The Christian Doppler Laboratory for Genetically Engineered Lactic Acid Bacteria

Christian Doppler Forschungsgesellschaft

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Background and objectives

Lactic acid bacteria (LAB) play an important role in the field of industrial food and feed fermentation. They are used to produce a variety of meat and dairy products, fermented vegetables and ensiled forage. Due to their economic importance there is a growing interest in the development of genetically engineered LAB strains.

One objective of our CD-laboratory is to improve industrial LAB strains, supplied by our business partner LACTOSAN GmbH, in terms of degradation and conversion of complex carbon hydrates. Another aim of our work is to develop a LAB based expression system to take advantage of the excellent secretion properties of some LAB. Therefore, we want to establish a molecular toolbox for a systems based, rational strain improvement approach, feasible for diverse LAB strains.

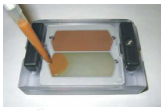
To get insight in its metabolic capacities and gene regulatory network, the whole genome sequence of one of our model strains, *Lactobacillus buchneri* RG03, was elucitated. The strains properties will be further investigated by means of microarray analysis and other molecular biology methods. Five other LAB species/strains have been investigated to find novel plasmids comprising new origins of replication which can be used to construct DNA-libraries and expression vectors. Several expression vectors have already been designed and tested regarding their plasmid copy numbers and seggregational stabilities. The five strains will also be analyzed in terms of identifying inducible/constitutive promoters of defined transcriptional activity which can be used to drive heterologous expression of chromosomally integrated or plasmid encoded target genes in LAB.

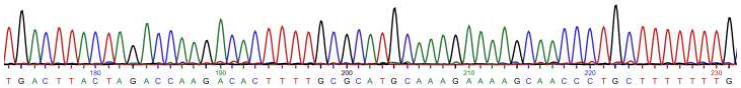
Whole genome sequence of *L. buchneri* RG03

In cooperation with the Center for Biotechnology (CeBiTec) in Bielefeld, Germany, the *L. buchneri* RG03 genome was sequenced by Roche 454-technology.

Number of sequenced bases:	298.627.005
Number of assembled bases:	297.063.664
Number of large contigs (> 500 bp):	37
Number of small contigs (< 500 bp):	5
All contigs:	2.523.672 bp
Number of predicted genes (GenDB):	2353
GC-contents:	44.18%

Table 1: Characteristics of the *L. buchneri* RG03 draft sequence.





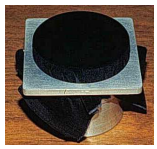
Transformation of industrial LAB

➢ Establishment of electroporation protocols

Introduction of novel genes into the target organism represents the crucial basis of genetic engineering. Therefore, we are working on efficient transformation protocols for our industrial LAB strains. Most successful was the transformation of *L. plantarum* RG02. With this strain we reached transformation efficiencies of 6x10e5 transformants/µg plasmid DNA.

➢ Stability of transformants

We study the stability of plasmid vectors with different origins of replication by replica plating. Later we will investigate the stability of chromosomally integrated target genes and pathways in our LAB strains.



➢ PCN

Relative plasmid copy numbers were determined by quantitative real time PCR.

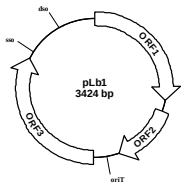
Novel LAB plasmids

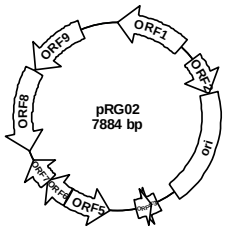
➢ Search for novel origins of replication

We dispose of several LAB strains bearing one or more plasmids. Four novel plasmids have already been sequenced completely and characterized.

Designation	Size	GC-content	Mode of repl.	PCN
pLb1	3424 bp	38.36%	RC	23
pLb2	2707 bp	38.60%	RC	143
pLb3	48646 bp	37.05%	Θ	4
pRG02	7884 bp	35.07%	Θ?	n. d.

Table 2: Sequenced plasmids.





Molecular toolbox for LAB

➢ Search for inducible/constitutive promoters

An aim of our work is to find inducible promoters and to elucidate their involvement into the regulatory network

➢ Identification of efficient signal peptides

This project deals with the identification of leader sequences of strongly secreted proteins by proteomics and an genomics based approaches. Most promising canditaes are, up to now, signal peptides of a muropeptidase and of several putative S-layer proteins.

➢ Search for optimal integration loci

Another objective of our studies wil be the identification of advantageous loci for genomic integration of expression cassettes into the host chromosome. Nontargeted integration of a reporter cassette into the host chromosome will show us if the reporter gets transcribed to a sufficient extent at its locus.

Conclusions and outlook

- **Cultivation of LAB was successfully established in our laboratory.**
- **The whole genome of *L. buchneri* RG03 was *de novo* sequenced by Roche 454-technology.**
- **For the first time *L. buchneri* plasmids have been sequenced and thus made available as a basis for vector construction.**
- **Efficient electroporation protocols for industrial LAB strains were established.**
- **Several expression and integration vectors were designed and characterized regarding their PCN and seggregational stability.**
- **A molecular toolbox comprising promoters, secretion signals and origins of replication, allowing efficient genomic engineering of industrial LAB, will be available.**
- **LAB strains with improved silage properties will be available.**
- **A LAB based expression system which allows large scale production of recombinant proteins will be established.**

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