

Pyrosequencing as tool for evaluating the bacterial biodiversity in cheese: study of Pico cheese microflora



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Aims

Our work was aimed at describing the bacterial biodiversity in an artisanal cheese of the Azores (Portugal) using a high-throughput sequencing methodology that is still underexploited in foods.

Methods

DNA was extracted from twenty-eight samples of cheese produced in artisanal cheesemaking units. A highly variable region of the 16S rRNA gene was amplified, and fragment libraries were obtained and sequenced by analyzing the pyrophosphate released during the process, with no bacterial cloning steps required [1]. Operational Taxonomic Units (OTU) were defined considering a cut-off value of 97% similarity of the 16S rRNA gene sequences. Rarefaction curves and nonparametric richness estimators Chao1, and Good's Coverage as well as Shannon diversity index were generated to evaluate sampling efforts.

Results

The rarefaction curve (Fig. 1) and richness estimators' values obtained (Table 1) reveal that the overall bacterial diversity is well represented in our samples. Four phyla and 53 genera were present. *Lactococcus* was the predominant genus (77.1% of the sequences) (Fig. 2). Some of the genera encountered had not been previously found in dairy environments (Table 2). A number of sequences (7.7%) could not be ascribed to a genus. Most of these belonged to the Enterobacteriales (Fig. 3).

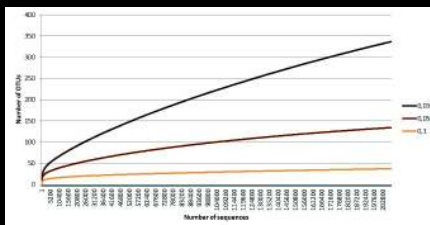


Fig. 1 – Rarefaction analysis for artisanal Pico cheese environment. Rarefaction is shown for OTUs with differences that do not exceed 3%, 5%, or 10%.

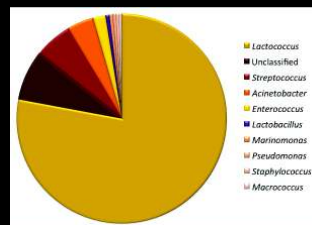


Fig. 2 – Genera Found in Pico cheese.

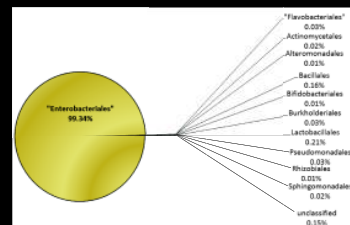


Fig. 3 – Identity (at the order level) of sequences that could not be ascribed to a genus.

Characteristics	Cheesemaker A			Cheesemaker B			Cheesemaker C		
	Day of ripening	Day of ripening	Day of ripening	Day of ripening	Day of ripening	Day of ripening	Day of ripening	Day of ripening	
No. of tags sequenced	22054	20795	22300	24603	28336	19673	17568	23981	22212
Coverage	0.999	0.998	0.998	0.998	0.999	0.998	0.998	0.998	0.999
No. of OTU observed	42	45	75	84	118	72	77	96	85
Shannon	0.25	0.26	0.66	0.60	0.60	0.60	0.52	0.66	0.63
Chao1	1.942	1.062	0.972	1.340	1.337	1.110	1.286	1.002	0.931
Good	161.20	189.00	141.11	162.21	174.10	134.00	140.91	210.81	186.10
ACE	118.88	219.14	265.78	235.97	274.96	200.65	211.67	484.42	205.17

Table 1 – Summary of the characteristics of the pyrosequencing data, number of total tags sequenced per producer and day of ripening and the observed richness, coverage and richness estimators at 97% similarity level.

Conclusions

Pyrosequencing revealed a considerable biodiversity in the Pico cheese bacterial community, including bacteria belonging to genera that may be of technological interest and genera that had not been previously described in cheese.

Significance of study

This is the first study published on Pico cheese biodiversity, an artisanal cheese that is threatened by the constraints of the market. Studies on Pico cheese microbiota are urgent and important to preserve its biodiversity, promote safety, enhance marketability and, ultimately, ensure its survival. Pyrosequencing proved to be a powerful tool for the evaluation of microbial biodiversity in cheese.

Phyla	Sub-Phyla	Order	Family	Genera
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides
	Floribacteria	Floribacteriales	Floribacteriaceae	Floribacterium
	Sphingobacteria	Sphingobacteriales	Sphingobacteriaceae	Nubaria
Firmicutes	Bacilli	Lactobacillales	Carobacteriaceae	Genomastella
	Clostridia	Clostridiales	Veillonellaceae	Zymophilus
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Paracoccus
	Betaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas
	Betaproteobacteria	Burkholderiales	Comamonadaceae	Vibrionaceae
	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Tubulifilum
		Kaistimonadales	Kaistimonadaceae	Lutibacter

Table 2 – Genera not previously encountered in dairy environments.

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References: [1] Mardis, ER (2008). Next-Generation DNA Sequencing Methods *Annu. Rev. Genomics Hum. Genet.* 9: 387 – 402

