DAIRY *PROPIONIBACTERIUM* STRAINS WITH POTENTIAL OXALATE DEGRADING ACTIVITY AND THE EFFECT OF EXOPOLYSACCHARIDE PRODUCTION

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BACKRAUND

Oxalate is a toxic compound that is found in a wide range of food such as fruits, vegetables, grains and nuts or produced by intestinal microflora from ascorbic acid. By reason of its highly oxidizing effects and the ability to combine with cations to form insoluble salts, this organic dicarboxylate is extremely toxic for most of life forms. High consumption of dietary oxalate can result in a number of pathologic disorders, including hyperoxaluria, urolithiasis, renal failure and kidney stone formation for human form (Amini*et al.* 2016; Turroni*et al.* 2007; Turroni*et al.* 2010). Hyperoxaluria is one of the main risk factors for renal stones, and the urine load of oxalate plays a pivotal role in calcium oxalate stone formation (Campieri et al. 2001; Ogawa et al. 2000). Specific treatments for hyperoxaluria are limited; these treatments are mostly based on dietary restrictions and may not be applicable to people who cannot identify causative dietary constituents in their own diet. Several studies have demonstrated that bacterial degradation of oxalate in the intestine decreases oxalate absorption and a reduction in the urinary oxalate level decreases the incidence of calcium oxalate stone formation (Campieri et al. 2001; Sidhu et al. 2001; Hoppe et al. 2006). Bacteria synthesize a number of polysaccharides which are defined by their location related to the cell. Those that are excreted outside the cell wall are called exocellular polysaccharides or EPSs (Ai et al. 2016). EPS is thought to protect microorganisms against bacteriophages, antibiotics, and toxic compounds (Ruas-Madiedo et al. 2002. Propionic acid bacteria (PAB) have widely been used as probiotic and starter cultures in dairy products.

The main objective of our study was to determine whether resistance to high oxalate concentrations and oxalate degrading activities of *Propionibacterium* spp. strains would be affected by the production of EPS.

MATERIALS AND METHODS

Bacterial strains and growth conditions: In the present study, five strains (*P. thoenii* AKP1, *P. freudenreichii* subsp*. freudenreichii* DO7, DO8, *P. jensenii* BDP6 and DO6) of *Propionibacterium* spp., which were identified and selected from 29 strains isolated from traditional homemade Turkish cheeses, exhibited gastrointestinal system tolerance, antimicrobial activity, resistance to different antibiotics, autoaggregation and coaggregation abilities. Propionibacteria species were cultured under anaerobic conditions for 48 h at 30 °C in Yeast Extract Sodium Lactate Broth (YEL) medium. To prepare of active cultures for all experiments, propionibacteria strains were activated by three successive transfers every 48 h in the YEL broth at 30 °C (Cummunis et al. 1909).

Testing strains for growth in oxalate and oxalate degrading capacity: The procedure for the determination of growth and oxalate-degrading capacity of *Propionibacterium* spp. strains was based on the method previously described by Campieri et al. (2001) with some modifications. Firstly strains were grown in pure culture on YEL broth for 48 h at 30 °C under anaerobic conditions. To ensure maximum synthesis of the enzymes involved in oxalate utilization, cultures for each oxalate concentration were initially transferred twice for 48 h in YEL medium containing 2 mM sodium oxalate and 500-µL aliquots of the culture broths (log 10.14 cfu/mL) were inoculated into 9.5 mL YEL-ox broths (5%) and were incubated at 30 °C for 72 h under anaerobic conditions. A media control (YEL-ox broth) was prepared as described above for each concentration but it was not inoculated. Optical density (600 nm) and plate counts (cfu/mL) were performed to determine growth of each strain in 10 and 20 mM YEL-ox which was compared to growth in YEL. The existing oxalate in the culture supernatants was measured through using an oxalate enzymatic experiment kit (Trinity Biotech, Bray, County Wicklow, Ireland) according to the manufacturer's instructions with the addition of activated charcoal to eliminate phenolic compounds (Federici et al. 2004). Data from three biological replicates that each was experimented in triplicate were averaged and the final values reported as the percentage of oxalate utilized. *L. acidophilus* NCFM was used as a positive control while *L. delbrueckii* DSM 20074 was used as the negative control in these experiments**.**

Exopolysaccharide (EPS) production: EPS production of *Propionibacterium* spp. strains were determined in YEL (for control) , 10 mM and 20 mM YEL-ox media (for toxic effect of oxalate). EPS concentration was estimated by the phenol/sulphuric acid method. The total EPS (expressed as mg/L) evaluated in each sample using glucose as standard (5-100 mg/L) (Dubois et al., 1956; Torino *et al.* 2001).
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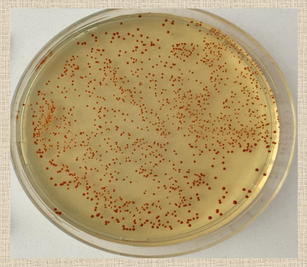


Figure 1. The growth of *P. thoenii* AKP1 strain in YEL medium (for control)

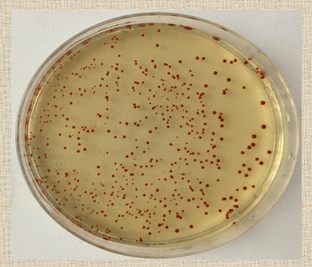


Figure 2. The growth of *P. thoenii* AKP1 strain in 20 mM YEL-OX medium

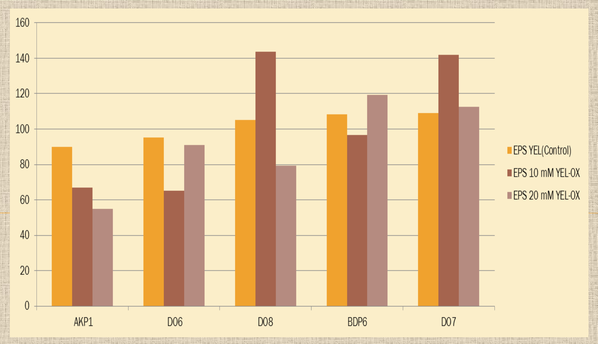


Figure 3. Comparison EPS production of *Propionibacterium species* in different growth media.

RESULTS

Testing strains for growth in oxalate and oxalate degrading capacity: All strains grew in the presence of 10 and 20mM sodium oxalate illustrating that oxalate at these concentrations are not toxic to *Propionibacterium spp.* strains. No major loss of viability was observed at YEL-ox media. All of strains were grown in the presence of 10 mM sodium oxalate while at a concentration of 20 mM, the growth of the strains was partially inhibited.

A high variability in the oxalate-degrading capacity was found in the different species and oxalate concentrations. The oxalate degradation behavior shown by the two P. *freudenreichii* subsp*. freudenreichii* strains tested was heterogeneous. *P. freudenreichii* subsp*. freudenreichii* DO8 strain showed 10.57-42.45% of oxalate consumption in both media respectively and *P. freudenreichii* subsp. *freudenreichii* DO6 strain exhibited 9.56-11.96% degrading activity. *P. thoenii* AKP1 and *P. freudenreichii* subsp. *freudenreichii* DO8 strains degraded oxalate at both concentrations in spite of the reduced growth in the presence of 20 mM sodium oxalate (Table 1 and 2).

Exopolysaccharide (EPS) production: To determine the toxic effect of oxalate on EPS production ability and viability, EPS production of *Propionibacterium* spp. strains was determined in YEL, 10 and 20 mM YEL-ox media. In this study, different amount of EPS were produced by different strains; however, EPS levels ranged 89.82 to 109.07 mg/L in YEL media and from 67.04 to 143.79 mg/L in 10 mM YEL-ox and from 55.00 to 119.14 in 20 mM YEL-ox media (Table 3). The high EPS-producing *P. jensenii* BDP6 and *P. freudenreichii* subsp. *freudenreichii* DO8 strains showed high oxalate degrading activity, whereas the low EPS- producing P. jensenii DO6 showed low oxalate degrading activity in both oxalate concentrations (Figure 3).

Table 1 . Viability (log cfu/mL) and oxalate degradation of *Propionibacterium* spp. strains after growth in 10 mM YEL-ox medium.

Strains	YEL (Control)			10 mMYEL-ox			Oxalate degrading %	Percentsurvival %
	pH	OD	Viablecounts (log cfu/mL)	pH	OD	Viable counts (log cfu/mL)		
AKP1	6.63±0.05	1.30±0.00	8.68±0.11	6.44±0.21	1.06±0.08	8.17±0.12	26.43±3.20	94.12
DO6	7.34±0.17	0.85±0.01	9.96±0.16	6.62±0.00	0.71±0.04	9.11±0.01	9.56±0.47	91.47
BDP6	7.01±0.05	1.00±0.04	9.94±0.05	6.61±0.02	0.96±0.07	9.19±0.13	22.90±1.54	92.45
DO7	7.4±0.02	0.59±0.03	10.10±0.16	7.29±0.33	0.58±0.10	10.64±0.17	21.48±3.32	100<
DO8	7.40±0.01	0.81±0.05	10.14±0.47	6.81±0.07	0.74±0.01	10.49±0.12	10.57±2.14	100<

Table 2. Viability (log cfu/mL) and oxalate degradation of propionibacterium after growth in 20 mMYEL-ox medium.

Strains	YEL (Control)			20 mMYEL-ox			% Oxalate degrading	Percent survival
	pH	OD	Viablecounts (logcfu/mL)	pH	OD	Viablecounts (logcfu/mL)		
AKP1	6.63±0.05	1.30±0.00	8.68±0.11	7.46±0.08	0.43±0.15	7.09±1.89	41.58±1.89	81.68
DO6	7.34±0.17	0.85±0.01	9.96±0.16	8.27±0.45	0.60±0.07	9.38±0.24	11.96±0.09	94.18
BDP6	7.01±0.05	1.00±0.04	9.94±0.05	7.76±0.00	0.92±0.00	9.26±0.37	24.20±5.10	93.16
DO7	7.40±0.02	0.59±0.03	10.10±0.16	7.97±0.07	0.58±0.06	9.18±0.40	11.56±3.88	90.89
DO8	7.40±0.01	0.81±0.05	10.14±0.47	6.84±0.08	0.54±0.01	9.30±0.45	42.45±6.90	91.71

Table 3. EPS production of propionibacteriumstrains in YEL and YEL-ox medium.

Strains	EPS Control (mg/L)	EPS 10 mM-YEL-ox (mg/L)	EPS 20 mM-YEL-ox(mg/L)
***P. thoenii* AKP1**	89,82±1,44	67,04±4,11	55,00±3,98
***P. jensenii* DO6**	95,09±1,70	65,08±3,61	90,92±2,45
***P. jensenii* BDP6**	108,19±1,18	96,46±2,37	119,14±0,49
***P. freudenreichii* subsp. *freudenreichii* DO7**	109,07±1,36	141,76±2,45	112,41±1,52
***P. freudenreichii* subsp. *freudenreichii* DO8**	105,28±3,27	143,79±0,39	79,45±1,59

CONCLUSIONS

To our knowledge, the interaction between the oxalate degrading ability and the EPS-producing capacity of *Propionibacterium* strains has not been examined until now. In this study, high EPS-producing strains showed an oxalate tolerance and oxalate degrading ability. A better understanding of the mechanisms related to EPS production and oxalate degrading activity can be used for preliminary screening in order to determine potentially probiotic bacteria applications for human or animal use. Further studies are needed to identify and characterize bacterial cell wall compositions and properties to understand their role in adhesion to oxalate, and their relation with degradation mechanisms.

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