Brazilian Journal of Microbiology (2005) 36:177-183
ISSN 1517-8382

PARTIAL CHARACTERIZATION OF RIBOSOMAL OPERONS OF *LACTOBACILLUS DELBRUECKII* UFV H2B20

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Submitted: April 05, 2005; Approved: June 28, 2005

ABSTRACT

Ribosomal operons are great tools for microbe community characterization and for microorganisms relationship study, particularly in the case of the acid lactic bacteria. The ribosomal operon of the probiotic strain *Lactobacillus delbrueckii* UFV H2b20 was partially characterized. A genomic library of this strain was constructed and the clones with partial ribosomal operon were sub-cloned using the shot-gun method for subsequent sequencing with the forward primer. The sequence analysis revealed that the 3' end of the rDNA 16S was following by the short spacer region 1 (16S-23S) and that the 3' end of the rDNA 23S was following by the short spacer region 2 (23S-5S), which preceded the rDNA 5S. In the flanking region of the rDNA 5S gene of this operon *rrn*, a region encoding six tRNAs was detected.

Key words: ribosomal operons, *Lactobacillus*, tRNA, acid lactic bacteria

INTRODUCTION

It has been established that phenotypic characteristics alone are not enough to allow a proper classification among the lactic acid bacteria. Thus, various reviews described the divergence of the results obtained using traditional phenotypic tests and the molecular taxonomy (10,11,15,25). The phylogenetic structure of this group is now well defined and is based on phenotypic division – important instrument of group identification and classification – as well as based on the data obtained from rRNA sequencing and DNA-DNA hybridization.

Ribosomal operons have acquired paramount relevance for the study of bacterial evolution and phylogeny. The 16S rRNA and 23S genes are the most widely used molecular chronometers for inferring microbial phylogeny and have been instrumental in developing a comprehensive view of the microbial systematic (9). The phylogenetic classification of prokaryotes with rDNA sequences is based on the assumption that the differences in sequences reflect the evolution of the organisms that they have been extracted from. For this reason, in the past few years, study based on the analysis of ribosomal sequences or genes encoding rRNA, were developed and used for to discriminate species of *Lactobacillus* or to identify different probiotic bacteria (18,24).

The sequences of multicopy rRNA genes are identical or nearly identical. The sequence of the different rrn operons existing in a given genome could vary up to 5% (4,16,23). The analysis of sequences found in the databank rrndb (Ribosomal RNA Operon Copy Number Database, http://rrndb.cme.msu.edu) revealed a variation of 1.23% between the operon sequences of *E. coli* and those of 14 others species (14).

The organization of the genes of the ribosomal operon is similar among the eubacteria. Basically, *rrn* operon contains the genes encoding the 16S, 23S and 5S ribosomal RNAs, which are organized as follows: 16S - spacer region 1 - 23S -spacer region 2 - 5S. The spacer regions are called "short" (S) when they do not encode any tRNAs, and called "long" (L) when a tRNA sequence is present (22). The presence of a tRNA encoding sequence within the spacer region 2 is not the nule in bacteria

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(5). Among the prokaryotes, the size, organization and number of *rrn* operons depends on the species. The highest copy number of *rrn* operons per genome (15 copy) was found in spore forming bacteria, like *Clostridium paradoxum*, while the lowest (one copy) was found in *Mycoplasma pneumonie* and *Rickettsia prowazekii* (13). In acid lactic bacteria, the number of operons varies from two in *Lactobacillus brevis* (14) to six in *Lactococcus lactis* (12,24) and *Lactobacillus delbrueckii* (17).

However, there is still limited knowledge of the organization of *rrn* operons in *Lactobacillus delbrueckii*. Because of its potential in the food industry as well as its clinical importance, the probiotic strain *Lactobacillus delbrueckii* UFV H2b20 has been studied in our laboratory. Preliminary analyses showed the existence of at least three copies of the *rrn* operon and polymorphism among the genes encoding the 16S rRNA. Intraspecific differences among industrial probiotic strains need to be established in order to monitor colonization in human and animal tests as well as their use in industrial products. We report here the partial characterization of a *rrn* operon of *L. delbrueckii* UFV H2b20 strain.

MATERIALS AND METHODS

Construction and screening of the genomic library of *Lactobacillus delbrueckii*

The genomic library of *Lactobacillus delbrueckii* UFV H2b20 was constructed in lambdaGEM®-11 phage (Promega - *Bam*H I Arms, Cat. nº B1901) according to the manufacturer's instructions. The 16S rDNA of the strain was previously cloned in pGEM®-T Easy (Promega), labelled using the Primer-It Fluor Fluorescence Labeling Kit as described by the manufacturer (Statagene), and used as probe to screen the genomic library. The genomic library was transferred to Nylon membranes which were pre-hybridized for 2h at 65ºC in pre-hybridization buffer (SSC 5X, SDS 0.5%, Denhardt solution 5X, salmon sperm DNA 100 mg/mL) and hybridized 4h at 65ºC in the pre-hybridization buffer containing the rDNA 16S probe (15 ng/mL). Kodak X-OMAT K films were exposed to the membranes for 30 min at room temperature, and developed according to manufacturer's instructions. Insert of the positive clones were subject to phage DNA extraction and DNA was digested by *Sac*I or *Xho*I according to the manufacturer's instructions (Promega), then analysed by Southern blot in the hybridisation conditions described above, using the 16S DNA probe. According to the restriction pattern and the Southern blot, five clones were selected and subcloned as 1.5 kb fragments in pBluescript by a shotgun method (Brazilian National Genome Project Consortium, 2003). Recombinant plasmids were used to transform the strain *E coli* (21).

Sequencing and sequence analysis

After transformation, about 200 colonies for each clone previously selected were pick-up and used for a subsequent sequencing. Plasmidic DNA was extracted and the inserts were sequenced with the primer forward M13 using the MegaBace 1000 DNA Analysis System (Molecular Dynamics & Life Science). Sequences were analysed using the Phred/Phrap software (www.phrap.org) and compared with the sequences available in the GenBank database (BLAST programs - www.ncbi.nlm.nih.gov). Contigs were defined by sequence overlapping as used for genome organization analysis (3).

RESULTS AND DISCUSSION

Based on the screening of a genomic library of *L. delbrueckii* using the rDNA 16S gene, 800 *rrn* operon sequences were obtained. These sequences were grouped in 50 different contigs with variable length (from 300 to 2000 pb). BLAST analysis of the contigs revealed a high homology with *L. delbrueckii* sequences already published (accession numbers: X15245, X68426, Z75476, AB035484, AF113602 and AB007908). We report here the analysis of two of these contigs, named C6 and C48. The contig C6 comprised 16S rDNA region, 16S-23S rDNA spacer, and the contig C48 comprised 23S rDNA, 23S-5S rDNA spacer, 5S rDNA region and tRNAs (Figs. 1A and B).

The sequence analysis of *L. delbrueckii* UFV H2b20 rDNA showed that the *rrn* operon organization in this strain was similar to the one observed in the most eubacteria: the 3'end of the 16S rDNA preceded the spacer region 1; the 3'end of the rDNA 23S preceded the spacer region 2 that was followed by rDNA 5S. The Fig. 2 shows the organization of the *rrn* region of *L. delbrueckii* UFV H2b20.

It has been demonstrated that *Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus helveticus* and *Lactobacillus curvatus* contained two main different types of *rrn* operon, named S and L. The *rrnS* operon was organized according to the following scheme: (5') 16S – spacer region 1S – 23S – spacer region 2 – 5S (3'), while the *rrnL* operon was described as the following: (5') 16S – spacer region 1L with ($tRNA^{Ile}$ $tRNA^{Ala}$) – 23S – spacer region 2 – 5S (3') (18). In our study (Fig.2), the spacer region 1 of *L. delbrueckii* UFV H2b20 was partially sequenced and was about 160 bp in length. It displayed 98.7% homology with the long spacer region 16S-23S of *L. delbrueckii* subsp. *bulgaricus* (accession number AB035484) but didn't show homology with the $tRNA^{Ala}$ and $tRNA^{Ile}$ encoding sequences observed in *L. delbrueckii* subsp. *bulgaricus* operons. Because the analysed sequence was partial – 160 bp compared to the total length of the spacer region 1 of the *Lactobacillus* group which comprised between 197 and 220 bp – we can not conclude if the spacer region 1 of *L. delbrueckii* UFV H2b20 contains or not any tRNA sequences. This was supported by the fact that, while most of the spacer regions 1 in the *Lactobacillus* group didn't contain tRNA sequences (8,17,18), they sometimes contain $tRNA^{Ala}$ or $tRNA^{Ile}$ or both. Sequence heterogeneity was found between the different rDNA

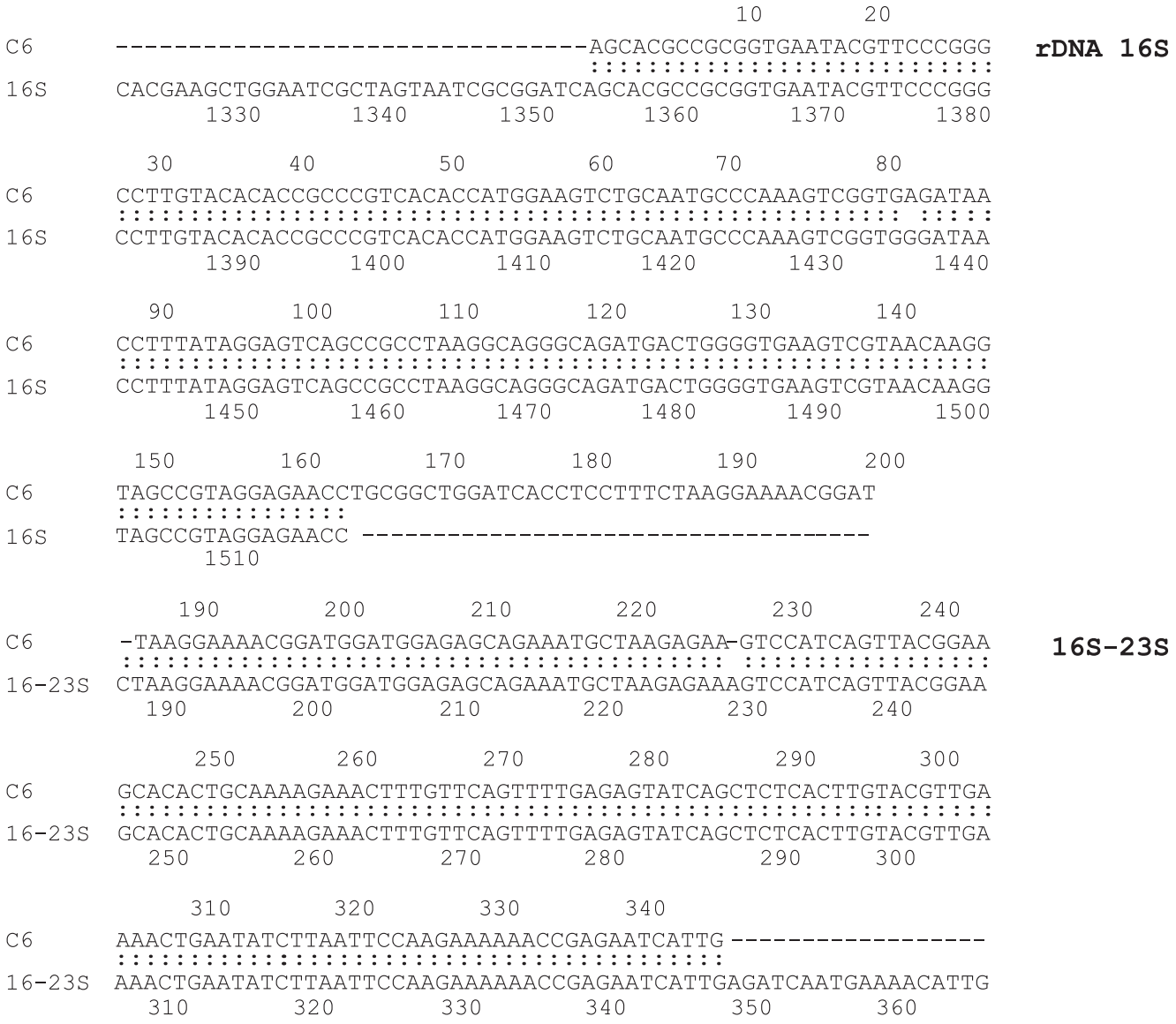
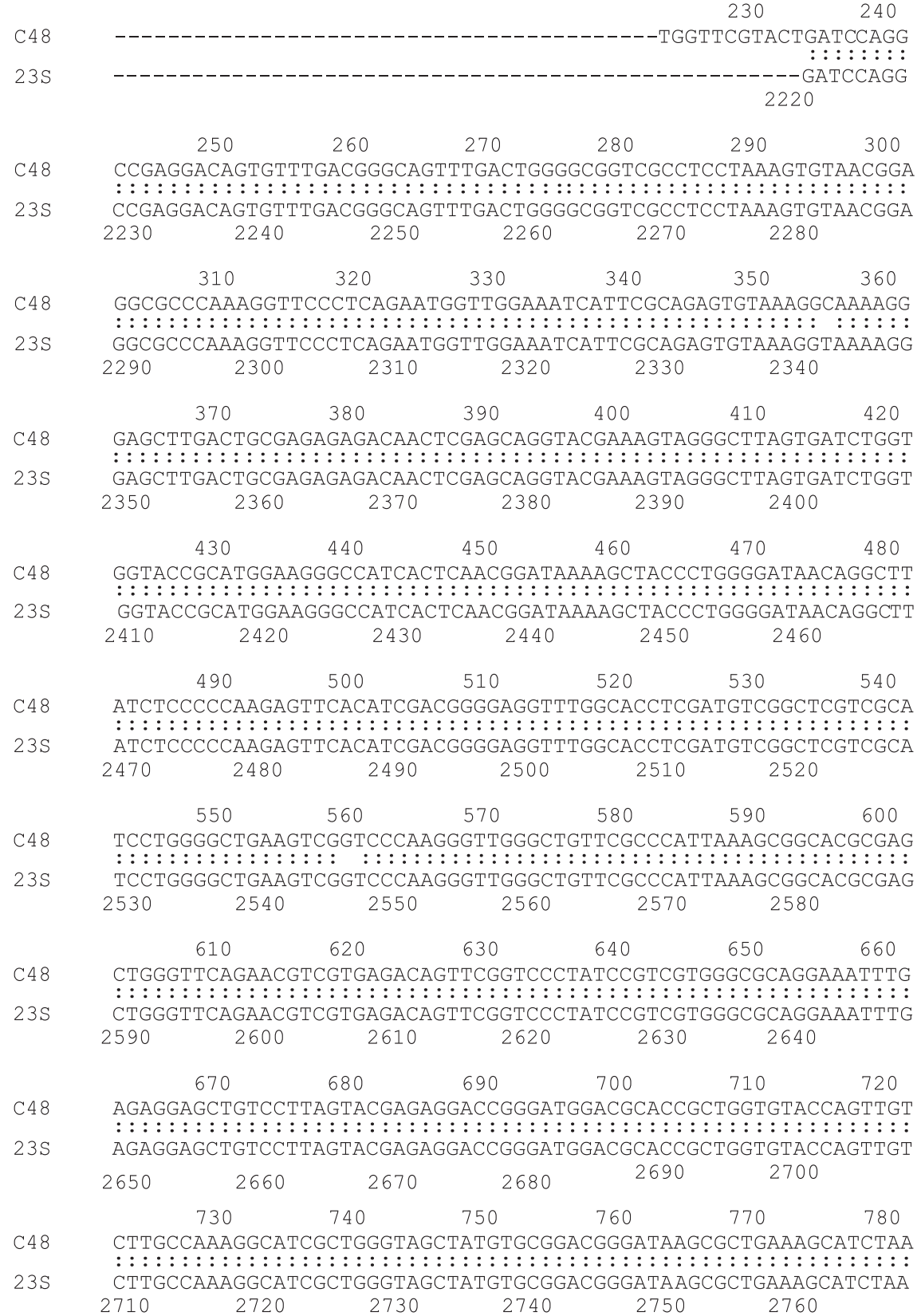


Figure 1A. Contig C6 - Sequence of the *rrn* region of *L. delbrueckii* UFV H2b20 which presents homology with the 3'end of the rDNA 16S of *L. delbrueckii* (AB007908) and with the spacer region 16S-23S of *L. delbrueckii* subsp. *bulgaricus* (AB035484).

encoded in the same genome (14) and the spacer regions were those which exhibited the highest degree of variation (2). The spacer region 23S-5S of *L. delbrueckii* UFV H2b20 was 71 bp in length and showed 95.7% homology with the corresponding region of *L. delbrueckii* (accession number X15245). The length of this region was in accordance with those of *L. acidophilus* (accession number Z75474; 69pb), *L. bulgaricus* (accession number Z75477; 71 pb) and *L. helveticus* (accession number Z75493; 71 pb). The obtained 5S rDNA of *L. delbrueckii* UFV H2b20 was 126 bp in length and displayed 98.6% of homology with the corresponding sequence of *L. delbrueckii* (accession number X15245). This length corresponds to the one observed for the *L. lactis* subsp. *cremoris* 117pb (1).

Sequences homologous to encoding elements associated to the *rrn* operons were also detected. We observed, at the 3' end of the rDNA 5S, 6 tRNA sequences which, in the following order, were homologous to the $tRNA^{Asn}$, $tRNA^{Pro}$, $tRNA^{Gly}$, $tRNA^{Arg}$, $tRNA^{Val}$ and $tRNA^{Asp}$ of *L. delbrueckii* (accession number X15245) (Fig.1B). Little information has been available about tRNA genes associated with *rrn* operon in


rDNA 23S
C48
23S
230
240
TGGTTCGTACTGATCCAGG
GATCCAGG
2220
250
260
270
280
290
300
CCGAGGACAGTGTTTGACGGGCAGTTTGACTGGGGCGGTCGCCTCCTAAAGTGTAACGGA
2230
2240
2250
2260
2270
2280
310
320
330
340
350
360
GGCGCCCAAAGGTTCCCTCAGAATGGTTGGAAATCATTCGCAGAGTGTAAAGGCAAAAGG
GGCGCCCAAAGGTTCCCTCAGAATGGTTGGAAATCATTCGCAGAGTGTAAAGGTAAAAGG
2290
2300
2310
2320
2330
2340
370
380
390
400
410
420
GAGCTTGACTGCGAGAGAGACAACTCGAGCAGGTACGAAAGTAGGGCTTAGTGATCTGGT
2350
2360
2370
2380
2390
2400
430
440
450
460
470
480
GGTACCGCATGGAAGGGCCATCACTCAACGGATAAAAGCTACCCTGGGGATAACAGGCTT
2410
2420
2430
2440
2450
2460
490
500
510
520
530
540
ATCTCCCCCAAGAGTTCACATCGACGGGGAGGTTTGGCACCTCGATGTCGGCTCGTCGCA
2470
2480
2490
2500
2510
2520
550
560
570
580
590
600
TCCTGGGGCTGAAGTCGGTCCCAAGGGTTGGGCTGTTCGCCCATTAAAGCGGCACGCGAG
2530
2540
2550
2560
2570
2580
610
620
630
640
650
660
CTGGGTTCAGAACGTCGTGAGACAGTTCGGTCCCTATCCGTCGTGGGCGCAGGAAATTTG
2590
2600
2610
2620
2630
2640
670
680
690
700
710
720
AGAGGAGCTGTCCTTAGTACGAGAGGACCGGGATGGACGCACCGCTGGTGTACCAGTTGT
2650
2660
2670
2680
2690
2700
730
740
750
760
770
780
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2720
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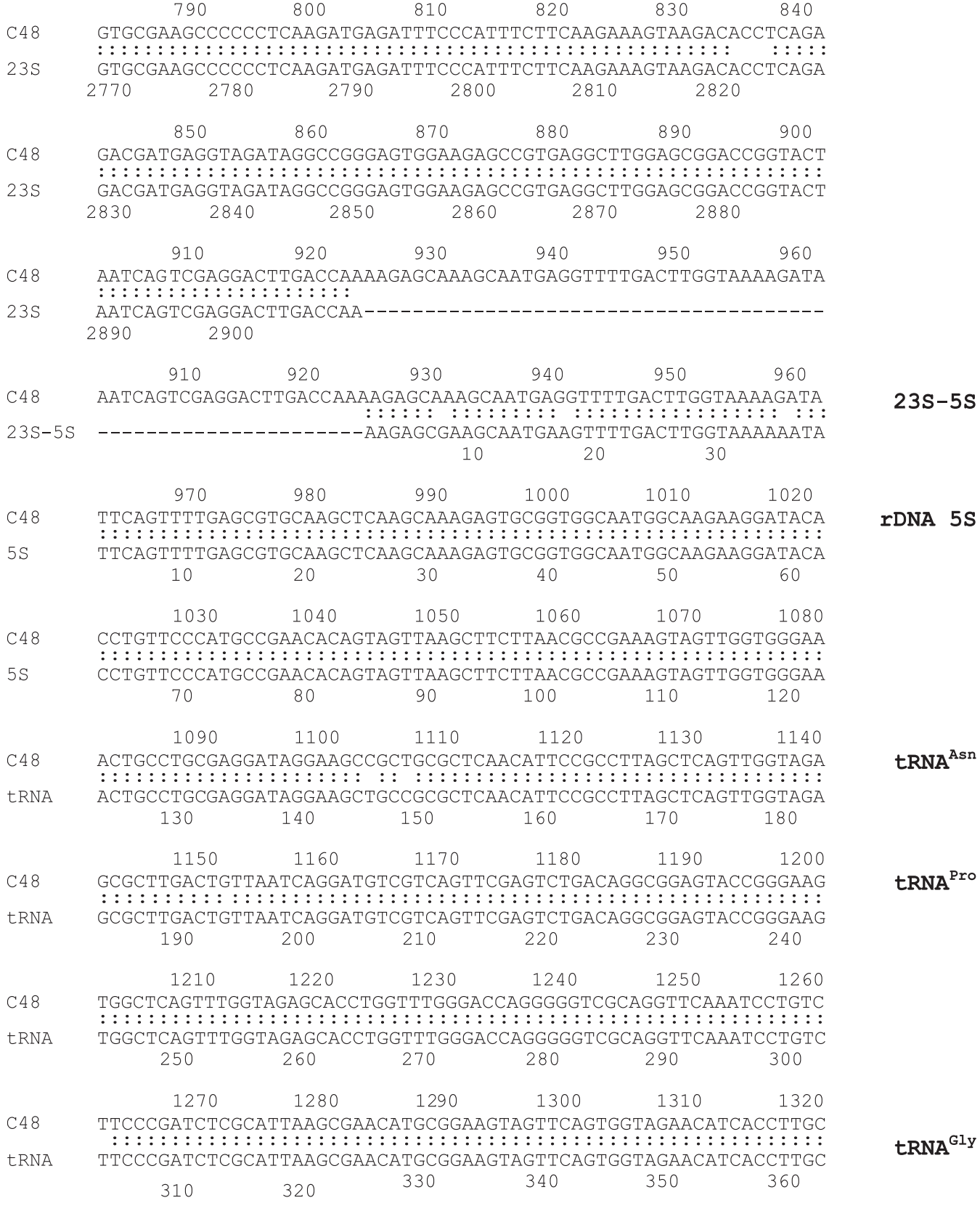
2750
2760


790 800 810 820 830 840
C48 GTGCGAAGCCCCCCTCAAGATGAGATTTCCCATTTCTTCAAGAAAGTAAGACACCTCAGA
23S GTGCGAAGCCCCCCTCAAGATGAGATTTCCCATTTCTTCAAGAAAGTAAGACACCTCAGA
2770 2780 2790 2800 2810 2820
850 860 870 880 890 900
C48 GACGATGAGGTAGATAGGCCGGGAGTGGAAGAGCCGTGAGGCTTGGAGCGGACCGGTACT
23S GACGATGAGGTAGATAGGCCGGGAGTGGAAGAGCCGTGAGGCTTGGAGCGGACCGGTACT
2830 2840 2850 2860 2870 2880
910 920 930 940 950 960
C48 AATCAGTCGAGGACTTGACCAAAAGAGCAAAGCAATGAGGTTTTGACTTGGTAAAAGATA
23S AATCAGTCGAGGACTTGACCAA----------
2890 2900
910 920 930 940 950 960
C48 AATCAGTCGAGGACTTGACCAAAAGAGCAAAGCAATGAGGTTTTGACTTGGTAAAAGATA
23S-5S ----------AAGAGCGAAGCAATGAAGTTTTGACTTGGTAAAAAATA
10 20 30
23S-5S
970 980 990 1000 1010 1020
C48 TTCAGTTTTGAGCGTGCAAGCTCAAGCAAAGAGTGCGGTGGCAATGGCAAGAAGGATACA
5S TTCAGTTTTGAGCGTGCAAGCTCAAGCAAAGAGTGCGGTGGCAATGGCAAGAAGGATACA
10 20 30 40 50 60
rDNA 5S
1030 1040 1050 1060 1070 1080
C48 CCTGTTCCCATGCCGAACACAGTAGTTAAGCTTCTTAACGCCGAAAGTAGTTGGTGGGAA
5S CCTGTTCCCATGCCGAACACAGTAGTTAAGCTTCTTAACGCCGAAAGTAGTTGGTGGGAA
70 80 90 100 110 120
1090 1100 1110 1120 1130 1140
C48 ACTGCCTGCGAGGATAGGAAGCCGCTGCGCTCAACATTCCGCCTTAGCTCAGTTGGTAGA
tRNA ACTGCCTGCGAGGATAGGAAGCTGCCGCGCTCAACATTCCGCCTTAGCTCAGTTGGTAGA
130 140 150 160 170 180
tRNA^{Asn}
1150 1160 1170 1180 1190 1200
C48 GCGCTTGACTGTTAATCAGGATGTCGTCAGTTCGAGTCTGACAGGCGGAGTACCGGGAAG
tRNA GCGCTTGACTGTTAATCAGGATGTCGTCAGTTCGAGTCTGACAGGCGGAGTACCGGGAAG
190 200 210 220 230 240
tRNA^{Pro}
1210 1220 1230 1240 1250 1260
C48 TGGCTCAGTTTGGTAGAGCACCTGGTTTGGGACCAGGGGGTCGCAGGTTCAAATCCTGTC
tRNA TGGCTCAGTTTGGTAGAGCACCTGGTTTGGGACCAGGGGGTCGCAGGTTCAAATCCTGTC
250 260 270 280 290 300
1270 1280 1290 1300 1310 1320
C48 TTCCCGATCTCGCATTAAGCGAACATGCGGAAGTAGTTCAGTGGTAGAACATCACCTTGC
tRNA TTCCCGATCTCGCATTAAGCGAACATGCGGAAGTAGTTCAGTGGTAGAACATCACCTTGC
310 320 330 340 350 360
tRNA^{Gly}

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                1330       1340       1350       1360       1370       1380
C48       CATGGTGGGGGTCGCGGGTTCGAATCCCGTCTTCCGCTTAACGCAGCAGCGTTAGCAAAC
          ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
tRNA      CATGGTGGGGGTCGCGGGTTCGAATCCCGTCTTCCGCTTAACGCAGCAGCGTTAGCAAAC
               370        380        390        400        410        420

                1390       1400       1410       1420       1430       1440
C48       AGAATATTCCAGCGCACCCATAGCGCAACTGGATAGAGTGTCTGACTACGAATCAGAAGG      tRNA^Arg
          :::::::: ::::::::::::::::::::::::::::::::::::::::::::::::::::
tRNA      AGAATATTTCAGCGCACCCATAGCGCAACTGGATAGAGTGTCTGACTACGAATCAGAAGG
               430        440        450        460        470        480

                1450       1460       1470       1480       1490       1500
C48       TTGTAGGTTCAAGTCCTACTGGGTGCATCGGAGGATTAGCTCAGCTGGGAGAGCATCTGC
          ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::      tRNA^Vac
tRNA      TTGTAGGTTCAAGTCCTACTGGGTGCATCGGAGGATTAGCTCAGCTGGGAGAGCATCTGC
               490        500        510        520        530        540

                1510       1520       1530       1540       1550       1560
C48       CTTACAAGCAGAGGGTCACAGGTTCGAGCCCTGTATCCTCCATATGGTCCATTGGAGCAG
          ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
tRNA      CTTACAAGCAGAGGGTCACAGGTTCGAGCCCTGTATCCTCCATATGGTCCATTGGAGCAG
                                                  580    590        600
               550        560        570
                                                                      tRNA^Asp
                1570       1580       1590
C48       TGGTCTATCTCGCCTCCCTGTCACGGAGGA----------------------------
          ::::::::::::::::::::::::::::::
tRNA      TGGTCTATCTCGCCTCCCTGTCACGGAGGAGATCGCGGGTTCAAATCCCGCATGGACCGT
                                                  640        650        660
               610        620        630
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Figure 1B. Contig C48 - Sequence of the *rrn* region of *L. delbrueckii* UFV H2b20 which presents homology with the 3'end of the rDNA 23S of *L. delbrueckii* (X68426) and with the spacer region 23S-5S, rDNA 5S and tRNAs of *L. delbrueckii* (X15245).

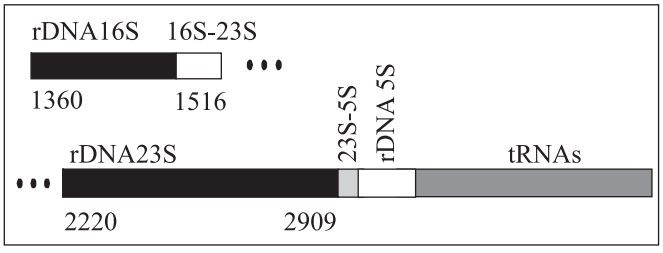

Figure 2. Organization of the genes in *L. delbrueckii* UFV H2b20. The tRNAs are $tRNA^{Asn}$, $tRNA^{Pro}$, $tRNA^{Gly}$, $tRNA^{Arg}$, $tRNA^{Val}$ and $tRNA^{Asp}$, in this order. The numbers indicate the gene positions of *L. delbrueckii* accession numbers: AB007908 and X68426.

Lactobacillus. However, in other species such as *Bacillus subtilis*, the 10 *rrn* operons were associated with groups of tRNAs: $tRNA^{Val}$, $tRNA^{Thr}$, $tRNA^{Lys}$, $tRNA^{Leu}$, $tRNA^{Gly}$, $tRNA^{Arg}$, $tRNA^{Pro}$, and $tRNA^{Ala}$ were found located between the *rrn*J and *rrn*W operons. In the 3' end of the *rrnE* operon, were found two tRNA genes, one for the methionine, the other for the aspartic acid (7, 20). In *Staphylococcus aureus*, the 3' ends of the main ribosomal operons were associated with tRNA encoding sequences (7). A tRNA for proline was also found beyond the rDNA 23S in the 3' end of an operon of *Streptococcus mutans* (19).

The special probiotic properties of *L. delbrueckii* UFV H2b20 have motivated the molecular characterization of this strain. Unique features of its *rrn* operons can be exploited to identify it among others of the same species, since *rrn* sequences are important tools for inter and intra-specific identification.

ACKNOWLEDGMENTS

Dr. J.T. Magalhães is supported by reseach grants from the FAPEMIG and the CNPq (Conselho Nacional de Desenvolvimento Científico). We are grateful to Dr. F. Micheli (CIRAD, Montpellier, France) for helpful discussion and advice on the manuscript. We also thank the team of the "Laboratório de Genética e Biologia Molecular" of the "Universidade Estadual de Santa Cruz" (UESC, Bahia, Brazil), and G.A.G. Pereira (UNICAMP, São Paulo, Brazil) for kindly providing assistance in the sequence analysis.

RESUMO

Caracterização parcial de operons ribossomais de *Lactobacillus delbrueckii* UFV H2b20

Operons ribossomais têm sido instrumentos importantes na caracterização de comunidades microbianas e no estudo de relacionamentos entre microrganismos, principalmente em bactérias do ácido láctico. Operons ribossomais da linhagem probiótica, *Lactobacillus delbrueckii* UFV H2b20, foram parcialmente caracterizados. Um banco genômico da linhagem foi construído e os clones, contendo parte do operon ribossomal, foram subclonados pelo método de "shot gun", para em seguida serem seqüenciados com primer "forward". As seqüências indicaram a presença da extremidade 3' do rDNA 16S seguida da região espaçadora curta 1 (16S-23S) e a presença da extremidade 3' do rDNA 23S seguido da região espaçadora 2 (23S-5S), que por sua vez precedia o rDNA 5S. Adjacente ao gene rDNA 5S deste operon *rrn* uma região codificadora de 6 tRNAs foi detectada.

Palavras-chave: operons ribossomais, *Lactobacillus*, tRNA, bactérias do ácido lático

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