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Molecular and Probiotic Characterizations of *Lactobacillus reuteri* DSM 12246 and Impact of pH on Biomass and Metabolic Profile in Batch-Culture

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Abstract

Lactobacillus reuteri is a powerful probiotic and adjunct functional culture candidate received a lot of scientific attention as it is one of the few endogenous "*Lactobacillus*" species found in the gastrointestinal tract of vertebrates, including humans, rats, pigs and chicken. The organism has been widely utilized as a probiotic in humans and animals for many years. In the present work *L. reuteri* DSM 12243; a high reuteri producer strain was molecularly characterized by 16SrRNA and RAPD analyses further, the probiotic properties including acid resistance, bile tolerance, adhesion to epithelial gastric cells, and antibiotic susceptibility were also assessed. Furthermore, the effect of pH on biomass production and metabolic profile of *L. reuteri* DSM 12246 in batch-culture was studied. The *L. reuteri* DSM 12246 showed a high similarity with *L. reuteri* strain I49 KR 36477 (100%) and type strain ATCC 55730 (99% of identity). The strain adhered well to $CaCO_2$ cells and showed to be a highly resistance to acid juice (pH 3.0), with 0.7 $\log_{10}$ cfu/ml reduction in count after 60 min exposition. There is no significant change in the cell count after exposure to bile salts. In batch-cultures, at low pH values both glucose consumption and metabolites were low while the production of lactic acid was noticeable. Maximum biomass was reached at pH 5.5, with growth rate of μ = 0.641/h. The switch in pH values from 3.7 to 6.7 resulted in raising of glucose depletion as well as in the yield of acetate and ethanol. It is concluded that *L. reuteri* DSM 12246 was deemed as a successful candidate to be used as potential probiotic.

Keywords

Lactobacillus reuteri DSM 12246, Probiotic, Acid and Bile Tolerance, Adherence, pH, Metabolic Profile

1. Introduction

Since Gerhard Reuter, in the 1960s discovered *Lactobacillus reuteri* species; which was known previously as "*Lactobacillus ferment* biotype II", a lot of research work has been triggered to isolate the culture from different sources, study the factors affecting their growth and conduct animal and human trails to evaluate they health impact as a powerful probiotic candidate. The organism was not only isolated from human intestinal and feces but also from many mammals and vertebrates. In human trails, *L. reuteri* were consistently isolated from same subjects over experimental period thus supported that the microbe is constitute stable component of the human microbiome [1].

Lactobacillus reuteri DSM 12246 has been found capable to produce high amount of reuterin; a broad spectrum antimicrobial substance derived from glycerol, able to cease the growth of different pathogens in meat and dairy products [2] [3]. Recently, reuterin production was optimized in semi-hard cheese using *L. reuteri*as adjunct culture [4]. Genotypic characteristics are becoming an indispensable figure of probiotic candidates in human to assign the probiotic effects to well-identified strains hence, avoid label misleading and maintain consumer confidence [5]. Recently, the defining of potential effects of probiotics on the intestinal microbiota, nutrition, immunity and mechanism of action are depending on genomic analysis of new target probiotics [6]. Selection criteria of probiotic strains include survival during transit and colonization of the human gastrointestinal tract [7].

The present study aimed to characterize the molecular and probiotic features of *L. reuteri* DSM 12246 further, investigate the effect of pH changes on fermentation parameters included biomass production and metabolic profile in batch-culture.

2. Materials and Methods

Microorganism, medium and inoculum preparation

Lactobacillus reuteri DSM 12246, previously known as *L. reueti* 12002 [8] was stored at −80˚C. For excremental trials, the culture was propagated in modified MRS (m-MRS) as previously described [8] for 16 hours at 37˚C.

Sequencing of 16S rRNA gene

The culture of *L. reuteri* DSM 12246 were subjected to molecular characterization by sequencing of 16SrRNA gene at AAT-Advanced Analytical Technologies srl., Piacenza, Italy. The culture was cultivated an aerobically (Anaerogen™; Oxoid, Basingstoke, Hampshire, UK) on MRS agar for 16 hours. To extract DNA, pure colonies were treated by Fast Prep-24 (MP Biomedicals, UK) inaccordance to manufacturer instructions. DNA was amplified using p10f, p1100r, p765f and p782r primers pair complementary to the conserved regions of 16S rRNA gene and PCR amplicons were used as templates for sequencing of the whole 16S rRNA gene (BMR Bio Molecular Research, Padova University, Italy). Six sequences of 16S rRNA gene of *L. reuteri* DSM 12246 strain were obtained.

Sequence results from each amplified fragment were aligned and assembled into contigs to obtain of the entire 1500-bp consensus sequence. Consensus sequence was compared with available sequences in GenBank using the BLASTN tool through the National Centre for Biotechnology Information (NCBI) server (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

RAPD-PCR analysis

DNA was extracted from pure colonies using FTA Starter pack (What man, Maidstone, UK) in accordance with manufacturer instruction. RAPD-PCR amplification using primer RAPD2 (5'-AGCAGCGTCG-3') was performed according to Fontana *et al.* [9] and the amplification product was first electrophoresed at 100 V on 2.5% agarosegel and then stained with ethidium bromide.

Determination of acid resistance

Fresh pellets of the strain *L. reuteri* DSM 12246 grown in MRS broth for 16 hours were centrifuged, washed with PBS buffer (50 mmol, pH 7). The cells resuspended at concentration of OD_{600} = 1 inacid solution containing pepsin A (1%) adjusted at pH 3 with HCl 1 M for 1 hour at 37˚C. The viable counts were determined before and after 60 minutes of acid treatment by plating on MRS agar (Oxoid) after serial 10-fold dilution in maximum recovery diluent. Plates were incubated an aerobically at 37˚C for 48 hours.

Resistance to simulated gastric and pancreatic juices

To determine the upper gastro intest in altransit tolerance of *L. reuteri* DSM 12246, a 16 hours culture was separated by centrifugation, washed and resuspended in PBS (pH 7.0), then were divided into two aliquots before inoculated in simulated gastric and pancreatic juices as described by Charteris *et al.* [10]. Bacterial suspension was inoculated at 37˚C in a fresh daily prepared simulated solution of gastric juice containing pepsin (0.3%; NaCl 0.5%) from porcinestomach mucosa (Sigma-Aldrich) at pH 2.0 and in pancreatic intestinal juice (pH 8) containing pancreatin USP (0.1%; NaCl 0.5%). To determine the survival rate, aliquots were taken directly after inoculation and intervals of 1, 90 and 180 minutes for gastric juice treatment and after 1 and 240 minutes in case of pancreatin juice. The viability was assessed by plating on MRS agar (Oxoid). Plates were incubated an aerobically at 37˚C for 48 hours.

Resistance to bile salt

Bile tolerance of *L. reuteri* DSM 12246 was evaluated according to Kaushik *et al.* [11] using Ox bile commercially available preparation (Fluka, Sigma-Aldrich; cat #70168). Fresh culture of *L. reuteri* DSM 12246 strain was inoculated in MRS broth (Oxoid) supplemented with 0.3% and 0.5% (w/v) of bile salts followed by incubation at 37˚C. Aliquots were withdrawn at 0, 1 and 2 hours intervals thenplated on MRS agar (Oxoid) and incubated an aerobically at 37˚C for 24 hours.

Adhesion to $CaCO_2$ cells

The adhesion to $CaCO_2$ cells test was performed in outsourcing service at Padova University (Italy). The ability of *L. reuteri* DSM 12246 to adhere to intestinal mucosa was determined on human $CaCO_2$ cells an intestinal epithelial cell line. Vital bacteria were labeled with fluorescent probe 5-carboxyfluorescein di-

acetate, Acetoxymethyl ester (5-CFDA, AM, Molecular Probes, Invitrogen) and ≅ 10^8 cfu/ml of culture were added to cell monolayers (MOI 1:10) in absence of antibiotics. Cells and bacteria were co-incubated 120 minutes at 37˚C and then non adhering bacteria were removed by washing three times in sterile PBS buffer. Cells were then treated with PBS-Triton 0.5%. Adhering bacteria were quantified by measuring the fluorescence at 485 nm excitation and 535 nm emissions, using a fluorescence plate reader (Infinite 200, Tecan). Assays were performed in duplicate three times. One-way ANOVA test followed by a Bonferroni's multi-comparison test was performed to determine statistical significance.

Antibiotic susceptibility profile

Strain sensitivity to antibiotics was evaluated by means of serial two-fold dilution procedures in agar as suggested by the EFSA Scientific Opinion [12]. Minimum inhibitory concentration (MICs) of nine antibiotics was determined for the strain according to the ISO 10932 IDF 223:2010 [13]. After incubating the plates under anaerobic conditions at 37˚C for 48 hours MIC values were read as the lowest concentration of an antimicrobial agent that inhibits bacterial visible growth. The test was performed as two replicates. The accuracy of susceptibility testing was monitored by parallel use of the quality control strain *L. paracasei* ATCC 334 which should use as internal standard according to the ISO 10932 protocol.

Effect of pH on biomass and metabolic profile in batch-culture

The culture was grown in modified MRS substrate (m-MRS) (sodium acetate and Tween 80 concentrations of 1.5 g/l and 1.2 g/l, respectively. Glucose was separately sterilized and added to the broth to give a final concentration of 1% (w/v).

Batch cultures experiments were carried out in Applikon fermenter connected with Applikon ADI 1020 control unit (Applikon® Biotechnology B.V., Delft, Netherlands). The fermenter conditions were set as 900 ml effective fermenter volume, 250 rpm stirring rate, continuous flushing with pure sterilized nitrogen (99.998% purity) to maintain anaerobic conditions and the temperature was set at 37˚C. The effect pH was done in m-MRS broth at pH rang of 3.7, 4.3, 5.0, 5.5, 6.0 and 6.7 which maintained by the addition of 1.5 mol NaOH or 1.5 mol H_3PO_4. Each batch was run for 12 hours and samples were withdrew interval of 2 hours [8].

Measurement of biomass concentration and growth rate

The growth was followed by measuring optical density at 600 nm (OD_{600}) using a Shimadzu UV-1201 spectrophotometer (Shimadzu Co., Japan). Samples were diluted appropriately to allow the OD_{600} to fall between values of 0.05 and 0.5. Cell dry weight (CDW) was calculated on the basis of measured optical density using a previously prepared standard curve of plotting OD_{600} values *vs* CDW. The specific growth rate was calculated by plotting OD_{600} *vs.* time.

Measurement of substrate components and metabolites

Glucose, acetate, lactate, and ethanol were measured using an HP1100 HPLC

(Agilent, USA). Collected samples were centrifuged to separate the pellet then filtered using 0.2 μ disc filters. Separation proceeded on a HPX-87H column (300 × 7.8 mm, 9 μ particle size, Bio-Rad, Richmond, CA)at 30˚C with a cation H1 Micro-Guard column (30 × 4.6 mm, Bio-Rad) at an eluent flow-rate of 0.6 ml/min of 10 mmol/l H_2SO_4. Metabolites were identified and quantified using an HP1047A refractive index detector in compared to retention times with those of standards (Sigma) [8].

3. Results and Discussion

Molecular characterization

Figure 1 presents the partial nucleotide sequence (length = 1565) of 16S rRNA of *L. reuteri* DSM 12246. Sequence similarity analyses were done using Blast searches for *L. reuteri* DSM 12246 against sequences available in gene bank. Phylogenetic tree construction multiple sequence alignments performed using ClustalWv1.81 with default based on 16S rRNA parameters. On the basis of the result multiple alignments a phylogenetic tree was calculated for each strain by applying the using the Neighbor-joining method implanted in MEGA software version 4.1.

Species identification of the strain was determined on the basis of the highest scores obtained by the alignment with known species. For strain DSM 12246 the highest similarity score was obtained with *L. reuteri* strain I49 KR 36477 (100%) and ATCC 5573016S ribosomal RNA gene (99% of identity). Meanwhile, *L. reutei* DSM 12246 showed lower similarity when compared to other *Lactobacillus* species; *i.e. L. sharpeae* NR044711, *L. rhamnosus* AB008211.1 and *L. casei* NR041893.1 (Figure 2).

RAPD patterns obtained for *L.reuteri* DSM 12246 is shown in Figure 3. Depend on used primer the *L. reuteri* DSM 12246 exhibited six amplified DNA fragments within size range between 240 to 600 bp. The RAPD primer was not directed against a particular genetic locus, this resulted in band patterns often exhibit a poor reproducibility. For this reason, based on accumulating evidence from previous studies, PFGE is considered best for strain molecular typing because of its sensitivity, consistency and accuracy.

NGTTTGATCCTGGCTCAGGATGAACGCCGGCGGTGTGCCTAATACATGCAAGTCGTACGCACTGGCCCAACTGATTGATGGTGC
TTGCACCTGATTGACGATGGATCACCAGTGAGTGGCGGACGGGTGAGTAACACGTAGGTAACCTGCCCCGGAGCGGGGGATAA
CATTTGGAAACAGATGCTAATACCGCATAACAACAAAAGCCACATGGCTTTTGTTTGAAAGATGGCTTTGGCTATCACTCTGGG
ATGGACCTGCGGTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCGATGATGCATAGCCGAGTTGAGAGACTGATCGG
CCACAATGGAACTGAGACACGGTCCATACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGGCGCAAGCCTGATGGA
GCAACACCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAGCTCTGTTGTTGGAGAAGAACGTGCGTGAGAGTAACTGTTCACG
CAGTGACGGTATCCAACCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGA
TTTATTGGGCGTAAAGCGAGCGCAGGCGGTTGCTTAGGTCTGATGTGAAAGCCTTCGGCTTAACCGAAGAAGTGCATCGGAAAC
CGGGCGACTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGGAATGCGTAGATATATGGAAGAACACCAGTGGC
GAAGGCGGCTGTCTGGTCTGCAACTGACGCTGAGGCTCGAAAGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGTCCATG
CCGTAAACGATGAGTGCTAGGTGTTGGAGGGTTTCCGCCCTTCAGTGCCGGAGCTAACGCATTAAGCACTCCGCCTGGGGAGTA
CGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCTACGCGAA
GAACCTTACCAGGTCTTGACATCTTGCGCTAACCTTAGAGATAAGGCGTTCCCTTCGGGGACGCAATGACAGGTGGTGCATGGT
CGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTTACTAGTTGCCAGCATTAAGTTGGG
CACTCTAGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGACGACGTCAGATCATCATGCCCCTTATGACCTGGGCTACA
CACGTGCTACAATGGACGGTACAACGAGTCGCAAGCTCGCGAGAGTAAGCTAATCTCTTAAAGCCGTTCTCAGTTCGGACTGTA
GGCTGCAACTCGCCTACACGAAGTCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTAC
ACACCGCCCGTCACACCATGGGAGTTTGTAACGCCCAAAGTCGGTGGCCTAACCATTATGGAGGGAGCCGCCTAAGGCGGGAC
AGATGACTGGGGTGAAGTCGTAACAAGGTAGCCGTAGGAGAACCTGCGGCTGGATCACCTCCA

Figure 1. Nucleotide sequence of 16S rRNA of *L. reuteri* DSM 12246.

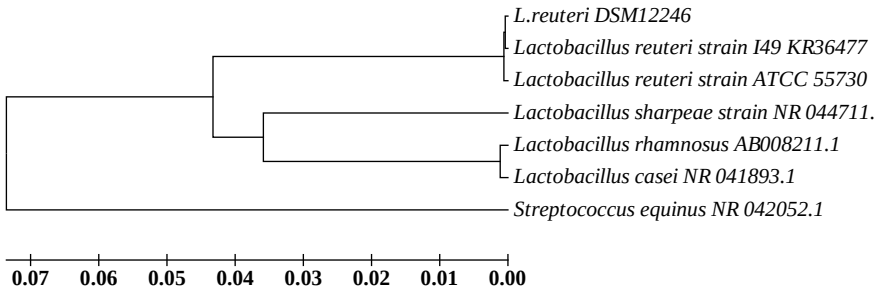

Figure 2. Phylogenetic tree analysis of *L. reuteri* DSM 12246 and the lineage of related species based on 16S rRNA nucleotide sequences.

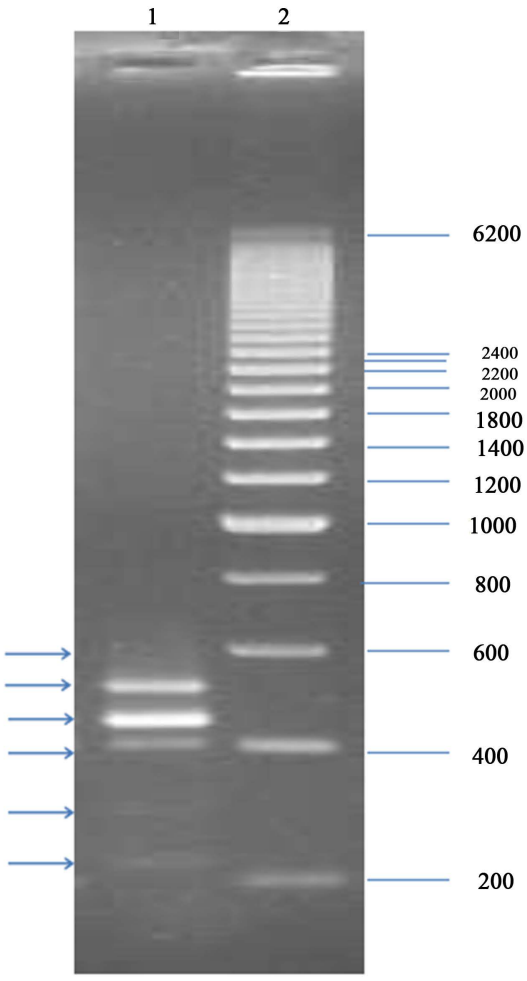

Figure 3. RAPD profile of *L. reuteri* DSM 12246 (lane 1) in compared to molecular weight marker 200 bp (Promega, Italy) (lane 2).

Probiotic characteristics

Resistance to gastrointestinal barriers

The effect of different gastrointestinal barriers against *L. reuteri* DSM 12246 is shown in Table 1. The viability of *L. reuteri* DSM 12246 was reduced by 0.7 $\log_{10}$ cfu/ml after one hour of incubation in acid juice. Exposed of *L. reuteri* DSM 12246 to simulated gastric juice caused a loss of viability by 3 $\log_{10}$ after three

Table 1. Probiotic characteristics and resistance to gastrointestinal biological barriers of *L. reuteri* DSM 12246.

Treatments	Time (min)	CFU/ml
Acid juice	0	6.3×10^8
	60	1.1×10^8
Simulated gastric juice	0	2.0×10^8
	1	2.0×10^8
	90	6.5×10^7
	180	1.6×10^5
Stimulated pancreatic juice	0	2.0×10^8
	1	1.4×10^8
	240	1.3×10^8
0.3% of bile salts	0	7.5×10^6
	60	7.0×10^6
	120	6.7×10^6
0.5% of bile salts	0	6.0×10^6
	60	6.0×10^6
	120	5.3×10^6

hours while, stimulated pancreatic juice did not demonstrate significant inhibitory effect as the viable count only decreased by 0.1 $\log_{10}$ cfu/ml. Similarly, *L. reuteri* DSM 12246 at concentration of 0.3% and 0.5% Ox bile salts were resistible only decreased by 0.05 and 0.1 $\log_{10}$ cfu/ml respectively.

Adhesion to $CaCO_2$ cells

The absolute number of adherent bacterial cells to $CaCO_2$ an intestinal epithelial cell line is presented in Figure 4. *L. reuteri* DSM 12246 showed better adhesion ability to human intestinal epithelial cells, as opposed to the negligible adhesion of *L. paracasei* and *L. gasseri*, although resulted significantly less efficient than *L. rhamnosus.*

Antibiotic susceptibility profile

Out of nine types of tested antibiotics, *L. reuteri* DSM 12246 showed to be sensitive to eight of it with equal or lower values than cut-off limits established by EFSA for *L. reuteri* (Table 2). In general, the reduced susceptibility of lactobacilli to aminoglycosides is thought to occur because of membrane impermeability [14]. It has also been documented that the presence of MRS medium may interfere with the susceptibility testing of specifically the aminoglycosides [15]. The MIC values for erythromycin were above to the EFSA cut-off for *L. reuteri* species (Table 2). *L. reuteri* DSM 12246 strain grew to higher dilutions of antibiotic tested for erythromycin (8 μg/ml).

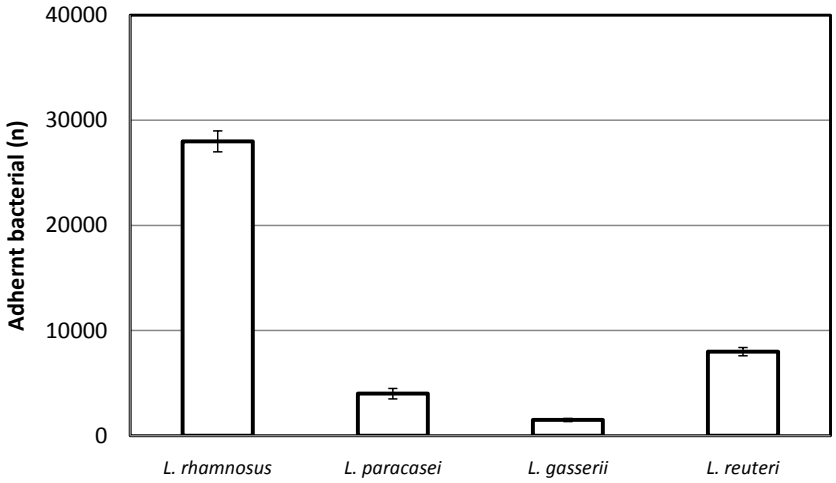

Figure 4. Number of adherent bacterial cells of *L. reuteri* DSM 12246 to $CaCO_2$ cells.

Table 2. Antibiotic susceptibility (MICs value, μg/ml) of *L. reuteri* DSM 12246 in agar dilution method.

Antibiotic	*L. reuteri* DSM 12246	[a]EFSA breakpoint valueof*L. reuteri*
Gentamycin	4	8
Kanamycin	64	64
Streptomycin	32	64
Tetracycline	8	16
Erythromycin	8	1
Clindamycin	4	4
Chloramphenicol	4	4
Ampicillin	1	2
Neomycin	8	8

[a]Adapted from Branton *et al.* [17].

Resistance to erythromycin is commonly attributed to *erm* genes. In a previous study Egervärn and collaborators [16] reported genetic characterization of 38 strains of *L. reuteri* from various human and animal habitats revealed that *tet* (W) and *erm* genes were found in almost all *L. reuteri* strains with atypical MICs for tetracycline and/or erythromycin and thus appear to be the most common resistance determinants within this species. Seven genes were found to be associated with antibiotic resistance, including a beta-lactamase, a MATE family multiantimicrobial extrusion protein, and 2 topoisomerase and 2 DNA gyrase genes associated with resistance to fluoroquinolones. These antibiotic resistance elements were demonstrated to be common to other lactobacilli [17]. The transferability of the tetracycline resistance gene *tet* (W) of *L. reuteri* as a donor of resistance genes in the human gut to related lactobacilli and unrelated genus of enterococci, and bifidobacteria was investigated meanwhile, no gene transfer was demonstrated under the tested conditions [18].

In most lactobacilli antibiotic genetic codes carried on plasmids hence, plasmid curing technique could be used to avoid any possibility of gene transformation. Curing plasmids (n = 2) from *L. reuteri* ATCC 55730 by protoplast technique resulted in a daughter antibiotic sensitive strain, DSM 17938 which is used in clinical trials [19]. The FDA evaluation considered *L. reuteri* strain DSM 17938 as GRAS [20].

Effect of pH on biomass and metabolites profile in batch culture

Glucose converted by heterofermentative lactic acid bacteria to produce equimolecular amounts of lactate, acetate and carbon dioxide or ethanol [21]. Changes in the culture conditions might lead in the prevalence of one of these metabolites. When *L. reuteri* was cultured in m-MRS maintained at pH rang of 3.7, 4.3, 4.7, 5.0, 5.5 and 6.7 in batch-culture results, several differences in the growth rate and metabolites were observed (Figure 5 and Figure 6 and Table 3). The specific growth rate reached its maximum at pH 5.5 (μ = 0.641/h) and reduced at lower and higher pH values. Similarly, biomass followed the same pattern (Figure 5).

Figures 6(a)-(f) presented the kinetics of both substrate consumption and end-productformation at different pH values. At low pH values of 4.3 and 4.7,

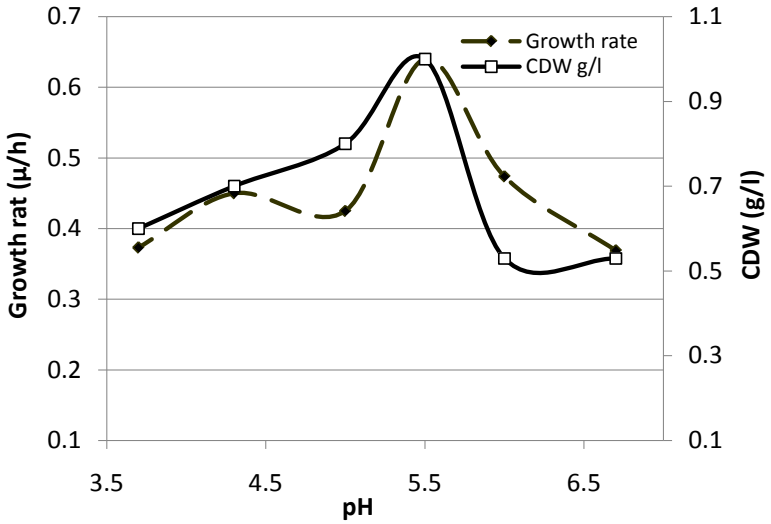

Figure 5. The specific growth rate and cell dry weight of *L reuteri* DSM 12246 during 12 h incubation in batch-culture at different pH values. growth rate; cell dry weight.

Table 3. Effect of pH on lactate, acetate and ethanol production in batch-culture of *L. reuteri* DSM 12246 grown under anaerobic conditions.

Yields	pH values					
	3.7	4.3	5.0	5.5	6.0	6.7
$Y_{lactate}$	29.7	25.0	45.0	49.0	55.5	68.5
$Y_{acetate}$	3.1	4.3	4.6	5.9	7.1	4.8
$Y_{ethanol}$	25.9	29.0	59.0	62.5	61.5	83.9

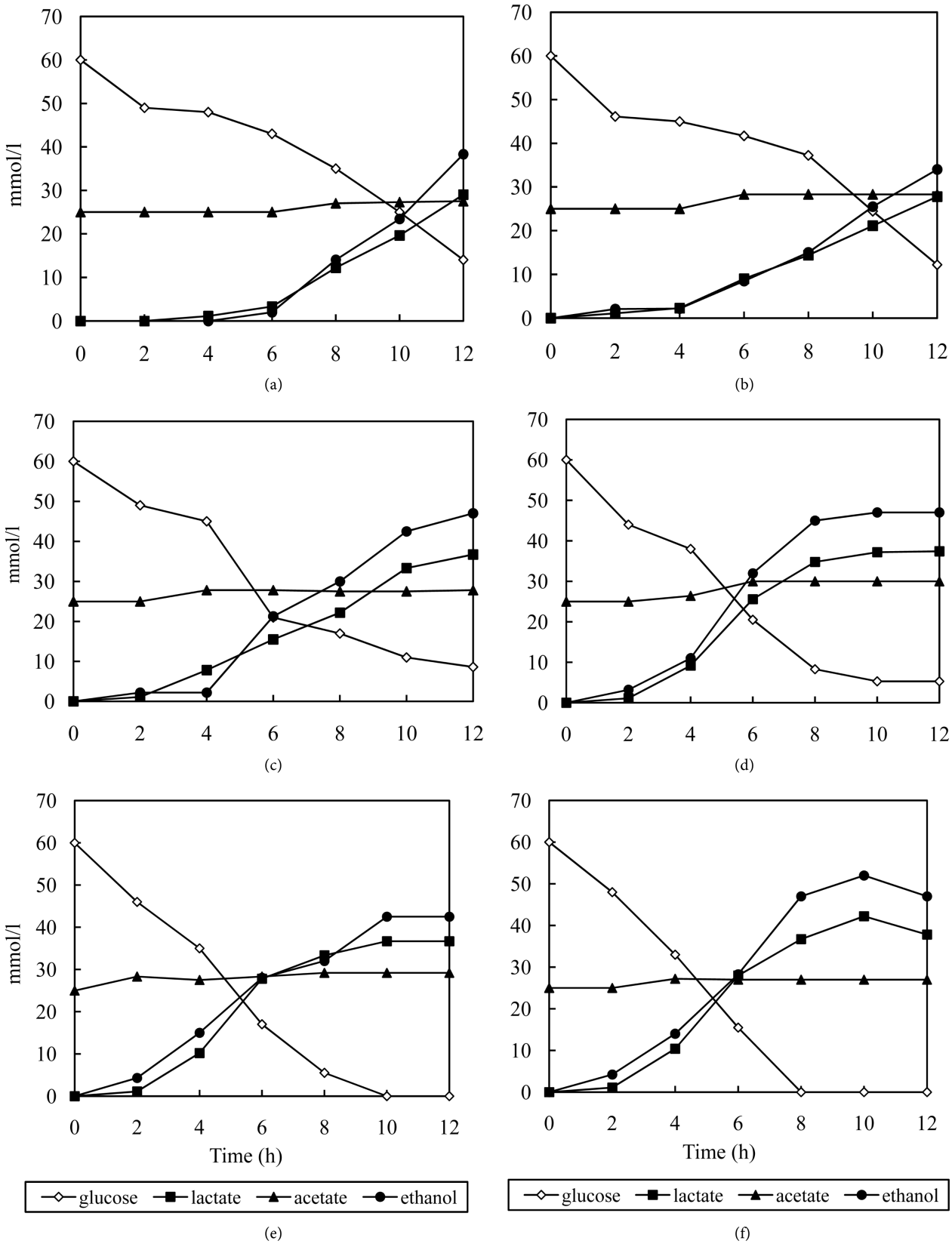

Figure 6. Effect of pH on metabolic profile by *L. reuteri* DSM 12246 in batch-culture. (a) pH 3.7; (b) pH 4.3; (c) pH 5.0; (d) pH 5.5; (e) pH 6.0; (f) pH 6.7. —◇— residual glucose; —■— lactate; —▲— acetate; —●— ethanol.

glucose utilization was low but kept efficient, 46 and 48 mmol residual sugar was found respectively at the end of incubation (**Figure 6(a)** and **Figure 6(b)**). Formate was not detected in any of the fermentations. Increasing pH to6.7 coincided with fast sugar depletion (8 hours) and the highest rate of glucoseutilization (**Figure 6(f)**). It is reported that heterofermentative lactobacilli (*i.e. L. buchneri* and *L. brevis)* cannot grow under anaerobic conditions on glucose, because they cannot reduce acetyl phosphate to ethanol, an essential product to maintain the overall redox balance [21]. In contradiction the behavior of *L. reuteri* DSM 12246 was quite different as ethanol was the major end-product at all pH values tested under anaerobic conditions. In agreement with present results, the same behavior of *L. reuteri* was reported by Ragout *et al.* [22].

At pH 4.3, culture began to produce lactate 2 hours earlier than ethanol at glucose depletion percent of 20% and 28% for each metabolite respectively (**Figure 6(a)**). Meanwhile, ethanol became a dominate metabolite after 12 hours. Acetate profile appeared not changed, a small increasing concentration being found after 8 hours and kept at the same level until the end of incubation time. A comparable trend was noticed at pH 4.7 with a typical plateaus were observed of both substrate consumption and metabolites formation (**Figure 6(b)**).

Ethanol production rate speeded up as pH culture increased (**Figure 6(c)** and **Figure 6(f)**). Meanwhile at the end of incubation, no significant change of final concentration was observed at pH's 5.0 to 6.7. In parallel with glucose depletion, the concentration of ethanol was 1.2 times that of the acids (**Figure 6(e)** and **Figure 6(f)**). A similar pattern of events was reported for other microorganisms [23]. It is suggested that the switch in metabolites from organic acids to ethanol synthesis might provide some protection of injured cells as a result of over-acidification in the growth medium [24].

Shifting the pH value from 3.7 to 6.7 led to increase the yield of ethanol per cell biomass ($Y_{x/p}$) by 3.24-foldafter 12 hours of fermentation (**Table 3**). The $Y_{lactate}$ also showed a significant increase from $Y_{pH3.7}$ = 29.7 to $Y_{pH6.7}$ = 68.5 mmol/g. The $Y_{acetate}$ remained fairly constant; ≅ 3.1 - 4.6 mmol/g at lower pH values while only increased at pH's 5.5 and 6.0 by 1.9 and 2.29-folds respectively (**Table 3**). This is might be due to acetate consumption by *L. reuteri* which previously produced it [22]. Form the present results it is postulated that *L. reuteri* DSM 12246 not only possess the ability to tolerate acid conditions but also to produce high amount of lactic acid in compared with other strains belong to same species [22]. These possibilities might provide the ability of the organism to dominate the growth in the surrounding environment, *i.e.* gastrointestinal. From the technological point of view, the ability of *L. reuteri* DSM 12246 to switch between metabolites during fermentation are of great importance since the development of flavour and texture profile during the production of dairy and sour dough are relay on the ratio between volatile and non-volatile compounds.

4. Conclusion

In addition to its high ability to produce antimicrobial reuterin, the present

study concludes that *L. reuteri* DSM 12246 is well molecular defined and characterized probiotic able to transit and colonize in GIT, fairly grow over a range of pH values while it preferred acidic environment and capable to switch between metabolites in order to not only provide cell protection but give the possibility to develop flavour and texture profile in fermented products and dominate the microbial community.

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