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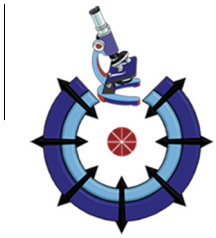

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ARTICLE

# 1,2 Propanediol utilization by *Lactobacillus reuteri* DSM 20016, role in bioconversion of glycerol to 1,3 propanediol, 3-hydroxypropionaldehyde and 3-hydroxypropionic acid

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1,3 Propanediol (1,3 PD);
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3-Hydroxypropionaldehyde (3-HPA)

**Abstract** The objective of the presented work is to demonstrate the metabolism of 1,2 propandiol by *Lactobacillus reuteri* and to elucidate the metabolites produced during the process. This Metabolic pathway is crucial for biotechnological applications using *L. reuteri* in bioconversion of glycerol to industrially important plate-form chemicals. *L. reuteri* grown on minimal media containing 1,2 propanediol was able to utilize the compound as a sole carbon and energy source. The growth of the bacteria was linear with time; however the specific growth rate was significantly low compared to bacteria grown on the same media in the presence of glucose.

The fermentation of 1,2 propanediol by *L. reuteri* in presence and absence of glucose was followed for 72 h and the metabolites produced during the process were detected using HPLC. 1,2 Propanediol was completely converted to propionaldhyde in a time dependent fashion, this process had a higher rate in presence of glucose. Consequently the produced propionaldhyde was converted to propionic acid and propanol in a skewed equimolar manner. In presence of glucose: acetic acid, lactic acid, succinic acid and ethanol were detected while in absence of glucose only minute amounts of acetic acid and lactic acid were detected which indicates presence of different metabolic pathways for glucose and 1,2 propanediol metabolism. Resting cells of *L. reuteri* induced in presence of 1,2

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propanediol have shown significant capabilities to convert aqueous glycerol to 1,3 propanediol, 3-hydroxypropionaldhyde and a compound proposed to be 3-hydroxypropionic acid as detected by gas chromatographic technique.



## 1. Introduction

Degradation of the common plant cell wall sugars fucose and rhamnose under anaerobic conditions produces 1,2 PD, which is used as a carbon and energy source by a number of enteric bacteria and by bacteria that grow in environments such as aquatic sediments where 1,2 PD is readily available due to the breakdown of plant material [13]. Catabolism of 1,2 PD requires a complex pathway that is ultimately provides the cell with propionyl-CoA, an electron sink, and ATP [13]. The proteins involved in 1,2 PD degradation are encoded by the genes of 1,2 PD utilization (*pdu*) operon [11]. These genes code for diol dehydratase and other proteins which have been involved in production of 3-HPA and 1,3 PD from glycerol [13,18,26].

Natural producers of 1,3 PD and 3-HPA from glycerol are of genera *Klebsiella* [10,19,37], *Clostridia* [6,18,24], *Citrobacter* [28], *Enterobacter* [38] and *Lactobacilli* [27]. Several Clostridial species such as non-pathogenic *Clostridium butyricum* [26], *Clostridium pasteurianum* [1] grow on glycerol and form 1,3 PD. Facultative anaerobes such as *Klebsiella pneumoniae* and *Citrobacter freundii* also appeared to be suitable for 1,3 PD and 3-HP production. Although it might be easier to handle facultative anaerobes, but since all these strains are classified as opportunistic pathogens, special safety precautions are required to grow them. Along with *K. pneumoniae*, other species of same genera have also been exploited for 1,3 PD and 3-HP production, such as *Klebsiella oxytoca* [37], *Lactobacillus brevis*, *Lactobacillus buchneri* [34].

*Lactobacillus reuteri* is a heterofermentative lactic acid bacterium and is frequently found in the gastrointestinal tract of humans and other animals. *L. reuteri* has been reported to exhibit "probiotic" properties. It has been extensively analyzed for probiotic applications, including its safe administration to healthy individuals, its ability to colonize the intestine, as a diarrhea therapeutic agent, as an inhibitor of bacterial pathogens, and the immunological modulation of the gastrointestinal mucosa [3,20,25,29,33,36]. Some strains of *L. reuteri* have the ability to produce and excrete the broad-spectrum antimicrobial compound reuterin, which is structurally identical to 3-HPA, during anaerobic metabolism of glycerol [5,31,32]. The probiotic effects of *L. reuteri* have been proposed to be largely associated with the production of reuterin, and this antimicrobial substance is also an effective food preservative agent [16,35].

*L. reuteri* have been also shown to use glycerol as an external hydrogen acceptor source during fermentation [22]. This study was undertaken to investigate the correlation between the utilization of 1,2 PD by *L. reuteri* and its potential for bioconversion of glycerol to 1,3 PD, 3-HPA and 3-HP.

## 2. Materials and methods

### 2.1. Bacteria and growth conditions

*L. reuteri* DSM 20016 was obtained from DSMZ culture collection (Germany). The stock culture was kept at −20 °C in solution containing MRS broth (Defco, England) and 20% glycerol. For inoculum preparation, 0.25 ml of stock culture was added to 100 ml MRS broth supplemented with 20 mM 1,2 propanediol, incubated at 37 °C for 8 h under anaerobic condition. This induction process was done once more before the culture being used for inoculation of the fermentation media.

### 2.2. 1,2 Propanediol metabolism

In a 500 ml conical flask, 250 ml Modified MRS (MOD-MRS) containing 100 mM 1,2 propanediol were inoculated with 1% of the pre-cultured *L. reuteri*. The micro-organism was left to grow anaerobically for 72 h at 37 °C. MOD-MRS composition (per liter) 5 g Bactopeptone, 4 g Lab-lemco (Meat Extract), 2 g Yeast Extract, 0.5 ml Tween 80, 1 g $K_2HPO_4$, 3 g $NaH_2PO_4 \cdot H_2O$, 0.6 g $CH_3COONa$, 0.3 g $MgSO_4 \cdot 7H_2$ and 0.04 g $MnSO_4 \cdot H_2O$. Ten milliliters samples were removed at different time intervals and pelleted. Supernatant was stored at −20 °C until tested for presence of metabolic products.

### 2.3. Effect of glucose addition on 1,2 PD metabolism

To the previously mentioned MOD-MRS media supplemented with 1,2 PD glucose were added (40 mM) at zero time. In a second set of experiments 40 mM glucose were added prior to 1,2 PD depletion from the media. In the third set of experiments glucose solution was fed batched so that 30 mM were added at zero time and the remaining 10 mM were added prior to 1,2 PD depletion from the media. Control experiments were performed by culturing *L. ruteri* cells on the same media without glucose addition. Samples (10 ml) were taken every 12 h and OD was measured to calculate cell density. Cells were centrifuged and supernatant was tested for production of propionic acid, propanol and propionaldehyde.

Biotransformation of glycerol to 1,3 propanediol by resting cells of *L. reutri* Biotransformation was performed anaerobically in a cooling thermomixer KTMR-133 (HLC-Biotech, Germany) set at 800 rpm and 37 °C unless mentioned otherwise reaction mixture (2 ml) volume contained resting cells of *L. reuteri* (20 mg) and glycerol (100 mM) in distilled water. At different time intervals samples were taken and quantitative determination of glycerol l,3-hydroxypropionaldyde and 1,3 propandiol was carried out.