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Publication date:
2012

Document version
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Isolation and Characterization of Antifungal Dairy Propionibacteria

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1 Introduction and aim

Dairy propionibacteria are important organisms in some fermented dairy products and some strains in addition have potential as bioprotective cultures (Lind et al. 2007). The mechanisms behind the activity are not fully elucidated but synergistic effects between produced metabolites are likely to play a role.

Our aim was to characterize the antifungal activity against foodborne fungi in propionibacteria isolated from raw milk Emmental cheeses.

2 Materials and methods

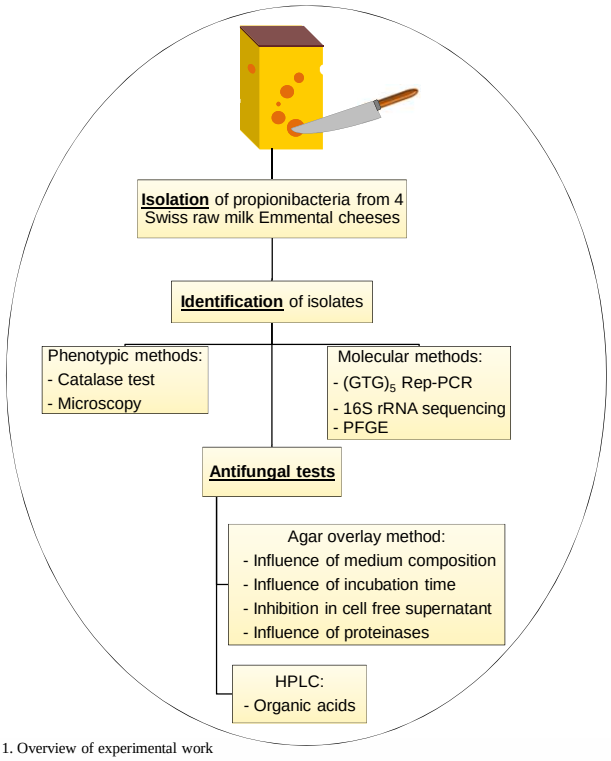

Figure 1. Overview of experimental work

3 Results

Isolation and identification of propionibacteria

- 40 propionibacteria isolates identified by Rep-PCR and 16S rRNA sequencing
- All isolates identified as *P. freudenreichii*
- PFGE was used to differentiate the isolates
- Restriction endonuclease *Spe* I was suitable for digesting propionibacteria (figure 2)

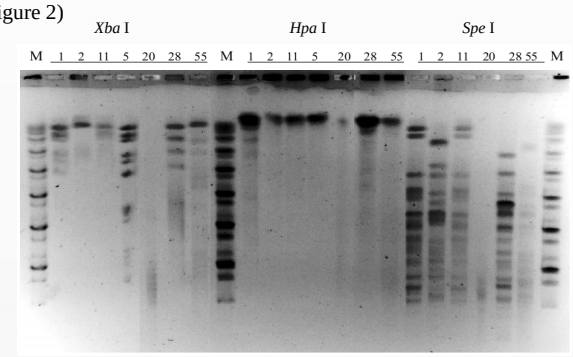

Figure 2. PFGE analysis of *Xba* I, *Hpa* I and *Spe* I fragments of genomic DNA from 7 propionibacteria isolates. Lane 1, 2, 11, 5, 20, 28 and 55 correspond to isolate number. M: Midrange II PFG marker.

Antifungal test of propionibacteria isolates

- Increased antifungal activity in growth media containing glycerol (figure 3)
- pH after fermentation in glucose and glycerol based media: ~ 4.5
- pH after fermentation in lactate + acetate and lactate based media: ~ 6.5-7.0
- None of the yeasts tested showed sensitivity to the propionibacteria isolates

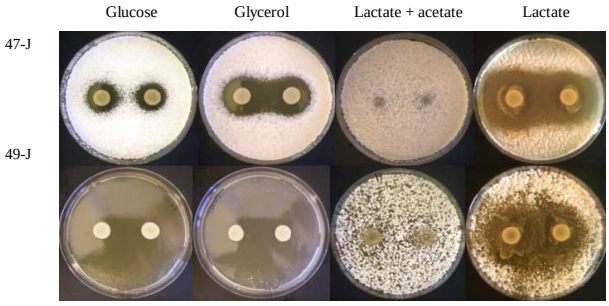

Figure 3. Inhibitory effect of spotted propionibacteria isolate 5 on two indicator moulds in an agar overlay assay. 47J: *Penicillium* sp. 47-J; 49J: *Penicillium* sp. 49-J.

- Increasing incubation time of propionibacteria → increased activity (table 1).
- Antifungal activity was stable up to 17 days, with only slight activity decrease

Table 1. Antifungal activity of isolate 1, 2, 5, 11, and 20 incubated anaerobically for 3 and 8 days prior to mould overlay.

Incubation time on sodium lactate agar	3 days				8 days			
Indicator mould	**47J**	**49J**	**161J**	**302**	**47J**	**49J**	**161J**	**302**
Isolate 1	+	+	-	*	+	++	+*	*
Isolate 2	*	*	-	-	+*	++	+*	+
Isolate 5	+*	++	+*	+	+*	++	++	+*
Isolate 11	+*	*	*	+	+*	++	+	+
Isolate 20	*	*	-	-	+*	++	++	+

47J: *Penicillium* sp. 47-J; 49J: *Penicillium* sp. 49-J; 161J: *Penicillium* sp. 161-J; 302: *Penicillium solitum* 302. Degree of inhibition is based on size of inhibition zone and is graded on a scale going from weak to strong inhibition : - = no inhibition; weak to strong inhibition: *, +, +*,++,++*,+++.

- Highest levels of produced propionate and acetate in lactate based media
- Highest amount of undissociated acids in glucose based media (table 2)

Table 2. Produced propionate and acetate by isolate 5 in supernatant

		Medium	
Concentration (mM)		**Glucose**	**Lactate**
End pH		4.49	6.77
Propionate	Total	45.93	64.09
	Dissociated	13.58	63.29
	Undissociated	32.35	0.80
Acetate	Total	22.69	31.42
	Dissociated	7.96	31.12
	Undissociated	14.73	0.30

- Treatment of isolate 5 with proteinases did not influence antifungal activity
- Inhibition were also observed in cell free supernatants

4 Conclusion

- Restriction endunuclease *Spe* I was suitable for grouping propionibacteria
- Carbon source affected antifungal activity, pH and acid production
- Antifungal activity was stable up to 17 days
- Increase in undissociated acids correlated with increased antifungal activity
- In the isolate with highest antifungal activity little effect of proteinase treatment was seen

5 References

Lind, H et al. (2007). FEMS Microbiology Letters 271, 310-315.