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Subspecies-specific distribution of intervening sequences in the *Bacillus subtilis* prophage ribonucleotide reductase genes

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Abstract

A collection of 212 gram-positive bacilli isolated from natural habitats was screened for the presence of intervening sequences (introns and intein-coding sequences) in the SPβ prophage-related ribonucleotide reductase genes *bnrdE* and *bnrdF*. Three novel configurations were identified on the basis of the presence of (i) intervening sequences in *bnrdE* and *bnrdF*, and (ii) an ORF in the *bnrdE-bnrdF* spacer. Analysis of the cell wall genetic determinants as well as of the incorporation of radio-labelled glycerol into cell wall allowed newly and previously identified *B. subtilis* strains with different configurations of *bnrdE/bnrdF* intervening sequences to be assigned to one of two subspecies. Strains apparently belonging to the subsp. *subtilis* contain three intervening sequences many of which are associated with the putative homing endonuclease activity. Strains of the subsp. *spizizenii* contain only one or two ORF-less group I introns. Introns occupying *bnrdF* are confined to the subspecies *subtilis*.

Keywords: Group I introns; Intron distribution; *Bacillus subtilis* subsp. *subtilis*; *Bacillus subtilis* subsp. *spizizenii*; Prophage; Ribonucleotide reductase

Introduction

Intervening sequences (IVSs) spliced from the precursor RNA (group I and group II introns) or from the precursor protein (inteins) occur only sporadically in bacteria and their phages. In bacterial genomes, group I introns generally interrupt tRNA genes [5]. In contrast, phage group I introns are inserted in protein coding genes, most of which are involved in DNA metabolism [5] and some in the host cell lysis [6,21].

Several group I introns encode a site-specific endonuclease, the so-called homing endonuclease (HE), that initiate horizontal transfer of an intron into allelic intron-minus sites, a process known as homing [11]. Similarly, the central endonuclease domain of inteins may confer mobility to intein DNA.

Despite the progress in understanding the mechanism of intron and intein splicing, the possible role of these

Abbreviations: HE, Homing endonuclease; IVS, Intervening sequence; Poly(GroP), Poly(glycerol phosphate); RR, Ribonucleotide reductase; WTA, Cell-wall teichoic acid

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IVSs remains an enigma. Although IVSs are generally regarded as selfish DNA, the prevalence of inteins and phage introns in genes involved in DNA metabolism suggested that splicing may play a role in a global regulatory mechanism involving DNA replication [4,5]. Therefore, it has been speculated that splicing elements might endow their host with a selective advantage over IVS-minus counterparts [4].

The ribonucleotide reductase (RR) genes of archaea, bacteria and phages are particularly susceptible to IVS acquisition [12,14,18]. RRs, ubiquitous in cellular organisms, but also encoded by many phages, supply deoxyribonucleotides through the reduction of the four ribonucleotides.

In *B. subtilis* SPβ-related prophages, the RR genes *bnrdE* and *bnrdF*, encoding the large and the small RR subunits, respectively, are highly variable in terms of the presence of IVSs [14]. In this contribution we show that, in addition to various configurations of splicing elements, the *bnrdE/bnrdF* tandem may contain different ORFs in the *bnrdE-bnrdF* spacer. The presence of HE-encoding IVSs appears to be subspecies-specific.

Material and methods

Bacterial isolation

Two hundred and twelve sporogenic aerobic bacteria were isolated from environmental sources including soil, manure, straw and hay. Samples were placed into a glass tube containing 10 ml sterile bidistilled water and vortexed. After 30-min incubation at room temperature, 800 µl of suspension was removed and heated for 10 min at 80 °C. One hundred µl of heat-treated samples were plated onto LB agar (Difco) and incubated at 37 °C for 24 h. For each sample, several isolates with morphologically distinct colonies were purified by two rounds of restreaking onto LB agar plates.

Identification of isolated strains S6, S72 and S114 was performed using the identification kit API 50 CHB (bioMérieux) according to the manufacturer's recommendations.

Oligonucleotides

The oligonucleotide primer pairs used in this study are listed in Table 1.

DNA isolation and PCR screening

To screen isolates for the presence of *bnrdE* and *bnrdF* [17] homologues, a colony from plates incubated for 24 h at 37 °C was suspended in 100 µl of sterile water and heated for 5 min at 95 °C. Suspensions were centrifuged at 15000*g* for 3 min. The top phase was removed to a new tube and used as template DNA. The PCRs included 20 pmol of each primer (Table 2a) and 23 µl of template DNA in a total volume of 50 µl of HotStarTaq Master Mix (Qiagen). Amplification consisted of an initial denaturation at 94 °C for 15 min, followed by 30 cycles of 94 °C for 20 s, 40 °C for 1 min,

Table 1. Oligonucleotide primer pairs used for PCR and RT-PCR

Oligonucleotide 1	Oligonucleotide 2
VL262 AGCAACATCTTTCTAACATTGGCTC	VL263 CAGTAAGTTTAAAGCCCATGCGTAC
VL286 CCTTAATGCTGGACGTAAGCGAAGA	VL287 GAATGCGGATAGAACATGTAATAAT
VL288 ATTATTACATGTTCTATCCGCATTC	VL289 GTTGTCAGTGAGTCAATTGCTATTC
VL308 ACGAAACAAGATCTACAGAAAATCA	VL311 ATTAAAGAGGTTTTCGATCACCATT
VL308 ACGAAACAAGATCTACAGAAAATCA	VL312 GTCTGGATGGAATACACTTAGATAA
VL314 CGAACACGTCTGAATGGTCTGCAAA	VL318 CGTTTAGACGGGTTTGCTACGATCA
VL315 TAGATAATGCCTTCAGATATGCCGA	VL318 CGTTTAGACGGGTTTGCTACGATCA
VL320 CGCCTTTCGTTGAATATTAAAGTCA	VL321 AGGACAAGCATCCCTCTTGGGTCGT
VL320 CGCCTTTCGTTGAATATTAAAGTCA	VL348 CTAGCCTTCTTTTGAAGAAACCTATTG
VL322 TTGACAAAAATTTATGACGCAGCAA	VL416 CAGCAATTAGAGAGGTTCACATTT
VL347 AATAAAAAAATTCAGTATACAGCAGCA	VL329 CATTGAATACATCCTTTCTCGGTAA
VL379 TGAATCGTCAATAGACGGCTA	VL380 CTTGTATTGAACTTCAACCAT
VL413 AATCGGATTTGAAGCGATGCA	VL414 TGGCGGTACTTATGGATAAAG
VL417 AGAGTAGCGAACTCGGTTGAACTA	VL329 CATTGAATACATCCTTTCTCGGTAA
VL421 GATATTTCACGTGCTATTGATATC	VL422 GTGTTTTAACTCGGACATCTTCAT
VL423 CAATTAGCCATTCTAAGAAGCGAA	VL425 TTTACAATGTTCATGGATCCAAGA
VL424 CAATTGGCCATTCTAAGAAGTGAA	VL425 TTTACAATGTTCATGGATCCAAGA
VL424 CAATTGGCCATTCTAAGAAGTGAA	VL426 TTTACAATGTTCATTGAGCCAAGA
VL427 GTTTCTTCAAAAGAAGGCTAGAAC	VL428 AAATCTCTTGAGCTAATAAACCGA
VL435 CTTGAAGCGTCTTTCTGCTGA	VL436 AACTGAATATCCGTTCCTTCA
VL588 ATGCCWTCTGTTCTTGGRAGGTAAA	VL589 CCGCTRAAYACATCAAAGCTTCC