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Introduction of Host-Controlled Modification and Restriction Systems of *Bacillus subtilis* IAM1247 into *Bacillus subtilis* 168

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Bacillus subtilis IAM1247 had two modification and restriction systems (*Bsu*1247I and *Bsu*1247II), the former producing an isoschizomer of *Pst*I endonuclease. A transformant clone was isolated which had *Bsu* 168, *Bsu*R, and *Bsu*1247I systems coexisting within a genome.

We previously reported the presence of typical type II restriction endonucleases of *Bacillus subtilis* and related species (7-9). In the present paper we describe studies on genetic determinants of these endonucleases. The studies were carried out by transformation of *B. subtilis* 168 with DNA prepared from the various strains.

B. subtilis IAM1247 is one of the strains which have the type II restriction endonucleases (9). Strains 101 (wild type of modification and restriction of strain 168, r_{168}^+ r_R^- m_{168}^+ m_R^-) (5), 1012 (a restriction-deficient [hsrM1] mutant of strain 168, r_{168}^- m_{168}^+) (5), and RM125 (a modification- and restriction-deficient mutant, r_{168}^- m_{168}^-) (12), derived from *B. subtilis* 168, were used as recipients in transformation experiments. Strain ISMR4 (r_{168}^+ r_R^+ m_{168}^+ m_R^+) and ISR11 (r_{168}^- r_R^+ m_{168}^+ m_R^+) are r_R^+ transformants of 101 and 1012, respectively, with DNA of *B. subtilis* R strain (r_R^+ m_R^+) (4). Enzymes of the modification and restriction system of strain 168 (*Bsu* 168 system) and those of R strain (*Bsu*R system) recognize different nucleotide sequences on DNA (4). Phage ϕ105C is a clear plaque mutant of temperate phage ϕ105 (6). Media, assay of phage titers, transformation procedures, and assay of type II restriction endonuclease activities were described previously (4, 6, 7, 9).

DNA of *B. subtilis* IAM1247 transforms the auxotrophic markers *purB6* and *leuA8* of *B. subtilis* 168 with an efficiency comparable to the efficiency observed with DNA of *B. subtilis* 168. From the cells of 101 and 1012 treated with IAM1247 DNA, we isolated transformants which acquired one of the restriction systems (*Bsu*1247I system) of IAM1247. They arose at a frequency about 20-fold less than the transformants of auxotrophic markers.

ISB8 and ISMB12 are r_{1247I}^+ transformants of 1012 and 101, respectively. IAM1247 has another modification and restriction system (the *Bsu*1247II system) which will be described later, and the transformation frequency of r_{1247II}^+ to strain 101 was about 100-fold less than the frequency of r_{1247I}^+.

Phage ϕ105C grown on IAM1247, as well as the phage grown on ISB8, was not restricted by ISB8, which restricts ϕ105C grown on RM125, ISR11, or 101. Phage ϕ105C grown on ISB8 was not restricted by ISMB12 and vice versa (Table 1). These results indicate that both ISB8 and ISMB12 have acquired a restriction activity specific to IAM1247. The results also indicate that the DNA sequences recognized by the *Bsu*1247I system are not modified by the enzyme specific to either the *Bsu* 168 or *Bsu*R system.

Since IAM1247 still restricted ϕ105C grown on either ISB8 or ISMB12 (Table 1), IAM1247 would have another restriction and modification system (the *Bsu*1247II system).

Phage ϕ105C grown on IAM1247 was restricted by either ISR11 or 101 (Table 1), indicating that enzyme of neither the *Bsu*1247I nor the *Bsu*1247II system modifies the DNA sequences recognized by the *Bsu*R or *Bsu* 168 system. ISMB12 was r_{168}^+ r_{1247I}^+ m_{168}^+ m_{1247I}^+ (Table 1), indicating that both the *Bsu* 168 and *Bsu*1247I systems can coexist in the transformant cell.

Then, we tried to construct the transformants which had all of the three modification and restriction systems. ISMRB9 is one of the r_{1247I}^+ transformants of ISMR4 with IAM1247 DNA. As shown on Table 1, ISMRB9 was r_{1247I}^+ r_{168}^+ r_R^+ m_{1247I}^+ m_{168}^+ m_R^+, and this indicates that all *Bsu*1247I, *Bsu* 168, *Bsu*R systems can coexist in a cell of transformant of *B. subtilis* 168.

TABLE 1. *Restriction and modification of ϕ105C r_{1247I}^{+} transformants and IAM1247*

Strain		Relative plating efficiency of ϕ105C grown on[a]:						
		ISB8 (m_B^+ m_C^-) (m_M^+ m_R^-)	ISMB12 (m_B^+ m_C^-) (m_M^+ m_R^-)	IAM1247 (m_B^+ m_C^+) (m_M^- m_R^-)	RM125 (m_M^-)	ISR11 (m_M^+ m_R^+)	101 (m_M^+)	ISMRB9 (m_M^+ m_R^+) (m_B^+ m_C^-)
ISB8	(r_B^+ r_C^-) (r_M^- r_R^-)	0.79	1.3	2.0	2.5×10^{-7}	7.9×10^{-7}	6.3×10^{-7}	1.6
ISMB12	(r_B^+ r_C^-) (r_M^+ r_R^-)	0.79	0.79	6.3×10^{-3}	4.0×10^{-8}	NT	6.3×10^{-7}	NT
IAM1247	(r_B^+ r_C^+) (r_M^-)	5.0×10^{-5}	6.3×10^{-5}	1.6	6.3×10^{-10}	NT	NT	3.2×10^{-6}
ISR11	(r_M^- r_R^+)	1.3×10^{-4}	1.3×10^{-4}	6.3×10^{-6}	6.3×10^{-6}	1.3	NT	1.3
101	(r_M^+)	1.3	0.79	6.3×10^{-3}	1.3×10^{-2}	0.79	0.63	1.3
1012[b]	(r_M^-)	1	1	1	1	1	1	1
ISMRB9	(r_B^+ r_C^-) (r_M^+ r_R^+)	1.0×10^{-4}	NT	4.0×10^{-8}	6.3×10^{-10}	1.3×10^{-7}	7.9×10^{-10}	1.0

[a] Abbreviations: m^-, m^+, modification deficient, proficient; r^-, r^+, restriction deficient, proficient; m_B, r_B, *Bsu* 1247I modification and restriction; m_C, r_C, *Bsu* 1247II modification and restriction; m_R, r_R, *Bsu*R modification and restriction; m_M, r_M, *Bsu* 168 modification and restriction; NT, not tested.

[b] The plating efficiency on 1012 is defined as 1.

Finally, we examined the site-specific endonuclease (type II restriction endonuclease) activities in cell-free extracts of the transformants and of IAM1247 by agarose-gel electrophoresis of the phage DNA treated with the extracts. The site-specific endonuclease of ISB8 gave digestion patterns the same as those given by the major site-specific nuclease activity of IAM1247 (compare lanes 5 and 8 with 4 and 7, respectively, in Fig. 1A). The DNA from ϕ105C grown on ISB8 was not cleaved by the site-specific nuclease in ISB8, indicating that these site-specific endonucleases are equal to the re-

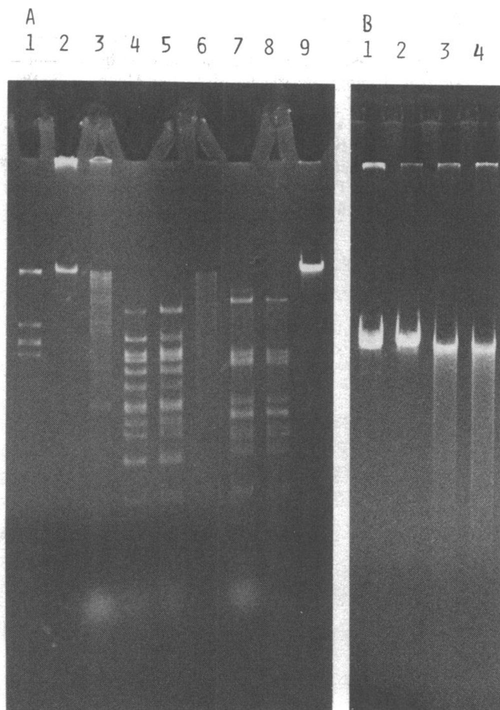

FIG. 1. *Electrophoretic profiles of DNA treated with the cell-free extract from an r_{1247I}^{+} transformant and B. subtilis IAM1247. DNAs (0.3 μg) from ϕ105C and Escherichia coli phage λ were incubated at 37°C for 50 min in reaction mixtures (90 μl) containing 50 mM Tris-hydrochloride buffer (pH 7.5), 10 mM $MgCl_2$, 0.2 mM EDTA, 5 mM 2-mercaptoethanol, and the cell-free extract (ca. 150 μg of protein). After phenol extraction and about threefold concentration by ethanol precipitation, 10 μl of the samples was loaded in the sample wells at the top of the 0.7% agarose gel slab and electrophoresed at 110 V for 45 min at room temperature. (A) Lane 1, EcoRI fragments of λ DNA; lane 2, untreated ϕ105C DNA; lane 3, ϕ105C [grown on ISB8 (m_{1247I}^{+})] DNA treated with the cell-free extract of IAM1247 (r_{1247I}^{+}, r_{1247II}^{+}); lane 4, ϕ105C (grown on 101) DNA treated with the cell-free extract of IAM1247; lane 5, ϕ105C (grown on 101) DNA treated with the cell-free extract of ISB8 (r_{1247I}^{+}); lane 6, ϕ105C [grown on ISB8 (m_{1247I}^{+})] DNA treated with the cell-free extract of ISB8; lane 7, λ DNA treated with the cell-free extract of IAM1247 (r_{1247I}^{+}, r_{1247II}^{+}); lane 8, λ DNA treated with the cell-free extract of ISB8 (r_{1247I}^{+}); lane 9, untreated λ DNA. (B) Lane 1, untreated ϕ105C DNA; lane 2, untreated λ DNA; lane 3, ϕ105C (grown on 101) DNA treated with the cell-free extract of 1012 (r^-); lane 4, λ DNA treated with the cell-free extract of 1012 (r^-).*

striction enzyme of *Bsu*1247I system. The DNA from ϕ105C grown on ISB8 treated with the cell-free extract of ISB8 exhibits a smear band in Fig. 1A (lane 6). This may be due to exonuclease activity in this cell-free extract. The cell-free extract of the parental strain (1012) of ISB8 also may contain exonuclease activity to give a smear band of treated DNA in gel electrophoretic profile (lanes 3 and 4 in Fig. 1B). DNA from ϕ105C grown on ISB8 was cleaved into fragments having discrete molecular weights by treatment with cell-free extracts of IAM1247 (lane 3 in Fig. 1A). This result shows IAM1247 has the second site-specific endonuclease, the activity of which is not significant under the conditions where the major nuclease of IAM1247 is active (compare lane 4 with 5 in Fig. 1A). The nucleotide sequence recognized by endonuclease R·*Bsu*1247I is $\begin{matrix}5'\text{-CTGCAG-3}'\\3'\text{-GACGTC-5-}\end{matrix}$ which is recognized by endonuclease R·*Pst*I of *Providencia stuartii* 164 (3, 10) (R. J. Roberts, personal communication).

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