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Systematic and Applied Microbiology 29 (2006) 358–367
SYSTEMATIC AND APPLIED MICROBIOLOGY
www.elsevier.de/syapm

Comparative and functional analysis of the rRNA-operons and their tRNA gene complement in different lactic acid bacteria

Maaike C. de Vries[a,b,*], Roland J. Siezen[a,c,d], Janneke G.E. Wijman[a,b], Yuan Zhao[a,b], Michiel Kleerebezem[a,d], Willem M. de Vos[a,b], Elaine E. Vaughan[a,b]

[a]*Wageningen Centre for Food Sciences, Wageningen, The Netherlands*
[b]*Laboratory of Microbiology, Wageningen University, Hesselink van Suchtelenweg 4, 6703 CT Wageningen, The Netherlands*
[c]*Center for Molecular and Biomolecular Informatics, University of Nijmegen, Nijmegen, The Netherlands*
[d]*NIZO Food Research, Ede, The Netherlands*

Received 15 June 2005

Abstract

The complete genome sequences of the lactic acid bacteria (LAB), *Lactobacillus plantarum*, *Lactococcus lactis*, and *Lactobacillus johnsonii* were used to compare location, sequence, organisation, and regulation of the ribosomal RNA (*rrn*) operons. All *rrn* operons of the examined LAB diverge from the origin of replication, which is compatible with their efficient expression. All operons show a common organisation of 5′-16S-23S-5S-3′ structure, but differ in the number, location and specificity of the tRNA genes. In the 16S-23S intergenic spacer region, two of the five *rrn* operons of *Lb. plantarum* and three of the six of *Lb. johnsonii* contain tRNA-ala and tRNA-ile genes, while *L. lactis* has a tRNA-ala gene in all six operons. The number of tRNA genes following the 5S rRNA gene ranges up to 14, 16, and 21 for *L. lactis*, *Lb. johnsonii* and *Lb. plantarum*, respectively. The tRNA gene complements are similar to each other and to those of other bacteria. Micro-heterogeneity was found within the rRNA structural genes and spacer regions of each strain. In the *rrn* operon promoter regions of *Lb. plantarum* and *L. lactis* marked differences were found, while the promoter regions of *Lb. johnsonii* showed a similar tandem promoter structure in all operons. The *rrn* promoters of *L. lactis* show either a single or a tandem promoter structure. All promoters of *Lb. plantarum* contain two or three −10 and −35 regions, of which either zero to two were followed by an UP-element. The *Lb. plantarum rrnA*, *rrnB*, and *rrnC* promoter regions display similarity to the *rrn* promoter structure of *Escherichia coli*. Differences in regulation between the five *Lb. plantarum* promoters were studied using a low copy promoter-probe plasmid. Taking copy number and growth rate into account, a differential expression over time was shown. Although all five *Lb. plantarum rrn* promoters are significantly different, this study shows that their activity was very similar under the circumstances tested. An active promoter was also identified within the *Lb. plantarum rrnC* operon preceding a cluster of 17 tRNA genes.

Keywords: Ribosomal DNA (*rrn*) operon; *Lactobacillus plantarum*; *Lactococcus lactis*; *Lactobacillus johnsonii*; tRNA; Promoter regulation

*Corresponding author. Laboratory of Microbiology, Wageningen University, Hesselink van Suchtelenweg 4, 6703 CT Wageningen, The Netherlands.
E-mail address: Maaike.deVries@wur.nl (M.C. de Vries).

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doi:10.1016/j.syapm.2005.11.010

Introduction

The use of rRNA and their encoding genes as target molecules has due to their universal distribution and high nucleotide sequence conservation become a widely used approach for the detection and classification of microbes [2]. The number of *rrn* operons located on a genome differs considerably between species [39]. It has been correlated to the rate with which microbes can respond to resource availability [22]. A high number of *rrn* operons on the genome together with high expression of the rRNA genes enables a high growth rate in bacteria [1].

The upstream regions of *rrn* operons usually contain highly efficient promoters, like the *Escherichia coli rrn* promoters, the most extensively studied to date, containing four major structures that improve promoter efficiency. Firstly, *E. coli rrn* operons contain tandem promoters, i.e. two −10 and −35 recognition sequences for RNA-polymerase, which is believed to increase the transcription rate by transcribing the genes simultaneously with multiple RNA-polymerase molecules [42]. Secondly, the UP-element (upstream-element), an AT-rich region upstream of the −35 region increases transcription 30–70-fold [34]. Thirdly, a binding site for FIS-protein (factor for inversion stimulation) is located upstream of the most upstream UP-element, which can lead to a 5–10-fold increased transcription level [12]. Finally, anti-termination-boxes at the start of the operon prevent RNA-polymerase dissociation from the DNA [16].

The genes within the *rrn* operons are in general organised in the order 5′-16S-23S-5S-3′ [25]. The intergenic spacer region between the 16S and 23S genes can contain between zero and two tRNA genes, usually encoding a tRNA-ile, tRNA-ala or tRNA-glu [28]. In the Gram-negative bacterium *E. coli*, most tRNA genes are independently transcribed from rRNA genes [31]; only one or two are situated following the *rrn* operon [29]. In several Gram-positive bacteria including *Bacillus subtilis* [35], *Staphylococcus aureus* [17] and *Lactococcus lactis* [9], several tRNA genes were found downstream of the 5S rRNA gene.

Until recently, the majority of studies on rRNA operons has been focussed on differences between operons of different species. The increasing amount of complete nucleotide sequence information of bacterial genomes provides easy access to the complete *rrn* operon information allowing the study of their intra-species differences [39]. In the present study, the *rrn* operons including promoter sequences, of lactic acid bacteria (LAB) of which the complete genome sequence is known, namely *Lactobacillus plantarum* [23], *Lactobacillus johnsonii* [32] and *Lactococcus lactis* [6], are compared. LAB are of industrial interest as starter cultures for the dairy industry and incorporated in functional foods, and they are often examined using molecular techniques based on ribosomal RNA [19,33,24]. In the present study, sequence comparison of the operon structure and promoter regions within an LAB strain revealed microheterogeneity within the rRNA structural genes and spacer regions, but large variations were encountered in the rRNA promoter regions of *Lb. plantarum* and *L. lactis*. Moreover, large differences were found in the presence and type of genetically linked tRNA genes of the species examined. rRNA promoter regulation was studied in *Lb. plantarum* using a reporter gene that revealed differential expression depending on the growth phase.

Materials and methods

The complete genomic sequences of *Lb. plantarum* WCFS1 [23], *Lb. johnsonii* NCC 533 [32], and *L. lactis* subsp. *lactis* IL1403 [6] were used in this study. The rRNA operons within one strain were compared to each other using the ERGO Bioinformatics Suite [30]. The promoter regions of the *rrn* operons were compared to each other and to well-characterised operons of other species.

Activity of the promoters of *Lb. plantarum* was tested using the low-copy promoter probe plasmid, pGKV210, which contains a promoterless chloramphenicol acetyltransferase (CAT) reporter gene [40]. All primers used in this study are shown in Table 1. A region of about 700 bp including the *rrn* promoter upstream of the operons was amplified by PCR using established protocols [36]. Where necessary, smaller regions were amplified to exclude open reading frames present. Reverse primers were designed from both the predicted transcriptional start point of the operon (−)(i.e. transcription start point, Fig. 2) and the start of the 16S rRNA gene (+) to investigate the influence of the first spacer including potential anti-termination boxes on transcription. The constructs were called pWUPX+ and pWUPX−, respectively, in which X represents the operon and +/− indicates presence or absence of the first spacer. pWUPX+ constructs were made of all operons. pWUPX− constructs were made only of *rrnC* and *rrnD*; due to the high activity of the *rrnA*, *rrnB*, and *rrnE* promoters, the constructs excluding the first spacer region showed instability and could not be used. *Eco*RI or *Bam*HI sites were built into the primers in order to allow cloning of the PCR products in the correct orientation preceding the CAT gene. A construct called pWUPtRNA was made of a possible promoter between the tRNA genes of *rrnC* (Fig. 3).

Plasmids were constructed in *E. coli* MC1061 using established protocols [36], and subsequently transformed into *Lb. plantarum* WCFS1 by electroporation