Butanol is cytotoxic to *Lactococcus lactis* while ethanol and hexanol are cytostatic

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**Abstract**

Lactic acid bacteria currently used extensively by the dairy industry have a superior tolerance towards short-chain alcohols, which makes them interesting targets for use in future bio-refineries. The mechanism underlying the alcohol tolerance of lactic acid bacteria has so far received little attention. In the present study, the physiological alcohol stress response of *Lactococcus lactis* subsp. *cremoris* MG1363 towards the primary, even-chain alcohols ethanol, butanol and hexanol, was characterized. The alcohol tolerance of *L. lactis* was found to be comparable to those reported for highly alcohol-resistant lactic acid bacteria. Combined results from alcohol survival rate, live/dead staining, and a novel usage of the β-galactosidase assay, revealed that while high concentrations of ethanol and hexanol were cytostatic to *L. lactis*, high concentrations of butanol were cytotoxic, causing irreparable damages to the cell membrane.

**INTRODUCTION**

To forsake modern society’s dependency on limited, non-renewable oil-reserves, recent approaches have aimed at constructing microbial hosts for the production of bio-alcohols as alternative fuels. Using several metabolic engineering strategies, production of the bio-alcohols ethanol and butanol have been shown feasible in a number of organisms such as *Escherichia coli*, *Saccharomyces cerevisiae*, *Pseudomonas putida* and *Bacillus subtilis* [1–3].

A major hurdle limiting the yield and titres of microbial bio-alcohol production is the toxicity of these compounds [4]. As ethanol and butanol are both short-chain alcohols there are similarities in how microbes respond to them. Through their hydrophobic nature, short-chain alcohols disrupt the phospholipid components of the cell membrane thereby increasing membrane fluidity and destroying the integrity of the cell [5–8]. A direct correlation between the potency of alcohols as biological effectors and their hydrophobicity has been found [9–11], suggesting that the alcohols primarily influence hydrophobic associations [8]. Ethanol stress, the most studied alcohol stress, has been shown to increase membrane permeability, allowing for the free passage of ions and charged molecules [8]. High concentrations of ethanol and butanol have been shown to interfere with the pH gradient of cell membranes, thereby increasing acid sensitivity in *S. cerevisiae* and *Clostridium* strains, respectively [5, 12, 13]. Introduction of saturated fatty acids can counteract the increased membrane fluidity associated with long-term ethanol stress [5, 8]. In *Lactococcus lactis* subsp. *cremoris* MG1363 an increase in membrane fluidity has likewise been observed as a consequence of sub-inhibitory ethanol stress [14]. To counteract long-term ethanol stress, *L. lactis* was found to upregulate shorter-chained fatty acids and downregulate longer-chained unsaturated fatty acids. The shorter-chained fatty acid C<sub>16:1</sub>n-7, known to decrease hydrophobic interaction between the free acyl chains and the proteins, was found to be upregulated under prolonged sub-inhibitory ethanol stress [14], in accordance with the suggested influence of ethanol on hydrophobic associations [8].

In the pursuit of an optimal production strain, various organisms have been screened for their alcohol tolerance [15–17], and it has here been found that lactic acid bacteria (LAB) intrinsically possess a superior tolerance towards ethanol compared to other micro-organisms [18, 19]. Gold et al. determined the ethanol tolerance of 31 *Lactobacillus* strains, and among these eight showed a significant growth increase in medium containing 16 % ethanol [16]. In comparison, *E. coli* is known to be extremely sensitive towards ethanol with marginal growth in concentrations above 6 %, and the obligate ethanol-producer *Zymomonas mobilis* shows minimum growth above 9 % ethanol [8]. In searching for butanol-tolerant strains, Knoshaug and Zang...
determined the butanol tolerance of a range of organisms deemed suitable for production of butanol [15]. From a total of 10 \textit{S. cerevisiae} strains, five \textit{E. coli} strains and three \textit{Lactobacillus} strains, only two lactobacilli showed an increase in optical cell density in the presence of 2.5\% \textit{n}-butanol. With one strain even showing growth in the presence of 3\% \textit{n}-butanol.

Although the superior alcohol tolerance of LAB have long been known [8, 19], no studies have characterized the mechanism behind. \textit{L. lactis} subsp. \textit{cremoris} MG1363 represents a suitable model organism for the analysis of alcohol tolerance in LAB because: (i) it has a simple fermentative metabolism in which glucose is converted almost exclusively into lactic acid [20]; (ii) a wide range of regulatory networks have been characterized, easing physiological and metabolic understanding [21, 22]; (iii) the genome sequence is known [23]; and lastly, (iv) a large array of genetic tools are available [24–26]. As a case study for its use as a biofuel producer, the ethanol production pathway of \textit{L. lactis} was changed from lactic acid to ethanol, reaching yields comparable to those reported for optimized strains of \textit{S. cerevisiae} [27].

In the present study, novel insight into the alcohol tolerance of LAB is achieved through characterization of growth physiology, cell survival and membrane integrity of \textit{L. subsp. cremoris} MG1363 when grown in the presence of the short-chain alcohols ethanol, butanol and hexanol.

\section*{METHODS}

\textbf{Strains and culture conditions}

\textit{Lactococcus lactis} subsp. \textit{cremoris} MG1363, a plasmid-free derivative of \textit{Lactococcus lactis} subsp. \textit{cremoris} NCD0712 [28] was used throughout. \textit{Lactococcus lactis} PRJ4621 is a derivative of \textit{Lactococcus lactis} subsp. \textit{cremoris} MG1363 containing the plasmid pAK80 with the synthetic promoter CP25 in front of \textit{lacLM} from \textit{Leuconostoc lactis} [29].

\textit{Lactococcus lactis} were grown in the chemically defined SA medium [30] modified by exclusion of acetate and supplemented with 2 \mu g ml\(^{-1}\) lipoic acid and 1\% glucose (GSAL), and incubated at 30\% C without aeration but with slow stirring to ensure homogeneity. Medium with excess buffering capacity was achieved by supplementing with twice the amount of the pH buffer MOPS as in regular GSAL (GSAL\(^{MOPS}\)).

To ensure statistical significance, all growth experiments were performed with a minimum of three biological replicates.

\textbf{Alcohol stress response}

The alcohol stress response of \textit{L. lactis} was determined by adding exogenous alcohol to cultures that had grown exponentially for at least 10 generations, ensuring a balanced pre-stress phenotype. By exposing growing cultures (OD\(_{450}\) = 0.4) to a range of ethanol (25–115 mg ml\(^{-1}\)), butanol (5–25 mg ml\(^{-1}\)) or hexanol (1–3 mg ml\(^{-1}\)) concentrations, the tolerance range of \textit{L. lactis} towards the specific alcohols was determined. The alcohol stress response was quantified through growth rates (h\(^{-1}\)), final cell density yields (OD\(_{450}\)) and pH. The amount of product produced and substrate consumed was quantified by HPLC following overnight stress inoculation.

\textbf{Quantification of extracellular glucose and end products}

To determine the extracellular metabolite levels in the growth medium, culture samples were passed through a 0.22 \mu m filter. Glucose, lactate, acetate, formate and ethanol were separated using an Ultimate 3000 HPLC system (Dionex) equipped with an Aminex HPX-87H heated column (Bio-Rad,) and a Shodex RI-101 detector (Showa Denko KK). The column temperature was set to 60\%C and 5 mM H\(_2\)SO\(_4\) was used a mobile phase with a flow rate of 0.6 ml min\(^{-1}\).

\textbf{Acid sensitivity assay}

Cultures grown in GSAL or in GSAL\(^{MOPS}\) were subjected to a range of exogenous ethanol (25–115 mg ml\(^{-1}\)), butanol (5–20 mg ml\(^{-1}\)) and hexanol (1–3 mg ml\(^{-1}\)) concentrations. Differences between final pH and between final yields of alcohol-stressed cultures grown in GSAL\(^{MOPS}\) compared to alcohol-stressed cultures grown in GSAL were used to quantify acid sensitivity.

\textbf{Survival rate under alcohol stress}

The survival rate of \textit{L. lactis} when exposed to the individual alcohols was determined by measuring the survival in c.f.u. after 120 min of alcohol stress. Concentrations used were: ethanol 55–120 mg ml\(^{-1}\); butanol 10–25 mg ml\(^{-1}\); and hexanol 2–3 mg ml\(^{-1}\). Aliquots were removed at 0 (reference), 10, 20, 40, 80, and 120 min after induction of alcohol stress, and the first six serial dilutions were plated on GM17 agar and incubated at 30\% C overnight [31]. The relative increase in c.f.u. compared to the reference was calculated.

\textbf{Quantification of the live/dead ratio}

The live/dead ratio of alcohol-stressed \textit{L. lactis} was determined \textit{in situ} using the BacLight LIVE/DEAD Bacterial Viability kit (L7012; Molecular Probes) and imaged using a Zeiss Axioplan fluorescence microscope with an RS Photometrics CoolSNAP camera. The filter had an excitation of 450–490 nm with long pass 520 nm enabling simultaneously visualization of SYTO 9 and propidium iodide (PI) dye. Exponentially growing \textit{L. lactis} was harvested and resuspended in cold GSAL medium to yield a final OD\(_{450}\) –1.5. Aliquots of the harvested cells were exposed to ethanol, butanol or hexanol for 5 min on ice before being mixed with the staining agents SYTO 9 and PI and then visualized by microscopy. A minimum of three samples of at least 500 fluorescent cells each were counted for each condition and used to determine the live/dead ratio.

\textbf{Assay of cell permeability towards ONPG}

The amount of ortho-nitrophenyl-\beta-galactoside (ONPG) passing the membrane of cells of \textit{L. lactis} PRJ4621 was measured as the rate of ortho-nitrophenol (ONP)
formation inside the cells. The strain _L. lactis_ PRJ4621 has a high constitutive β-galactosidase activity, and the rate of ONP formation was determined using the standard protocol described by Miller with the following modifications [32]. Exponentially growing cells OD<sub>450</sub>~0.4 were either harvested for β-galactosidase assay or incubated at 30°C for 1 h with ethanol (120 mg ml<sup>−1</sup>), butanol (25 mg ml<sup>−1</sup>) or hexanol (2.5 mg ml<sup>−1</sup>). The non-stressed harvested cells were washed and lysed with chloroform, left untreated or subjected to either ethanol (120 mg ml<sup>−1</sup>), butanol (25 mg ml<sup>−1</sup>) or hexanol (3 mg ml<sup>−1</sup>) for 10 min at 30°C prior to addition of ONPG and subsequent quantification of β-galactosidase activity. The alcohol-stressed cells were harvested and washed followed by treatment with chloroform or left untreated for 10 min at 30°C. ONPG was then added and the rate of ONP formation was quantified.

**RESULTS AND DISCUSSION**

Tolerance of _L. lactis_ towards primary alcohols categorizes _L. lactis_ as a highly butanol-tolerant strain

The tolerance range of _L. lactis_ subsp. cremoris MG1363 against the primary alcohols ethanol, butanol and hexanol was determined by exposing exponentially growing _L. lactis_ to a broad range of exogenous alcohol concentrations (Fig. 1). All growth experiments were performed in the chemically defined SA medium [30] supplemented with 1% glucose (GSAL), to avoid evoking the purine-sensitive phenotype of _L. lactis_ [33]. Fig. 1(a–c) shows the growth curves obtained for the first 100 min after addition of increasing concentrations of ethanol, butanol or hexanol. The specific growth rates obtained under alcohol stress as a function of the final alcohol concentration in the growth medium revealed, as expected, an inverse correlation to the normal pH threshold is reached, doubling the buffering capacity only insignificantly increased the pH level (0.1 pH unit), as shown in Fig. 3(a–c, filled circles) for the stressed cultures (0 mg ml<sup>−1</sup>). The excess biomass production upon doubling the buffering capacity of the medium (right axis) is shown as a function of alcohol concentration in the second type of plot (open circles). Plots from all three alcohol stress conditions showed that higher alcohol concentrations follow model 2, since the increase in biomass yield is reduced to zero and the final pH is elevated. Butanol at 15 mg ml<sup>−1</sup> has the most severe effect on the increase in final pH (1.6 pH units).

Growth cessation was not caused by sugar starvation, as an excess of glucose was found to be present in the medium (data not shown). Furthermore, the higher pH observed at higher alcohol concentrations was not accompanied by a shift in the general metabolism since the cultures performed homolactic fermentation throughout the experiment (data not shown).

**Butanol is cytotoxic while ethanol and hexanol are cytostatic to _L. lactis_**

Alcohol could be either cytostatic or cytotoxic to the lactococcal cell. If alcohol is cytotoxic, it inhibits cell growth as long as the stress is applied, but the inhibition is reversed when the stress disappears. In contrast, if alcohol is cytotoxic, the inhibition is not reversed. The survival rate of _L. lactis_ exposed to the three alcohols was determined by quantifying the number of c.f.u. at various time points before and after the onset of alcohol stress. Only alcohol concentrations that had been found to inhibit growth to less than half-maximal growth rates were used. Fig. 4(a–c) show the growth in optical density before and after addition of ethanol, butanol or hexanol, respectively. Fig. 4(d–f) show the relative c.f.u. determination (survival) of _L. lactis_ exposed to ethanol, butanol or hexanol, respectively.

Interestingly, it could be noted from all curves in Fig. 4(d–f) that even though a steady increase in OD<sub>450</sub>~0.4 was observed for ethanol, butanol and hexanol at 75, 15 and 2.5 mg ml<sup>−1</sup>, corresponding to 10, 2, and 0.3% (v/v), respectively, indicating a high level of alcohol resistance.

**Alcohol does not induce increased acid sensitivity**

To assess if alcohol stress causes increased acid sensitivity in _L. lactis_, a growth-based assay was devised in which the buffering capacity of the medium could be increased by doubling the concentration of the buffering agent. This assay was used to distinguish between two contrasting models; one where the alcohol stress causes an increased acid sensitivity (model 1), or one where the alcohol stress does not cause an increased acid sensitivity (model 2). If the final OD was determined by a pH threshold (model 1), then a doubling of the buffering capacity of the medium would result in a doubling of the final OD of the culture reaching the same pH threshold. If, in contrast, the final OD was determined by other alcohol-induced thresholds (model 2), then a doubling of the buffering capacity would have no effect on the final yield, but would result in a higher final pH. In Fig. 3(a–c) a set of growth experiments has been represented in two types of plots. The first type of plot shows the increase in final pH upon doubling the buffering capacity of the medium (left axis) as a function of alcohol concentration. Since unstressed cultures use the excess buffering capacity to increase biomass production and continue fermentation until the normal pH threshold is reached, doubling the buffering capacity only insignificantly increased the pH level (0.1 pH unit), as shown in Fig. 3(a–c, filled circles) for the unstressed conditions (0 mg ml<sup>−1</sup>). The excess biomass production upon doubling the buffering capacity of the medium (right axis) is shown as a function of alcohol concentration in the second type of plot (open circles). Plots from all three alcohol stress conditions showed that higher alcohol concentrations follow model 2, since the increase in biomass yield is reduced to zero and the final pH is elevated. Butanol at 15 mg ml<sup>−1</sup> has the most severe effect on the increase in final pH (1.6 pH units).
**Fig. 1.** Stress response of *L. lactis* towards exogenous ethanol, butanol and hexanol. (a–c) Growth of *L. lactis* in GSAL medium in the presence of various concentrations of (a) ethanol, (b) butanol and (c) hexanol was monitored by measuring OD$_{450}$. Exogenous alcohol was added in mid-exponential phase (OD$_{450} ~0.4$). (d–f) Growth rates with one standard deviation, µ (h$^{-1}$), were calculated from the growth curves a minimum of three biological replica experiments (a–c) and plotted against the final concentrations of (d) ethanol, (e) butanol and (f) hexanol. (a) Concentrations of ethanol in mg ml$^{-1}$: 25 (filled circles), 55 (open circles), 75 (filled squares), 95 (open squares) and 115 (filled triangles). (b) Concentrations of butanol in mg ml$^{-1}$: 5 (filled circles), 10 (open circles), 15 (filled squares), 20 (open squares), and 25 (filled triangles). (c) Concentrations of hexanol in mg ml$^{-1}$: 1.0 (filled circles), 1.5 (open circles), 2.0 (filled squares), 2.5 (open squares) and 3.0 (filled triangles).
the number of c.f.u. under heat stress of L. lactis [34], and phase-contrast microscopy revealed that L. lactis forms chains when dividing during ethanol, butanol and hexanol stress (data not shown). As shown in Fig. 4(e), high butanol concentrations caused severe cell death with a fast death rate at a concentration of 25 mg ml\(^{-1}\). In contrast, high concentrations of ethanol (Fig. 4d) and hexanol (Fig. 4f) did not lead to cell death, suggesting that butanol is cytotoxic to L. lactis whereas both ethanol and hexanol are cytostatic. It should be noted that no decrease in optical density was observed for butanol-treated L. lactis cells; thus butanol is not suspected to cause cell lysis.

To substantiate the claim that high butanol concentrations are cytostatic, the cytotoxicity of the three alcohols was investigated in situ using live/dead staining. Live/dead staining uses a double-labelling technique with the green-fluorescent SYTO 9 and the red-fluorescent propidium iodide (PI). PI is frequently used in lactococcal research to distinguish dead from living cells as only cells with irreparably damaged membranes are stained [35–37], while SYTO 9 stains the DNA of both living and dead cells. Exponentially growing cells in GSAL medium were harvested and subjected to ethanol, butanol or hexanol at 120, 25 or 3 mg ml\(^{-1}\), respectively, for 5 min on ice before addition of the live/dead stain. Fluorescence microscopy imaging was subsequently used to determine the ratio of dead versus live cells (Fig. 5).

By inspection of the staining pattern in Fig. 5, it became clear that the ratio of dead cells was higher after butanol addition than after addition of ethanol or hexanol, in accordance with the results from the survival determination. Quantification of the live/dead ratio of L. lactis showed that ethanol at 120 mg ml\(^{-1}\) and hexanol at 3 mg ml\(^{-1}\) slightly lowered the percentage of live cells from 100 % in untreated cells to 86±8 % and 91±2 %, respectively. In contrast, butanol at 25 mg ml\(^{-1}\) decreased the fraction of living cells to 35±8 %. Even when the butanol concentration was reduced to 21 mg ml\(^{-1}\), a low survival rate was observed (25±17 %),

**Fig. 2.** Final yield, as defined by OD\(_{450}\), of L. lactis grown in GSAL and one standard deviation, shown as a function of increased ethanol (a), butanol (b) and hexanol (c) concentrations. Exogenous alcohol was added in mid-exponential phase (OD\(_{450}\) ~0.4), and final yield was measured after overnight incubation.
confirining that high butanol concentrations are toxic towards *L. lactis*.

To confirm the observation that high concentrations of butanol cause damage to the cell membrane, the ability of ONPG to cross the membrane under ethanol, butanol and hexanol stress was determined. *L. lactis* strain PRJ4621, with a high constitutive β-galactosidase activity, was used for detection of intracellular ONPG by conversion to ONP. Chloroform is usually used to permeabilize cells prior to determination of β-galactosidase activity in bacteria [32], and Fig. 6 shows that non-permeabilized cells produce very little ONP. Exposure to chloroform for 10 min resulted in high ONP production (1000 Miller units). Treatment with ethanol at 120 mg ml\(^{-1}\) for 10 min had no effect, while exposure to butanol or hexanol for 10 min (at 25 mg ml\(^{-1}\) and 2.5 mg ml\(^{-1}\), respectively) resulted in permeabilization close to that of chloroform (Fig. 6). The obtained β-galactosidase activity in *L. lactis* PRJ4621 was comparable to those observed in the literature [29].

Surprisingly, both butanol and hexanol caused permeabilization of the membrane towards ONPG, since only butanol was found to be cytotoxic. It was therefore hypothesized that the damage by hexanol could be reversible and thus cytostatic, while the damage by butanol was irreversible leading to cytotoxicity. To test this theory, exponentially growing cells of *L. lactis* PRJ4621 were first subjected to ethanol, butanol or hexanol (120, 25, or 2.5 mg ml\(^{-1}\), respectively) for 1 h. The stressed cells were then harvested, washed and assayed for production of ONP. The maximal ONP production rate was found for each condition by assaying a chloroform-permeabilized sample. The relative ONP production rate between untreated and chloroform-permeabilized cells for each stress condition is shown in Fig. 7. For unstressed cells, the intracellular ONP production rate was approximately 60-fold elevated by chloroform permeabilization. In contrast, for butanol-treated cells, the chloroform permeabilization only resulted in 5-fold elevation of the ONP production rate, showing that the cells are already highly permeable to ONPG. It is evident from Fig. 7 that the hexanol permeabilization is partially reversed, since chloroform is needed for ONPG to enter the cell. The 28-fold elevated ONP production between chloroform-treated and non-treated hexanol-stressed cells is almost half of the observed 59-fold change for the control condition. This supports the suggestion that butanol is cytotoxic because it creates irreversible damage to the membrane that results in increased permeability to small impermeable molecules. Due to the irreversible nature of butanol-induced membrane disruption, it could be hypothesized that butanol forms stable pores in the membrane, while the larger hexanol forms chains that align with the phospholipids. Upon removing hexanol stress, the aligned hexanol chains may distribute evenly between the cell membrane and cytosol resulting in a partial reversal of the permeabilization.

**Conclusion**

Combined results from the alcohol survival rate, live/dead staining through SYTO 9 and PI, and novel usage of the β-
Fig. 4. Relative survival of *L. lactis* grown in GSAL after addition of ethanol (a, d), butanol (b, e) or hexanol (c, f). Exogenous alcohol was added in mid-exponential phase (OD<sub>450</sub>~0.4 m, reference time point). Relative survival and standard deviation were determined by measuring changes in c.f.u. in triplicate experiments, following the onset of stress relative to the reference condition. (a, d) Concentrations of ethanol in mg ml<sup>-1</sup>: 55 (filled circles), 75 (open circles), 95 (filled squares), and 120 (open squares). (b, e) Concentrations of butanol in mg ml<sup>-1</sup>: 10 (filled circles), 20 (open circles), 22.5 (filled squares), and 25 (open squares). (c, f) Concentrations of hexanol in mg ml<sup>-1</sup>: 2.0 (filled circles), 2.5 (open circles), 2.75 (filled squares), and 3.0 (open squares).
galactosidase assay, revealed that while high concentrations of ethanol and hexanol were cytostatic to L. lactis, high concentrations of butanol were cytotoxic through disruption of the cell membrane.

For the future industrial use of LAB as butanol producers, the cytotoxic effect of butanol needs to be addressed before selecting production strains. In this regard, L. lactis is unsuitable unless the cytotoxicity of butanol is diminished. Adaptive laboratory evolution has been initiated to provide butanol-resistant strains for the further characterization of a L. lactis strain with improved butanol tolerance.

**Fig. 5.** In situ live/dead imaging of L. lactis (a), or L. lactis exposed to 120 mg ml⁻¹ ethanol (b), 25 mg ml⁻¹ butanol (c) or 3 mg ml⁻¹ hexanol (d). Exponentially growing cells were harvested and subjected to ethanol, butanol or hexanol stress before staining with SYTO 9 and propidium iodide. Cells with physically intact membranes are shown in green while cells with damaged membranes are shown in red.

**Fig. 6.** Intracellular production of ONP from ONPG with one standard deviation in a Lac⁺ derivative of L. lactis. Exponentially growing cells of a L. lactis Lac⁺ derivative (PRJ4621) were harvested and permeabilized with chloroform, ethanol (120 mg ml⁻¹), butanol (25 mg ml⁻¹) or hexanol (2.5 mg ml⁻¹) following by determination of ONP production from ONPG.

**Fig. 7.** Relative ONP production rate in a Lac⁺ derivative of L. lactis. Exponentially growing cells of a L. lactis Lac⁺ derivative (PRJ4621) were subjected to either non-stressed, ethanol (120 mg ml⁻¹), butanol (25 mg ml⁻¹) or hexanol (2.5 mg ml⁻¹) stress for one hour following which the cells were either permeabilized with chloroform or not, and ONP production from ONPG was determined. The relative difference in ONP production between the chloroform-permeabilized and non-permeabilized cells with one standard deviation is shown.

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**Conflicts of interest**
The authors declare that there are no conflicts of interest.

**References**


34. Chen J, Shen J, Ingvar Hellgren L, Ruhdal Jensen P, Solem C. Adaptation of Lactococcus lactis to high growth temperature leads to a dramatic increase in acidification rate. Sci Rep 2015;5:14199.


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