RESEARCH LETTER

Genetic engineering of *Lactobacillus diolivorans*

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Received 28 March 2013; revised 22 April 2013; accepted 27 April 2013. Final version published online 23 May 2013.

DOI: 10.1111/1574-6968.12168

Editor: Ezio Ricca

Keywords
DNA methylation; *Lactobacillus* transformation; 1,3-propanediol production; plasmid replication origin.

Abstract

In this study, we developed a toolbox for genetic manipulation of *Lactobacillus diolivorans*, a promising production organism for 1,3-propanediol from glycerol. Two major findings play a key role for successful transformation of this organism: (1) the absence of a native plasmid, because a native plasmid is a major obstacle for transformation of *L. diolivorans*, and (2) the absence of DNA methylation. A suitable expression plasmid, pSHM, for homologous and heterologous protein expression in *L. diolivorans* was constructed. This plasmid is based on the replication origin repA of *L. diolivorans*. The native glyceraldehyde-3-phosphate dehydrogenase promoter is used for constitutive expression of the genes of interest. Functional expression of genes in *L. diolivorans* was shown with two examples: production of green fluorescent protein resulted in a 40- to 60-fold higher fluorescence of the obtained clones compared with the wild-type strain. Finally, the homologous overexpression of a putatively NADPH-dependent 1,3-propanediol oxidoreductase improved 1,3-propanediol production by 20% in batch cultures.

Introduction

Due to new applications, including the production of polytrimethylene terephthalate (PTT) used for fibre, carpet and apparel fabrication, 1,3-propanediol has become a bulk chemical over the last decade. Concomitantly, the efforts to produce this substance microbially have been increased (Johnson & Taconi, 2007; Kaur *et al.*, 2012). While a major part of these efforts is based on glycerol as carbon source, to the best of our knowledge, the only industrial-scale production process today uses glucose as substrate and a recombinant strain of *Escherichia coli*. Titres of up to 135 g L^{-1} 1,3-propanediol in a fed-batch process have been reported (Nakamura & Whited, 2003). However, a surplus on the glycerol market due to the increasing biodiesel production together with the dramatic decrease in glycerol prices makes this substance an interesting substrate for the production of chemicals (Da Silva *et al.*, 2009; Clomburg & Gonzalez, 2013). Strains of *Lactobacillus diolivorans* were first described to produce 1,3-propanediol from glycerol when they have been isolated as spoilage organisms from cider (Garai-Ibabe *et al.*, 2008). We could show that *L. diolivorans* is a good natural producer of 1,3-propanediol with great industrial potential. A titre of 85 g L^{-1} has been obtained from glycerol in a fed-batch process (Pflügl *et al.*, 2012). In addition to process optimization, the ability for genetic engineering of the strain would be advantageous for further improvement of this natural producer (Sauer & Mattanovich, 2012). Genetic engineering tools for lactic acid bacteria (LAB) have been developed extensively (Sørvig *et al.*, 2003; Teresa Alegre *et al.*, 2004; Kim *et al.*, 2005; Peterbauer *et al.*, 2011; Spath *et al.*, 2011). However, tools for manipulation of LAB are usually strain specific. Standard transformation procedures with plasmids proven to be functional in a variety of *Lactobacilli* were unsuccessful for *L. diolivorans*. Nevertheless, based on the tools already available, we developed a toolbox for genetic engineering of this organism. The genetic engineering tools were evaluated by heterologous expression of green fluorescent protein. Furthermore, a putatively NADPH-dependent 1,3-propanediol oxidoreductase was homologously overexpressed to increase the 1,3-propanediol production in *L. diolivorans*.

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Materials and methods

Bacterial strains and growth conditions

Lactobacillus diolivorans DSM 14421 and LMG 19668 used in this study were cultivated at 30 °C. MRS medium supplemented with 3% (w/v) glucose was used for cultivations (De Man *et al.*, 1960). In case of 1,3-propanediol production experiments, MRS medium was additionally supplemented with 3% (w/v) glycerol. MRS plates supplemented with 2% (w/v) glucose and 10 μg mL^{-1} erythromycin were used in the transformation procedure. *Escherichia coli* Top10 (Invitrogen) was used for cloning procedures, and *E. coli* K12-ER2925 (New England Biolabs) was used for amplification of unmethylated plasmids. *E. coli* was cultivated at 37 °C either in LB medium or on LB plates with kanamycin as selection marker (10 μg mL^{-1}).

Identification of the replication origin from *L. diolivorans* for plasmid construction

The ampicillin and kanamycin resistance cassettes from pSTBlue-1 (Novagen) were replaced by the erythromycin cassette from pSIP 409 (Sørvig *et al.*, 2005). Then, genomic DNA (including endogenous plasmids) from *L. diolivorans* DSM 14421 was digested Sau3AI and cloned into the BamHI cut vector. After amplification in *E. coli*, this library was transformed into *L. plantarum* ATCC 8014.

Construction of an expression plasmid for protein production in *L. diolivorans*

Figure 1 shows the expression plasmid pSHM constructed for protein production in *L. diolivorans*. Each fragment of the plasmid was amplified by PCR and enzymatically assembled by the method of Gibson *et al.* (2009) (Table 1). The assembled plasmid was transformed into *E. coli* K12 ER 2925 by standard electroporation to obtain nonmethylated plasmid DNA for the transformation of *L. diolivorans* LMG 19668. Plasmid DNA was isolated from *E. coli* with a Plasmid Maxi Kit (Qiagen) from overnight cultures grown on LB supplemented with 10 μg mL^{-1} kanamycin.

Transformation procedure

An optimized transformation procedure was developed for *L. diolivorans* LMG 19668. In brief, 50 mL MRS medium supplemented with 2% (w/v) glucose was inoculated with 1.5 mL cryo culture of *L. diolivorans* LMG 19668 and incubated overnight at 30 °C without shaking. 500 mL MRS medium supplemented with 2% (w/v) glucose and 1% (w/v) glycine was inoculated with 50 mL overnight culture and incubated for 4 h at 30 °C without shaking. After this incubation, the cells were harvested by centrifugation for 10 min at 9000 ***g*** and 4 °C and were kept cold (4 °C in the centrifuge or on ice) from that point on. Cells were washed five times with 100 mL 0.3 M sucrose, the third washing step additionally containing 50 mM EDTA, pH 8. The cells were finally resuspended in 1 mL of an electroporation buffer containing 272 mM sucrose, 7 mM sodium phosphate and 1 mM $MgCl_2$, pH 7.4. An 80-μL aliquot together with 20 μL of DNA solution (DNA amount: 5 μg) was mixed and loaded into an electroporation cuvette with 4 mm gap width. Electroporation was carried out at 2500 V, 200 Ω and 25 μF. Immediately after the electroporation, the cells were mixed with 900 μL of MRS medium supplemented with 2% (w/v) glucose and 0.3 M sucrose and incubated for 2 h at 30 °C without shaking. The cell suspension was then plated on MRS plates containing 10 μg mL^{-1} erythromycin and incubated at 30 °C for 3 days.

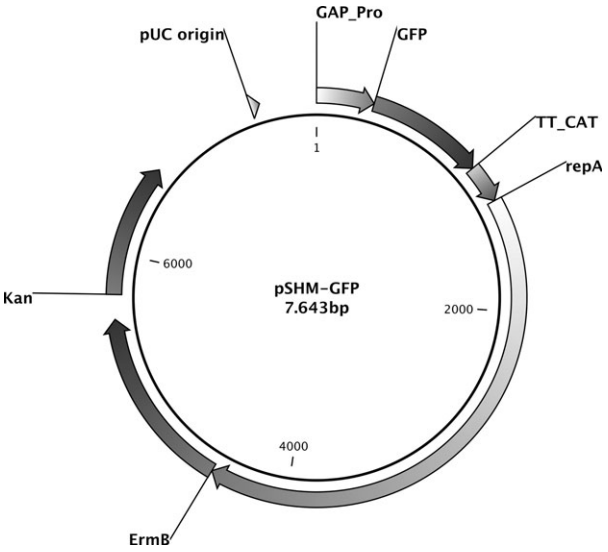

Fig. 1. Plasmid pSHM contains (1) the replication origin from *Lactobacillus diolivorans*, repA, (2) the origin of replication (pUC origin) for *Escherichia coli* from pUC 19, (3) the kanamycin resistance cassette (Kan) from pSTBLUE, (4) the erythromycin resistance cassette (ErmB) from pSIP 409, (5) an expression cassette for gene expression containing the promoter of the glyceraldehyde 3-phosphate dehydrogenase (GAP_Pro) from *L. diolivorans* DSM 14421 and the terminator of the chloramphenicol resistance cassette (TT_CAT) from pC194. The PCR-amplified coding sequence (cds) of the respective target gene (cds – gene of interest) was cloned into this cassette by enzymatic assembly (Gibson *et al.*, 2009). For expression of green fluorescent protein, the coding sequence for GFP was PCR-amplified from plasmid pET30a_GFPmut3.1, and the putative NADPH-dependent 1,3-propanediol oxidoreductase was PCR-amplified from genomic DNA from *L. diolivorans* LMG 19668.

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Table 1. Primers for amplification of DNA fragments used for the enzymatic assembly of pSHM+GFP and pSHM+PDO-DH(NADPH)

Fragment	Primer
GAP	FW: GATAACAATTTCACACAGGAAACAGCGAAACATCATTTAATAATGCGCTCG
	REV (GFP): TAAACAGTTCTTCGCCTTTGCTCATACTAAGTTTTCCTCCTTTAGGAAAT
	REV (PDO-DH(NADPH)): AATTCGAATTTCTTCCATACTAAGTTTTCCTCCTTTAGGAAAT
TT_CAT	FW (GFP): CATGGCATGGATGAACTGTATAAATAATATGAGATAATGCCGACTGTACTTTTTAC
	FW (PDO-DH(NADPH)): ACAAGAAAGTAATCCGTTAATATGAGATAATGCCGACTGTACTTTTTAC
	REV: GCTTCTTTGCGTAACTCGTTAATTCGTCGGCATAGCGTGAGCTATTAAGC
repA	FW: GCTTAATAGCTCACGCTATGCCGACGAATTAACGAGTTACGCAAAGAAGC
	REV: CACCGAGTTCAAAGACGCTAAAGAGCCTTAGAAGCAAACTTAGAGTGTGTT
ErmB_BL	FW: AACACACTCTAAGTTTGCTTCTAAGGCTCTTTAGCGTCTTTGAACTCGGTG
	REV: GTATATATGAGTAAACTTGGTCTGACAGTTACCTCCATTCCCTTTAGTAACGTGTAACTT
KAN + ori	FW: AAGTTACACGTTACTAAAGGGAATGGAGGTAACTGTCAGACCAAGTTTACTCATATATAC
	REV: CGAGCGCATTATTAAATGATGTTTCGCTGTTTCCTGTGTGAAATTGTTATC
PDO-DH (NADPH)	FW: ATTTCCTAAAGGAGGAAAACTTAGTATGGAAGAAATTCGAATT
	REV: GTAAAAAGTACAGTCGGCATTATCTCATATTAACGGATTACTTTCTTGT
GFP	FW: ATTTCCTAAAGGAGGAAAACTTAGTATGAGCAAAGGCGAAGAACTGTTTA
	REV: GTAAAAAGTACAGTCGGCATTATCTCATATTATTTATACAGTTCATCCATGCCATG

FW, forward primer; REV, reverse primer.

Fluorescence measurements

The cell suspension of an overnight culture at 30 °C without shaking was centrifuged, washed with PBS once and resuspended in the same buffer. Samples with an optical density of 0.5 and 1.0 were prepared. The detection of fluorescence from GFP was carried out with a TECANreader Infinite®200, using an excitation wavelength of 488 nm and an emission wavelength of 520 nm. Each sample was measured in triplicate.

Flow cytometry measurements

Flow cytometric analyses were performed on a FACSCalibur system (Becton Dickinson, Franklin Lakes, NJ) with 488-nm excitation from a 15-mW air-cooled argon-ion laser. Fluorescence emission was acquired through a 530/30-nm band-pass filter (FL1) in logarithmic mode. Acquisition of forward scatter (FSC) and side scatter (SSC) was performed in linear mode. Cell debris was excluded from the data acquisition by gating related to SSC and FSC; 10 000 cells were measured for every sample. For analysis, 50 mL overnight culture was centrifuged, washed once with PBS and resuspended in PBS to a cell density of *c.* 8×10^5 cells per mL.

HPLC measurements

Metabolites produced during cultivations were quantified by HPLC analysis (Shimadzu, Korneuburg, Austria) with a Rezex ROA-Organic Acid H^+ column (300 × 7.8 mm, Phenomenex) and a refraction index detector (RID-10A, Shimadzu). The column was operated at 60 °C, 1.0 mL min^{-1} flow rate and 0.004 M H_2SO_4 as mobile phase.

Cell disruption and enzymatic assays

The activities of NADPH- and NADH-dependent 1,3-propanediol oxidoreductases were measured in the crude extract from *L. diolivorans* cultures. Therefore, 50 mL overnight cultures grown on MRS supplemented with glucose and glycerol was collected by centrifugation, washed once with Tris–HCl buffer, pH 6.5, and resuspended in the same buffer in 1/10 of the original volume. Cell disruption was carried out by sonication with a Branson Sonifier 250 (Emerson Electric), setting 3% and 50% intervals, for 2 min. Cell debris was collected by centrifugation. The protein amount of the supernatant was determined by a Bradford protein assay (Bradford, 1976) to equilibrate the amount of protein used in all enzyme assays. 1,3-propanediol oxidoreductase activity was determined as described previously, except that the assay was carried out in the reductive direction using 3-HPA as substrate (Saint-Amans *et al.*, 2001). In brief, the decrease in absorbance due to oxidation of NADH was tracked for 180 s. The reaction mixture contained 2 mM NADH, 30 mM $(NH_4)_2SO_4$, 100 mM 3-HPA and 100 mM potassium bicarbonate (pH 9.0) with 2 mM DTT.

Results

Transformation of *L. diolivorans*

Attempts have been made to transform *L. diolivorans* DSM 14421 and LMG 19668 with a broad range of suitable expression vectors for LAB such as pTRKH3 and pSIP409 (O'Sullivan & Klaenhammer, 1993; Sørvig *et al.*, 2005). However, no transformants were obtained with any of the plasmids tested. DNA preparation from both

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strains showed that *L. diolivorans* LMG 19668 does not contain native plasmids, whereas *L. diolivorans* DSM 14421 harbours two native plasmids with sizes of 44 and 55 kb, respectively.

To construct a suitable expression vector, DNA from *L. diolivorans* DSM 14421 was digested and cloned into the pSTBlue vector to isolate a functional replication origin for plasmids in *L. diolivorans*.

The obtained library was transformed into *L. diolivorans* DSM 14421, *L. diolivorans* LMG 19668 and *L. plantarum* ATCC 8014. No transformants were obtained for any of the *L. diolivorans* strains. However, few transformants were obtained for *L. plantarum* (*c.* 10^1 per μg DNA). Plasmid preparation confirmed the presence of an episomal plasmid in each transformant. One of these plasmids, designated p22, was chosen for sequencing of the insert. The obtained sequence showed a high similarity with sequences from other *Lactobacilli* for the plasmid replication origin repA. Thus, the presence of a functional replication origin on plasmid p22 suitable for *Lactobacilli* could be shown.

Additionally, the impact of the methylation of the DNA used for transformation of *L. diolivorans* was studied in more detail. To this end, plasmid p22 was transformed into *E. coli* K12 ER 2925, a strain lacking dam and dcm methylases, hence producing unmethylated DNA. DNA prepared from this source was used for transformation of both *L. diolivorans* strains, DSM 14421 and LMG 19668. Still, no colonies were obtained for DSM 14421, the strain containing native plasmids. However, for LMG 19668 – a plasmid-free strain – a small number of transformants (*c.* 10^1 per μg DNA) were obtained.

Construction of expression vector pSHM and heterologous expression of GFP in LMG 19668

The expression vector pSHM for heterologous and homologous gene expression was constructed as described in materials and methods and is shown in more detail in Fig. 1. Based on the genome sequence of *L. diolivorans* DSM 14421, which we obtained in between, the optimal plasmid replication origin repA was designed for pSHM. Furthermore, the endogenous promoter for the glyceraldehyde-3-phosphate dehydrogenase was identified and used for the expression cassette on pSHM. The glyceraldehyde-3-phosphate dehydrogenase promoter was chosen because this enzyme is considered to be strongly and constitutively expressed in LAB.

Transformation of pSHM+GFP into *L. diolivorans* LMG 19668 resulted in *c.* 10^1 transformants per μg DNA. Significantly increased fluorescence values confirmed a high production of green fluorescent protein in *L. diolivorans* (Table 2).

Table 2. Fluorescence of *Lactobacillus diolivorans* LMG 19668. The fluorescence of cells from a wild-type culture (untransformed) was compared with cells from two of the obtained clones. All cultures were adjusted to two different optical densities. PBS used for washing and resuspension of the cells was measured as blank

L. diolivorans LMG 19668	OD_{600} = 1.0	OD_{600} = 0.5
Wild type	121 ± 5	101 ± 1
pSHM-GFP clone 1	7461 ± 190	3908 ± 30
pSHM-GFP clone 2	4788 ± 94	2483 ± 15
Blank	78 ± 1	

Plasmid stability of recombinant *L. diolivorans* LMG 19668 cultures carrying the plasmid pSHM+GFP was investigated by flow cytometric single-cell fluorescence analysis.

To this end, *L. diolivorans* LMG 19668 carrying pSHM+GFP was incubated overnight with and without antibiotic selection pressure and re-inoculated two times. Figure 2 shows dot plots of FL1 (GFP fluorescence) vs. forward scatter (correlating to cell size), representing the development over 3 days. The plasmid turned out to be quite stable with and without selection pressure, resulting in elevated fluorescence of *c.* 70% to 92% of the cells.

Expression of a putative NADPH-dependent 1,3-propanediol oxidoreductase

The gene encoding the putatively NADPH-dependent 1,3-propanediol oxidoreductase (PDO-DH (NADPH)) from *L. diolivorans* LMG 19668 was PCR-amplified and cloned into pSHM. Plasmid pSHM+PDO-DH (NADPH) was transformed into *L. diolivorans* LMG 19668. A small number of transformants were obtained. The clones were tested under production conditions for their 1,3-propanediol production potential (Table 3).

Comparison of the growth behaviour shows an initially lower specific growth rate for the PDO-DH overexpressing clones compared with the wild type ($\mu = 0.18\ h^{-1}$ for #2 and #9, $\mu = 0.20\ h^{-1}$ for wild type). However, the final biomass obtained from the cultivations of the PDO-DH overexpressing clones was comparable with the wild-type strain (OD_{600} = 16.5, 16.4 and 15.5 for #2, #9 and wild type, respectively). Lactic acid, acetic acid and ethanol produced during the cultivations were at the same level as in the wild type. However, lower glycerol concentrations were observed in the supernatants from the PDO-DH overexpressing clone cultures compared with the wild type, while the 1,3-PDO concentration was increased for the PDO-DH overexpressing clones by about 20% (Table 3).

To confirm a higher activity of NAPDH-dependent 1,3-propanediol oxidoreductase and to assess the co-factor requirement, enzyme activity assays were carried out (Table 4).

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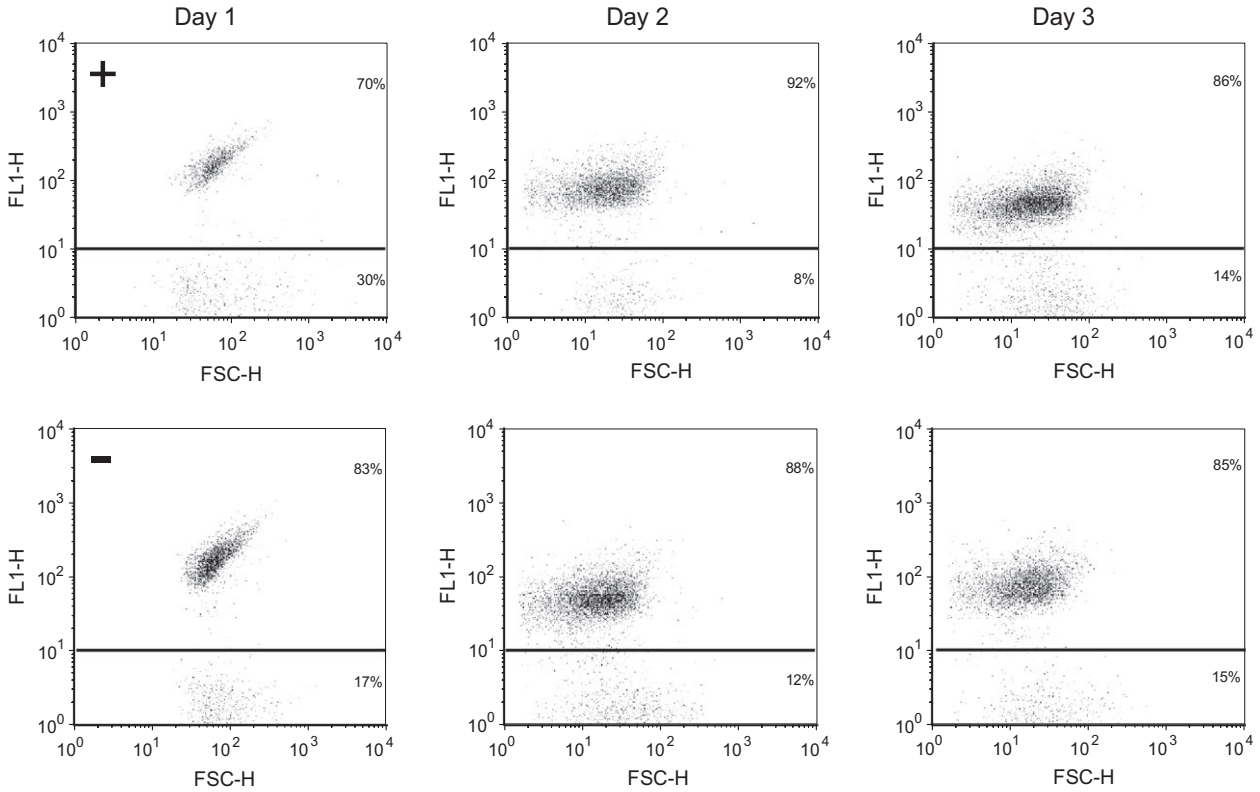

Fig. 2. Flow cytometric analysis of *Lactobacillus diolivorans* LMG 19668 pSHM+GFP cultures grown three times overnight on MRS + 2% (w/v) glucose with and without erythromycin, respectively. (+) Dot plots showing cells of *L. diolivorans* LMG 19668 pSHM+GFP grown with antibiotic selection pressure and (−) dot plots showing cells of *L. diolivorans* LMG 19668 pSHM+GFP grown without antibiotic selection pressure. The upper gate contains cells with elevated fluorescence, indicating production of green fluorescent protein and thereby the presence of the plasmid. The lower gate contains cells with basal fluorescence, indicating no production of green fluorescent protein and thereby the absence of the recombinant plasmid. Dot plots are given for each analysed time point over 3 days, and the percentage of the cells in each gate is given.

Table 3. Final 1,3-propanediol concentrations, volumetric (Q_p) and specific (q_p) productivities of the two best clones expressing PDO-DH (NADPH) compared with a wild-type strain of *Lactobacillus diolivorans* LMG 19668 (untransformed)

L. diolivorans LMG 19668	1,3-PDO [g L^{-1}]	Q_p [g L^{-1} h^{-1}]	q_p [g g^{-1} h^{-1}]
Wild type	9.6 ± 0.1	0.42 ± 0.01	0.14 ± 0.01
pSHM+PDO-DH (NADPH) #2	11.7 ± 0.1	0.53 ± 0.01	0.19 ± 0.01
pSHM+PDO-DH (NADPH) #9	11.7 ± 0.2	0.53 ± 0.01	0.19 ± 0.01

The values represent the mean of six individual experiments.

Table 4. Total NADPH- and NADH-dependent activities of 1,3-propanediol oxidoreductases in cultures of wild-type strain *Lactobacillus diolivorans* LMG 19668 (untransformed) and clones #2 and #9 expressing pSHM+PDO-DH (NADPH)

L. diolivorans LMG 19668	NADPH [U mg^{-1}]	NADH [U mg^{-1}]
Wild type	0.12	0.14
pSHM+PDO-DH (NADPH) #2	0.25	0.22
pSHM+PDO-DH (NADPH) #9	0.30	0.33

The values given represent the mean of two individual experiments.

Discussion

Two major factors were identified to be of importance for the successful transformation of *L. diolivorans*: first, the absence of native plasmids in the target strain and second the absence of *E. coli* type methylation of the DNA used for transformation.

Lactobacillus diolivorans showed to be recalcitrant to genetic manipulation with vectors like pTRKH3 or pSIP409 used for transformation of other lactic acid bacteria. Strain DSM 14421 containing native plasmids turned out to be generally untransformable with plasmid p22 using the same replication origin as one of the strain's native plasmids. This implies that two plasmids with the same replication origin cannot be present in a cell at the same time. However, the presence of a native plasmid turned out not to be the sole reason for unsuc-

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cessful transformation of *L. diolivorans*. Also the plasmid-free strain LMG 19668 could only be transformed when plasmid p22 originated from an *E. coli* strain producing unmethylated (dam^- and dcm^-) DNA. Therefore, methylation of DNA also plays a key role in successful transformation of *L. diolivorans*. This suggests that plasmid DNA carrying a wrong methylation pattern is less suitable for transformation of *L. diolivorans* than DNA carrying no methylation pattern whatsoever. Assuming the presence of a restriction modification system in *L. diolivorans*, DNA with the wrong methylation pattern seems to be degraded and therefore results in no transformants, whereas DNA with no methylation seems to be either degraded by the cell's restriction system or methylated by the cell's DNA modification system. In the latter case, positive transformants carrying the transformed plasmid are obtained. Considering these points, a transformation procedure for genetic manipulation of *L. diolivorans* with a transformation efficiency of *c.* 10^1 per μg DNA could be established. Heterologous expression of green fluorescent protein from plasmid pSHM+GFP was successfully demonstrated. Two clones tested revealed a 40- and 60-fold higher fluorescence compared with the wild type, respectively. This clearly indicates functional expression of GFP under the native glyceraldehyde 3-phosphate promoter. Plasmid stability of 70% to 92% could be shown via flow cytometry. High plasmid stability in case of incubation of the recombinant strain without antibiotic selection pressure indicates that once a plasmid is introduced to *L. diolivorans* LMG 19668, it is quite stably retained. Nevertheless, some cells loose the plasmid what implies that curing is possible when desired.

The conversion of glycerol into 1,3-propanediol in bacteria occurs in two steps. First, glycerol is converted into 3-hydroxypropionaldehyde (3-HPA) by glycerol dehydratase. Subsequently, 3-HPA is reduced to 1,3-propanediol. This step requires NADH as cofactor. The availability of the cofactor NADH is considered a limiting factor in conversion of 3-HPA to 1,3-propanediol and therefore glycerol in general. However, the second step may also be carried out by enzymes using NADPH instead of NADH as cofactor. Analysis of the genome sequence of *L. diolivorans* revealed that such an enzyme is also present in *L. diolivorans* LMG 19668. NADPH in heterofermentative lactic acid bacteria is provided by the phosphoketolase pathway upon degradation of glucose. The enzymes of the pathway in *L. diolivorans* have been assayed enzymatically for their activity with $NADP^+$ as cofactor. It could be shown that the 6-phosphogluconate dehydrogenase uses both NAD^+ and $NADP^+$ as cofactor, therefore providing the organism with NADPH as well as NADH (data not shown).

Homologous expression of PDO-DH (NADPH) in *L. diolivorans* LMG 19668 increased 1,3-propanediol concentrations by *c.* 20% compared with the wild-type strain. Furthermore, the enzymatic activity of the NADPH-dependent 1,3-propanediol oxidoreductase of the clones was 2- to 2.5-fold increased. However, also the NADH-dependent activity increased 1.6- and 2.4-fold, indicating that the enzyme might not strictly discriminate between these cofactors. The other metabolites produced during the cultivation, in particular lactic acid and ethanol, did not differ from the wild type. Considering that the expression of PDO-DH (NADPH) showed an increased activity of 1,3-propanediol oxidoreductase for both NADH and NADPH, the unchanged metabolite concentrations except for increased 1,3-propanediol concentrations can be explained. This is in contrast to what has been reported by Vaidyanathan *et al.* (2011) that *L. reuteri* expressing NADPH-dependent alcohol dehydrogenase YqhD from *E. coli* showed increased lactic acid and ethanol production. It has been suggested that the overexpression of YqhD led to a preference for utilization of NADPH over NADH for the reduction of 3-HPA to 1,3-propanediol due to a lower activity of the native NADH-dependent 1,3-propanediol oxidoreductase. The surplus NADH was suggested to be used for lactate and ethanol production. Because the clones we obtained did not show a lower but higher NADH-dependent 1,3-propanediol oxidoreductase activity, this suggests that the overexpressed gene, although annotated as NADPH-dependent, in fact is using both NADH and NADPH as cofactor for the conversion of 3-HPA to 1,3-propanediol.

In this study, for the first time, the transformation of *L. diolivorans* could be shown. Functional expression of proteins, both homologously and heterologously, could be achieved. Furthermore, genetic engineering of the strain improved the 1,3-propanediol production capacities. Ongoing engineering efforts could lead the way to a powerful production strain for 1,3-propanediol.

Acknowledgements

This work was financially supported by Vogelbusch GmbH, Vienna, Austria. A patent application comprising the data of this study has been filed. The authors are indebted to Stefanie Müller for excellent technical support and to G.H. Sodeck, J. Gatterer and J. Modl for fruitful discussions. L. Axelsson is thankfully acknowledged for provision of the plasmid pSIP 409 and *L. plantarum* NC8.

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