15, 16, 20–23). Comparative studies of competent and non-competent strains of the same species might reveal the functional significance of natural transformation and its impact on the evolution of bacteria. We therefore performed a pilot study to examine the clonal lineages, genotypes, and disease association of competent and non-competent *A. actinomycetemcomitans* strains.

Sixty clinical *A. actinomycetemcomitans* strains originating in Finland (n = 38) and the US (n = 22) and seven laboratory strains (ATCC 29522, 29523, 29524, and 33384, strains JP2, Y4, and HK1651) were included in this study. The clinical isolates were recovered from cultured subgingival plaque samples and identified by conventional means (4) and by 16S rRNA-based polymerase chain reaction (PCR) analysis (1). The periodontal diagnoses of the subjects included aggressive periodontitis (localized and generalized forms), chronic periodontitis, and no periodontitis.

A. actinomycetemcomitans strains were serotyped by the PCR method previously described (11, 27). Arbitrarily primed-PCR (AP-PCR) was used to distinguish genotypes of *A. actinomycetemcomitans* strains as described earlier (3, 6, 18).

The natural competence of *A. actinomycetemcomitans* was determined by an agar-based transformation assay as described by Wang et al. (29). Briefly, *A. actinomycetemcomitans* was cultured on sTSB agar (Trypticase Soy Broth supplemented with 0.1% yeast extract, 5% heat-inactivated horse serum and 1.5% agar) at 37°C in 5% CO_2 overnight, and adjusted with Trypticase Soy Broth to 1×10^9

colony-forming units (CFU)/ml. A 20-μl aliquot of the bacterial suspension was spotted onto a prewarmed sTSB agar plate and spread in a small area (diameter of ~10 mm). After incubation for 2 h, 10 μl of the donor DNA at a concentration of 100 μg/ml was mixed with the *A. actinomycetemcomitans* cells. The mixture was further incubated for 5–6 h, washed off the agar, and plated onto a suitable selective agar to enumerate the transformants.

Both recombinant DNA and chromosomal DNA of *A. actinomycetemcomitans* were used as donor DNA for transformation. The recombinant DNA, designated *pilA′*-Spe^r-*pilC′*, contains a cloned fragment of *pilABC* of *A. actinomycetemcomitans* with the *pilB* gene replaced by a Spe^r cassette (30). The DNA fragment *pilA′*-Spe^r-*pilC′* possesses two copies of the *A. actinomycetemcomitans* uptake signal sequence (USS), which enhances the DNA uptake by competent cells (29). Natural competence was also determined using genomic DNA of a Nal^{r}Rif^r mutant of *A. actinomycetemcomitans* strain D17S (29). Transformation frequency was calculated as the number of transformants/CFU. The lowest detection limit of the transformation frequency was 2×10^{-7}. Competence was defined as a transformation frequency of 10^{-7} or higher.

Table 1 shows the competence of *A. actinomycetemcomitans* strains in relation to their serotype and the periodontal status of the donor subjects. Since the study strains had been selected based on their serotype and mainly from patients with aggressive periodontitis, the distribution frequency of the serotypes is not likely to represent the natural population of the *A. actinomycetemcomitan*s species. The seven laboratory strains were listed separately because these strains may have lost certain phenotypes (e.g. natural competence) in the adaptation to laboratory growth conditions.

Competent *A. actinomycetemcomitans* strains were found in three serotypes: a, d, and e. The proportions of the competent strains were 67% (12/18) for serotype a, 50% (3/6) for serotype d, and 75% (3/4) for serotype e. None of the strains of serotypes b, c, and f (21, 14, and 3 strains, respectively) were competent for transformation. The transformation frequencies of competent *A. actinomycetemcomitans* strains varied from 5×10^{-3} to 4×10^{-6} (median 1.5×10^{-4}). Seventeen of the 18 competent *A. actinomycetemcomitans* strains exhibited a greater than 10^{-5} transformation frequency, which is at least 100 times greater than the detection limit of the assay. The status of competence or non-competence of *A. actinomycetemcomitans* was dichotomous.

No significant association was found between aggressive and chronic periodontitis in the frequency distribution of competent *A. actinomycetemcomitans* strains (Fisher exact probability test, $P > 0.05$) (Table 1). There were too few strains in subjects without periodontitis to determine whether the prevalence of competent strains differed between patients with or without periodontitis. The results of the AP-PCR genotype analysis of 15 competent strains and 8 non-competent strains are shown in Table 2. Among the limited number of strains examined, we did not detect association between competence and genotype of *A. actinomycetemcomitans*.

The population structure of *A. actinomycetemcomitans* is clonal (3, 9, 10, 12, 19). The major clonal lineages of *A. actinomycetemcomitans* (>80% of all strains in the nature) are represented by serotypes a, b, and c (3, 9, 10, 12, 19). In this study, serotype a strains were predominantly competent for transformation, while none of serotypes b and c strains of *A. actinomycetemcomitans* was transformable. We have excluded several of the following simplistic explanations for the observations:

- the donor DNA may not be bound and taken up efficiently by *A. actinomycetemcomitans* serotypes b and c strains;
- the donor DNA may not recombine into the genome of *A. actinomycetemcomitans* serotypes b and c strains;
- the restriction and modification systems of *A. actinomycetemcomitans* serotypes b and c strains may degrade the donor DNA originating from other serotypes.

All donor DNA used for transformation contained USS sites, which should have enhanced the uptake and binding of DNA by competent *A. actinomycetemcomitans* strains irrespective of their serotypes. The donor recombinant DNA *pilA′*-Spe^r-*pilC′* was derived from a highly conserved gene cluster *pilABCD* of *A. actinomycetemcomitans* (30), and should have posed no problem for recombination. The transformation assays in this study were performed with PCR-amplified DNA (data not shown), cloned DNA (from the *Escherichia coli* host) and chromosomal DNA of *A. actinomycetemcomitans*. No significant variations were seen in the transformation frequencies with respect to the sources of the donor DNA (data not shown). Also, it has been reported that natural transformation of *H. influenzae* was not affected by either type I or type II restriction enzymes (26).

It is possible that all *A. actinomycetemcomitans* strains are competent for transformation under some growth conditions, and thus the results are of no great biological relevance. On the other hand, the results may imply significant biological differences between competent (serotype a) and non-competent (serotypes b and c) *A. actinomycetemcomitans* clones. Competent *A. actinomycetemcomitans* serotype a strains maintain a functional transformation system for a purpose that is either not met or met by the use of different strategies in non-competent serotypes b and c strains. Moreover, competence is ostensibly a mechanism for gene acquisition to improve genetic fitness of bacteria (5, 13–15). The ability

Table 1. Competence of *A. actinomycetemcomitans* strains in relation to the serotype characteristics and periodontal status of the donors of the strains

Periodontal status of the donors of the strains or source of strains	No. of competent strains/Total strains in serotype							
	a	b	c	d	e	f	x[a]	Total
Aggressive periodontitis	7/12	0/10	0/8	2/4	1/1	0/3	0	10/38
Chronic periodontitis	4/4	0/2	0/5	0/1	2/3	0	0	6/15
No periodontitis	0/1	0/4	0	1/1	0	0	0/1	1/7
Common laboratory strains	1/1[b]	0/5[c]	0/1[d]	0	0	0	0	1/7
Total	12/18	0/21	0/14	3/6	3/4	0/3	0/1	18/67

[a]Could not be serotyped by PCR analysis.
[b]ATCC29523.
[c]ATCC29522, ATCC29524, HK1651, JP2, Y4.
[d]ATCC33384.

Table 2. The AP-PCR analysis of selected competent and non-competent *A. actinomycetemcomitans* strains

	AP-PCR genotype (no. of isolates)	
Serotype	Competent	Non-competent
a	I (11)	I (4)
d	XXII (1), V (1)	V (3)
e	VI (1)	XI (1)

and the mechanisms for adaptation to drastic environmental changes may differ between competent and non-competent *A. actinomycetemcomitans* strains. The functional significance of natural competence and its impact on the genome of *A. actinomycetemcomitans* remain to be determined.

Acknowledgments

This research was supported by NIDCR grant R01 DE12212, and by Finnish Dental Society and Finnish Cultural Foundation, and Umeå University Research Grants.

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