Gram-positive bacteria with a high DNA G+C content are characterized by a common insertion within their 23S rRNA genes

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An insertion of about 100 bases within the central part of the 23S rRNA genes was found to be a phylogenetic marker for the bacterial line of descent of Gram-positive bacteria with a high DNA G + C content. The insertion was present in 23S rRNA genes of 64 strains representing the major phylogenetic groups of Gram-positive bacteria with a high DNA G + C content, whereas it was not found in 23S rRNA genes of 55 (eu)bacteria representing Gram-positive bacteria with a low DNA G + C content and all other known (eu)bacterial phyla. The presence of the insertion could be easily demonstrated by comparative gel electrophoretic analysis of *in vitro*-amplified 23S rDNA fragments, which contained the insertion. The nucleotide sequences of the amplified fragments were determined and sequence similarities of at least 44% were found. The overall similarity values are lower than those of 16S and 23S rRNA sequences of the particular organism. Northern hybridization experiments indicated the presence of the insertion within the mature 23S rRNA of *Corynebacterium glutamicum*.

Introduction

The conserved character of rRNAs is manifested in a common core structure of the homologous molecules. At the same time there is enough variation of primary and higher order structure at well known positions or regions to allow comprehensive phylogenetic investigations. In many cases signature nucleotides or sequences could be defined which are specific for phylogenetic groups of organisms (Larsen *et al.*, 1985; Woese, 1987). Besides the primary structure signatures, the presence or absence, length and shape of higher order structure elements (helices) often correlate with phylogenetic groups. A remarkable degree of length variation can be seen, especially in 23S rRNAs or rRNA genes, which results from the presence or absence of often large structure elements or sequence stretches (Larsen *et al.*, 1985; Höpfl *et al.*, 1989). The presently available data on 23S rRNA gene sequences indicate that there are group-specific insertions or deletions. The terms insertion and deletion are used to denote additional or missing bases in comparison to the 23S rRNA gene sequence of *Escherichia coli*. However, many of the large insertions are highly variable in occurrence and structure. Burgin *et al.* (1990) demonstrated this variability for extra sequences in 23S rRNA genes of two *Salmonella* species which the

authors called intervening sequences. These intervening sequences were found at two different positions. They can form helical structures and expand helices 25 and 45 (Höpfl *et al.*, 1989) of premature 23S rRNA. Even within a particular strain only part of the multiple rRNA operons contain one or both of the intervening sequences. The sequence similarity between the extra elements of helix 45 of two different *Salmonella* species is only 77% while the overall similarity of the 23S rRNA genes is 97%. Recently Skurnik & Toivanen (1991) reported intervening sequences corresponding to those of salmonella (helix 45) in the 23S rRNA genes of *Yersinia enterocolitica* strains. The sequence similarities between the intervening sequences of highly virulent *Yersinia* strains and those of *Salmonella* strains are higher (about 90%) than the similarities between the corresponding sequences of highly and poorly virulent *Yersinia enterocolitica* strains (about 60%). Additional sequence stretches are inserted at two homologous positions of 23S rRNA genes of the closely related species *Rhodobacter capsulatus* (Höpfl *et al.*, 1988, 1989) and *Rhodobacter sphaeroides* (Dryden & Kaplan, 1990). In both cases the lengths of the intervening sequences of the two species are remarkably different. It has been shown that the large intervening sequences in 23S rRNA genes of *Salmonella* (Burgin *et al.*, 1990), *Yersinia* (Shurnik & Toivanen, 1991) and *Rhodobacter* (Dryden & Kaplan, 1990) are removed during maturation of the rRNAs. After

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Table 1. *Organisms used in this study*

Organism	Strain*	Abbrevi- ation	DNA G + C content (%)	23S-rRNA insertion	
				Length (bp)	G + C content (%)
<i>Actinomyces suis</i>	DSM 20639 ^{Ta}	Asu39	55.0	116	48.8
<i>Actinoplanes digitatis</i>	DSM 43149 ^T	Adi49	73.0	95	58.0
<i>Arthrobacter citreus</i>	DSM 20133 ^{Tb}	Aci33	62.9	107	60.7
<i>Arthrobacter citreus</i>	ATCC 21348 ^b	Aci48	ND	108	50.5
<i>Arthrobacter globiformis</i>	DSM 20124 ^{Tb}	Ag124	62.0	107	58.9
<i>Arthrobacter pascens</i>	ATCC 13346 ^b	Ag146	ND	107	60.7
<i>Arthrobacter ramosus</i>	CCM 1646 ^T	Ara46	62.2	106	63.1
<i>Aureobacterium testaceum</i>	DSM 20166 ^T	Ate66	65.4	113	59.3
<i>Aureobacterium testaceum</i>	DSM 20526	Ate26	ND	113	59.3
<i>Bifidobacterium adolescentis</i>	DSM 20083 ^{Tb}	Bad83	58.9	102	61.7
<i>Bifidobacterium bifidum</i>	DSM 20456 ^{Tb}	Bbi56	58.0	100	70.5
<i>Bifidobacterium infantis</i>	DSM 20088 ^{Tb}	Bin88	60.8	104	64.2
<i>Brevibacterium linens</i>	DSM 20425 ^T	Bli25	63.4	102	57.0
<i>Brevibacterium</i> sp.	DSM 20165	Bke65	65.9	99	60.6
<i>Cellulomonas biazotea</i>	DSM 20112 ^T	Cbi12	72.2	107	64.3
<i>Cellulomonas cellulans</i>	DSM 20106	Cce06	76.0	107	67.0
<i>Cellulomonas fimi</i>	DSM 20113 ^T	Cfi13	71.7	107	67.8
<i>Cellulomonas flavigena</i>	DSM 20109 ^T	Cfi09	72.7	107	61.6
<i>Cellulomonas gelida</i>	DSM 20110	Cge10	73.8	110	65.2
<i>Cellulomonas turbata</i>	DSM 20577 ^T	Ctu77	70.5	106	62.2
<i>Cellulomonas uda</i>	DSM 20107 ^T	Cud07	72.0	107	67.0
<i>Clavibacter michiganense</i>	DSM 20134 ^b	Cmi34	70.7	107	52.7
<i>Corynebacterium callunae</i>	DSM 20147 ^{Tb}	Cca47	51.2	104	47.7
<i>Corynebacterium glutamicum</i>	AS 019 ^c	Cgl19	53.2	104	48.6
<i>Corynebacterium glutamicum</i>	DSM 1412 ^c	Cgl14	54.3	104	47.7
<i>Corynebacterium glutamicum</i>	DSM 20137 ^c	Cgl37	52.9	104	48.6
<i>Corynebacterium glutamicum</i>	DSM 20163 ^c	Cgl63	56.0	104	48.6
<i>Corynebacterium glutamicum</i>	DSM 20297 ^c	Cgl97	54.4	104	47.7
<i>Corynebacterium glutamicum</i>	DSM 20300 ^{Tc}	Cgl00	53.4	104	46.8
<i>Corynebacterium glutamicum</i>	DSM 20411 ^c	Cgl11	54.0	104	46.8
<i>Corynebacterium glutamicum</i>	DSM 20412 ^c	Cgl12	53.7	104	46.8
<i>Curtobacterium flaccumfaciens</i>	DSM 20141 ^b	Cfl41	73.7	111	58.6
<i>Frankia</i> sp.	Ag45/mut15 ^d	Fsp45	ND	95	59.0
<i>Frankia</i> sp.	AgB1.9 ^d	Fsp19	ND	86	54.9
<i>Frankia</i> sp.	Ar1.3 ^d	Fsp13	ND	94	55.6
<i>Gardnerella vaginalis</i>	DSM 4944 ^T	Gva44	42–44	102	43.9
<i>Geodermatophilus obscurus</i>	ATCC 20578 ^T	Gob78	72.9	106	64.9
<i>Micrococcus luteus</i>	CCM 169 ^T	Mlu69	72.8	107	63.4

processing the rRNAs remain fragmented with in the ribosome. Fragmented 23S rRNAs seem to be widespread among the Proteobacteria (Pace, 1973), but have also been reported for representatives of other bacterial phyla (Hsu *et al.*, 1990). While for eucarya and archaea (Woese *et al.*, 1990) true introns in rRNA genes have been described (Garret *et al.*, 1991), none of the extra sequences in rRNA genes of (eu)bacteria known so far are introns.

Besides several smaller insertions, an additional large insertion was found in the 23S rRNA genes of some representatives of the phylogenetic group of Gram-positive bacteria with a high DNA G+C content, namely *Frankia* sp. (GenBank accession no. M55343), *Micrococcus luteus* (Regensburger *et al.*, 1988; Höpfl *et al.*, 1989), eight *Streptomyces* species (Pernodet *et al.*, 1989; Stackebrandt *et al.*, 1991; Kim *et al.*, 1991) and 14 *Mycobacterium* species (Liesack *et al.*, 1991). This

insertion comprises up to 107 additional bases and is located between helices 54 and 55 of the 23S rRNA. Complete 23S rRNA sequences have been published for *Frankia* sp. (GenBank accession no. M55343), *Micrococcus luteus* (Regensburger *et al.*, 1988), *Streptomyces ambofaciens* (Pernodet *et al.*, 1989) and *Streptomyces griseus* (Kim *et al.*, 1991). The similarities between the insertion sequences of the four species are 54%–79% in comparison with overall 23S rRNA gene similarities of 81.8%–95.1%. This again indicates, that the insertion belongs to the variable regions of the 23S rRNA gene. However, since insertions of comparable lengths were found at homologous positions of the 23S rRNA genes of only moderately related *M. luteus*, streptomycetes and mycobacteria, we decided to investigate the presence and structure of this insertion in 23S rRNA genes of various representatives of the Gram-positive bacteria with a high DNA G+C content.

Table 1 (continued)

Organism	Strain*	Abbreviation	DNA G + C content (%)	23S-rRNA insertion	
				Length (bp)	G + C content (%)
<i>Micrococcus luteus</i>	ATCC 10240	Mlu40	65.0	107	63.4
<i>Micrococcus lylae</i>	DSM 20315 ^{Tb}	Mly15	67.1	109	57.9
<i>Micrococcus nishinomiyaensis</i>	DSM 20448 ^{Tb}	Mni48	67.8	103	56.6
<i>Micrococcus roseus</i>	DSM 20447 ^{Tb}	Mro47	69.0	109	65.8
<i>Micrococcus sedentarius</i>	DSM 20317	Mse17	67.2	103	58.3
<i>Micrococcus</i> sp.	MAW 2 ^e	Msp02	ND	109	59.6
<i>Micrococcus varians</i>	DSM 20033 ^T	Mva33	69.0	111	59.5
<i>Mycobacterium gordonae</i>	-f	Mgo31	ND	105	59.1
<i>Nocardia</i> sp.	ATCC 4275 ^d	Nsp75	ND	101	57.5
<i>Nocardia vacinii</i>	ATCC 11092 ^{Td}	Nva92	ND	100	57.1
<i>Nocardiopsis dassonvillei</i>	DSM 43111 ^{Tb}	Nda11	69.0	101	52.8
<i>Nocardioides simplex</i>	DSM 20130 ^T	Nsi30	71.7	106	60.4
<i>Propionibacterium freudenreichii</i>	DSM 20271 ^T	Pfr71	64.0	99	57.7
<i>Renibacterium salmoninarum</i>	DSM 20767 ^{Tu}	Rsa67	53.0	108	49.6
<i>Rhodococcus chlorophenolicus</i>	DSM 43826 ^{Td}	Rch26	ND	105	61.8
<i>Rhodococcus erythropolis</i>	DSM 43066 ^T	Rer66	67-71	100	54.3
<i>Rhodococcus fascians</i>	DSM 20669 ^{Tb}	Rfa69	ND	107	53.6
<i>Streptomyces albireticuli</i>	ATCC 19721 ^{Tb}	Sal21	ND	101	62.3
<i>Streptomyces albus</i>	ATCC 3004 ^{Td}	Sal04	ND	102	58.9
<i>Streptomyces goshikiensis</i>	ATCC 23914 ^{Td}	Sgo14	ND	102	55.1
<i>Streptomyces griseoruber</i>	ATCC 23919 ^{Td}	Sgr19	ND	103	57.4
<i>Streptomyces purpureus</i>	DSM 43360 ^b	Spu60	ND	102	57.0
<i>Streptomyces</i> sp.	ATCC 13962 ^d	Ssp62	ND	103	55.6
<i>Streptomyces</i> sp.	ATCC 19828 ^d	Ssp28	ND	103	57.4
<i>Streptomyces</i> sp.	DSM 40225 ^b	Ssp25	ND	100	60.0
<i>Streptosporangium roseum</i>	DSM 43021 ^b	Sro21	71.0	100	57.0

ND, Not determined.

* Source of cells or DNAs: a, N. Weiss (DSM, Braunschweig, Germany); b, A. Regensburger and c, M. Ehrmann (Technical University, München, Germany); d, D. Hahn (University Wageningen, The Netherlands); e, W. E. Kloos (State University, Raleigh, North Carolina, USA); f, E. Böttger (Medical University, Hannover, Germany); g, F. Fiedler (University München, Germany). ATCC, American Type Culture Collection (Rockville, MD, USA); CCM, Czechoslovak Collection of Micro-organisms (Brno, Czechoslovakia); DSM (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany). Superscript T indicates the type strain.

Methods

Organisms and culture conditions. The organisms investigated in the present study are listed in Table 1. Cellulomonads, *Nocardioides simplex* DSM 20130 and *Rhodococcus erythropolis* DSM 43066^T were cultured as described in the Catalogue of Strains of the DSM (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany). Cells of *Renibacterium salmoninarum* ATCC 33209 were kindly provided by F. Fiedler (University München, Germany), cells of *Eubacterium suis* DSM 20639 by N. Weiss (DSM, Braunschweig, Germany) and cells of *Frankia* strains by D. Hahn (Wageningen, The Netherlands).

Purification of DNA. DNAs were purified according to Marmur (1961). In addition, DNAs were kindly provided by E. Böttger (Hannover, Germany), M. Ehrmann (Technical University München, Germany), D. Hahn (Wageningen, The Netherlands), and A. Regensburger (Technical University München, Germany).

In vitro amplification of rRNA genes. Either purified genomic DNA (0.1–1 µg) or whole cells (one colony) were used for *in vitro* amplification of rRNA genes (rDNA) applying the polymerase chain

reaction technique (Saiki *et al.*, 1988) in combination with a 23S rRNA gene-specific primer pair (23InsV, 5'-MADGCGTAGNCGAWGG-3' and 23InsR, 5'-GTGWC GGTTT NBGGTA-3'). The amplification was carried out using the Taq Polymerase kit of Boehringer and a PolyChain Thermocycler (Polygen). The cycling parameters were as follows: 1 min denaturation at 95 °C, 2 min annealing at 52 °C, and 3 min extension at 72 °C. After 33 cycles, a final incubation followed for 5 min at 72 °C. The lengths of the amplified fragments were checked by agarose gel electrophoresis (3%, w/v, agarose) in comparison with the corresponding rDNA fragments of *E. coli* and *Rhodobacter capsulatus*, and a length standard (123 bp ladder, Gibco BRL).

DNA and RNA sequencing. The amplified rDNA fragments were separated by agarose gel electrophoresis and purified using the USBioClean MP kit (USB). The DNA fragments were either sequenced directly or after cloning (Chen & Seeburg, 1985) in the vector pBluescript (Stratagene) using the Sequenase Version 2.0 Sequencing Kit (USB). rRNA was purified and sequenced using reverse transcriptase as described by Embley *et al.* (1988).

Hybridization. Southern and Northern hybridizations were carried out as described by Sambrook *et al.* (1989).

Fig. 1. Potential secondary structure model of Domain III of the 23S rRNA of *C. glutamicum* AS019. The insertion is shown as a secondary structure element which is located between helices 54 and 55. The target sites of the amplification primers (23InsV, 23InsR) and of the insertion-specific hybridization probe (Cgl1875) are marked by solid lines.

Results and Discussion

The 23S rRNA genes of 64 representatives of the major phylogenetic groups within the line of descent of Gram-positive bacteria with a high DNA G+C content were investigated with respect to presence, length and structure of an insertion in domain III (Höpfel *et al.*, 1989), which had been detected in the corresponding genes of *M. luteus* (Regensburger *et al.*, 1988; Höpfel *et al.*, 1989) and eight *Streptomyces* species (Pernodet *et al.*, 1989; Stackebrandt *et al.*, 1991). Fig. 1 shows a potential secondary structure model of domain III of the 23S rRNA of *Corynebacterium glutamicum*. The insertion is located between helices 54 and 55 (Höpfel *et al.*, 1989). The part of domain III comprising helices 54–59 is a rather variable region within the 23S rRNA (Höpfel *et al.*, 1989) which is flanked by short conserved sequence stretches. These conserved regions of the 23S rRNA gene served as target sites for a primer pair used for the *in vitro* amplification of rDNA coding for this portion of the 23S rRNA. The target sites are marked in Fig. 1. The resulting rDNA fragments (about 375 bp) can easily be distinguished from homologous fragments lacking the approximately 100 bp insertion by agarose gel electrophoresis. Fig. 2 is a photograph of electrophoretically

separated rDNA amplicates of various Gram-positive bacteria with a high DNA G+C content. Besides the length standards, a mixture of homologous *in vitro*-amplified rDNA fragments from *E. coli* and *R. capsulatus* were included for comparison. The insertion is missing in the 23S rRNA genes of the latter organisms (Brosius *et al.*, 1981; Höpfel *et al.*, 1988, 1989). In comparison with the *E. coli* 23S rRNA sequence a further 103 bases are deleted in the 23S rRNA of *R. capsulatus* from helices 55, 56, 58 and 59 (Höpfel *et al.*, 1988, 1989). Therefore, the amplicate of the rDNA of *R. capsulatus* is even shorter than that of *E. coli*. The amplified rDNA fragments of all Gram-positive bacteria with a high DNA G+C content comprised about 375 bp indicating the presence of about 100 extra base pairs. The insertion was detected in the 23S rRNA genes of all 64 Gram-positive bacteria with a high DNA G+C content investigated (Table 1), and was missing in 55 complete 23S rRNA sequences representing Gram-positive bacteria with a low DNA G+C content and all (eu)bacterial phyla (Table 2). In addition, the presence of the sequence in all of five putative 23S rRNA genes of *C. glutamicum* was demonstrated by Southern hybridization of genomic DNA restriction fragments to a site-specific oligonucleotide Cgl1875 (5'-TATTGGGTGGTTAGTATC-3') which binds within the insertion sequence. The target site is marked in Fig. 1.

In order to see whether these additional bases are inserted at a particular homologous position in the 23S rRNA genes of the different organisms, the nucleotide sequences of all rDNA fragments were determined. The nucleotide sequences have been deposited at the Genbank sequence data base under accession numbers M85086 – M85141. In all 23S rRNA genes investigated the additional bases are inserted at a homologous position. The position within domain III of 23S rRNA is shown in the potential secondary structure model of that domain of *C. glutamicum* (Fig. 1). An alignment of a selection of sequences is shown in Fig. 3. The corresponding sequences of the following pairs or groups of strains are identical: *Streptomyces griseoruber* ATCC 23919^T and *Streptomyces* sp. ATCC 19828; *M. luteus* ATCC 10240 and CCM 169^T; *C. glutamicum* DSM 20411 and DSM 20412; *C. glutamicum* DSM 1412 and DSM 20297; *C. glutamicum* AS019, DSM 20137, DSM 20163 and DSM 20300^T; *Aureobacterium testaceum* DSM 20166^T and DSM 20526. The average length of the insertion is 104 bp and varies between 86 (*Frankia* sp. AgB1.9) and 116 bp (*Actinomyces suis*). The similarities of the inserted sequences are 44% (*Actinomyces/Bifidobacterium*) and higher. Table 3 lists similarity values of the insertion sequences from representatives of the major phylogenetic groups of Gram-positive bacteria with a high DNA G+C content. Mean values are given for

Fig. 2. Gel electrophoretic separation of *in vitro*-amplified rDNA fragments. The abbreviations are defined in Table 1. Std, standard size markers; Ec/Rc, mixture of amplified rDNA fragments of *E. coli* and *R. capsulatus*.

those groups of which more than one member have been investigated. The similarity values between the corresponding rRNA regions of the phylogenetic groups are in the range of 44–81% and intra-genus similarities are 61% and higher. Lowest overall sequence similarity values of complete 23S or 16S rRNAs from members of the major line of descent of Gram-positive bacteria with a high DNA G+C content are 70%. The inserted sequence together with the flanking regions of domain III comprises the largest coherent variable part of the 23S rRNA molecule. Thus, the type of insertion investigated here is a variable element too, but in comparison with the enterobacterial insertions (Burgin *et al.*, 1990; Skurnik & Toivanen, 1991) the sequences are rather conserved.

Invariant and conserved residues were found within the primary structures of the insertions and are shown as a consensus sequence in Fig. 3. Some group-specific primary structure elements (signatures) can be detected: AAC (position 8–10 in Fig. 3) is unique for bifidobacteria, the combination of TA (108, 109) and TAACA (124–128) is characteristic for cellulomonads and the combination TT (87, 88) and T (119) occurs only in corynebacteria.

Potential secondary structures were derived from the insertion sequences (Fig. 4). A common pattern of helical and non-helical regions may be seen. The lengths of these regions are conserved within phylogenetic groups and vary between the groups. The overall similarity of these potential structure elements is evident. However, *in vivo* presence and/or stability has not been proven experimentally.

The conserved primary structure elements and the similarity of the secondary structure models indicate that these insertions in the 23S rRNA genes of Gram-positive bacteria with a high DNA G+C content may be derived from a single ancestral event.

Since all investigations were done at the gene level, it has to be clarified whether these secondary structure elements are stable and retained in the mature 23S rRNA, or are removed by processing, as was demonstrated for the enterobacterial intervening sequences. The presence of the insertion within cellular 23S rRNA was shown by reverse transcriptase sequencing of the particular region of the molecules from *M. luteus* (Höpfel *et al.*, 1989) and *C. glutamicum* (this study). A Northern hybridization was performed with the insertion-specific probe Cgl1875 and the amplification primer 23InsR

Table 2. *Organisms which do not contain the 23S rRNA gene insertion investigated in this study*

Proteobacteria, alpha subclass	Gram-positive bacteria with a low DNA G + C content
<i>Bradyrhizobium japonicum</i> *	<i>Bacillus globisporus</i> *
<i>Pseudomonas diminuta</i> *	<i>Bacillus licheniformis</i> *
<i>Rhizobium lupini</i> *	<i>Bacillus stearothermophilus</i> †
<i>Rhodobacter capsulatus</i> †	<i>Bacillus subtilis</i> †
<i>Rhodobacter sphaeroides</i> †	<i>Heliobacterium chlorum</i> ‡
<i>Rhodopseudomonas palustris</i> *	<i>Lactobacillus delbrueckii</i> *
Proteobacteria, beta subclass	<i>Lactococcus lactis</i> *
<i>Pseudomonas cepacia</i> †	<i>Listeria monocytogenes</i> *
<i>Thiobacillus cuprinus</i> *	<i>Mycoplasma hyopneumoniae</i> *
Proteobacteria, gamma subclass	<i>Mycoplasma pneumoniae</i> *
<i>Acinetobacter calcoaceticus</i> *	<i>Pectinatus frisingensis</i> *
<i>Escherichia coli</i> †	<i>Peptococcus niger</i> *
<i>Klebsiella pneumoniae</i> *	<i>Staphylococcus aureus</i> *
<i>Leuconitrix mucor</i> *	<i>Staphylococcus carnosus</i> *
<i>Pseudomonas aeruginosa</i> †	<i>Streptococcus oralis</i> *
<i>Ruminobacter amylophilus</i> †	<i>Streptococcus thermophilus</i> *
<i>Salmonella arizonae</i> §	Cyanobacteria
<i>Salmonella typhimurium</i> §	<i>Anacystis nidulans</i> †
Proteobacteria, delta subclass	<i>Spirulina platensis</i> *
<i>Nannocystis exedens</i> *	<i>Propionigenium</i>
<i>Stigmatella aurantiaca</i> *	<i>Propionigenium modestum</i> *
Proteobacteria	Green non-sulphur bacteria
<i>Wolinella succinogenes</i> *	<i>Chloroflexus aurantiacus</i> *
Cytophaga/Flavobacterium/Bacteroides	<i>Herpetosiphon giganteus</i> *
<i>Bacteroides fragilis</i> *	Thermotogales
<i>Flexithrix dorotheae</i> *	<i>Thermotoga maritima</i> †
<i>Flavobacterium odoratum</i> †	<i>Fervidobacterium islandicum</i> *
<i>Flexibacter flexibilis</i> †	Spirochaetes
<i>Taxebacter ocellatus</i> *	<i>Leptospira interrogans</i> †
Deinococci	<i>Spirochaeta aurantia</i> *
<i>"Deinonema" sp.</i> *	Green sulphur bacteria
<i>Thermus thermophilus</i> †	<i>Chlorobium vibrioforme</i> *
Planctomycetes	<i>Chlorobium limicola</i> †
<i>Pirellula marina</i> †	Chlamydiae
	<i>Chlamydia trachomatis</i> §

* W. Ludwig and others (unpublished results).

† Genbank accession nos: J01695, K02663, K00637, M10606, M62805–M62807, M31904, M32365, X00007, X00343, X00512, X06458, X12612, X07408, X14249, X16368, X53853–X53855.

‡ C. R. Woese (Urbana, USA) personal communication.

§ D. Lane (Boston, USA) personal communication.

which was used as a common probe for (eu)bacteria (Fig. 5). rRNAs of *C. glutamicum* and *R. capsulatus* were analysed in comparison. The amplified 23S rDNA fragments of *C. glutamicum*, *E. coli* and *R. capsulatus* were included for length comparison and as standards for the intensities of the hybridization signals. No major amounts of precursor nor of fragmented rRNA were detected by gel electrophoresis in the case of *C. glutamicum*. In contrast, the 23S rRNA of *R. capsulatus* was fragmented (Fig. 5a). The (eu)bacterial probe hybridized to the non-fragmented *C. glutamicum* 23S

rRNA, one of the *R. capsulatus* 23S rRNA fragments, and to the amplified rDNAs (Fig. 5b). The insertion-specific probe hybridized only to the non-fragmented 23S rRNA and the amplified rDNA fragments of *C. glutamicum* (Fig. 5c). The intensity of the hybridization signals of the insertion-specific probe/RNA hybrids is not diminished in comparison with that of the (eu)bacterial probe/RNA hybrids. These results indicate that the insertion is present in non-fragmented mature 23S rRNA of *C. glutamicum*, and that a splicing event is highly unlikely. However, the presence in mature 23S

Fig. 4. Potential secondary structure models of the insertion sequences in domain III of representatives of the major phylogenetic groups of Gram-positive bacteria with a high DNA G+C content.

Fig. 5. Comparative gel electrophoretic and hybridization analyses of *C. glutamicum* and *R. capsulatus* 23S rRNA. (a) Gel electrophoretic separation of cellular RNAs and amplified insertion rDNA. (b) Hybridization to the (eu)bacterial probe 23InsR. (c) Hybridization to the insertion-specific probe Cg11875. Lanes: 1, RNA of *C. glutamicum*; 2, RNA of *R. capsulatus*; 3, amplified rDNA of *C. glutamicum*; 4, amplified rDNA of *R. capsulatus*; 5, amplified rDNA of *E. coli*.

rRNA of other Gram-positive bacteria with a high DNA G+C content as well as its potential position and function within ribosomes remain to be analysed.

The type of insertion found in the 23S rRNA genes of Gram-positive bacteria with a high DNA G+C content seems to be substantially different from that found in all or part of the rRNA genes of a few strains of *Salmonella* and *Yersinia* (Burgin *et al.*, 1990; Skurnik & Toivanen, 1991). The intervening sequences described by these authors are unstable elements which are lost during the processing of precursor rRNA resulting in fragmented 23S rRNA molecules. These elements may be exchanged between the different genes within the individual and may be transferred to other strains or species (Burgin *et al.*, 1990). It is highly likely that they were acquired very recently during the course of evolution. In contrast, the ubiquitous distribution among the various lines of descent of the Gram-positive bacteria with a high DNA G+C content, together with the obvious structural similarities indicate that the insertion described here is a characteristic marker of this major phylogenetic group. In consequence the element may have been introduced in early evolutionary times. Since a comparable insertion could not be detected in any 23S rRNA genes of 16 representatives of Gram-positive bacteria with a low DNA G+C content, nor in those from 39 members of other bacterial phyla, it is unlikely that it is a remnant that was deleted from the other genes.

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