STUDY OF VIRULENCE FACTORS IN ENTEROCOCCI ISOLATED FROM CHEESE

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INTRODUCTION

Enterococcus are part of the natural microflora of several traditional cheeses, where they play important roles toward development of suitable organoleptic characteristics throughout ripening. Beneficial roles have accordingly led to inclusion of enterococcal strains in a number of starter cultures (Giraffa, 2002).
A major issue of concern when using bacterial cultures in food products is their safety, when they are supposed to be consumed live and in large quantities (Eaton and Gasson, 2001). Many lactic acid bacteria are used as probiotics, but the case of enterococci remains controversial because of their association with human infections. The main aspect of *Enterococcus* pertains to their capacity to accumulate several virulence factors and antibiotic resistances.

The aim of this work was to investigate the occurrence of some virulence factors (viz. cytolysin, adhesins) in enterococci of food origin (isolated from Terrincho cheese). Screening for cytolysin genes (*cylL*$_L$, *cylL*$_S$, *cylM*, *cylB* and *cylA*), surface adhesin genes (*efaA*$_{fs}$, *efaA*$_{fm}$, *esp*), aggregation protein gene (*agg*) and extracellular metalloendopeptidase gene (*gelE*) was performed in 32 enterococci isolates of 5 different species (*E. gallinarum*, *E. faecalis*, *E. durans*, *E. casseliflavus*, *E. faecium* and *E. durans*). The assays were made via PCR detection.

MATERIALS AND METHODS

Thirty two enterococci, selected because of their different properties, were isolated from a traditional Portuguese cheese, and were assessed for presence of virulence genes

The following virulence genes were amplified by PCR using primers and conditions described previously (Eaton and Gasson, 2001; Semedo *et al.*, 2003)

Gene	Primer	Product size (bp)	
➢ *esp*	TE34 / TE36	933	The primers used for amplification of these genes were described by Eaton and Gasson (2001)
➢ *agg*	TE3 / TE4	1553	
➢ *gelE*	TE9 / TE10	419	
➢ *efaA*$_{fs}$	TE5 / TE6	705	
➢ *efaA*$_{fm}$	TE37 / TE38	735	
➢ *cylM*	CylM1 / CylM2	2940	The primers used for amplification of cytolysin genes were developed by Semedo *et al.* (2003)
➢ *cylB*	CylB1 / CylB2	2020	
➢ *cylA*	CylA1 / CylA2	1282	
➢ *cylL*$_L$	CylL$_L$1 / CylL$_L$2	253	
➢ *cylL*$_s$	CylL$_S$1 / CylL$_S$2	240	

PCR AMPLIFICATION

Performed in a 25 µl-volume reaction, containing:
2U Taq polymerase
0.1 mM deoxynucleoside triphosphates (dNTPs)
PCR buffer (pH 8.4, 2.5 mM $MgCl_2$)
0.5 µM of each primer
250 ng enterococcal DNA

CYCLING PARAMETERS

Initial cycle of 95ºC for 3 min
35 cycles of 95ºC for 1 min
35 cycles of 54ºC/55ºC* for 1 min
35 cycles of 72ºC 5 min
and final extension step of 72ºC for 7 min

* 54ºC for *efaA*$_{fm}$ and 55ºC for the remainer

RESULTS AND DISCUSSION

The gene for the cell wall-associated protein Esp was only present in two (i.e. 6%) of the strains, both *E. faecalis* isolates (see Figure 1). All strains were negative for *cylL*$_L$, *cylM*, *cylB*, and *agg* genes (results not shown). The cell wall adhesin *efaA*$_{fs}$ was detected in all *E. faecalis* strains, as well as *efaA*$_{fm}$ in *E. faecium* isolates (see Figures 2 and 3). Only one isolate (*E. durans* 17) possessed the *cylLs* determinant, and another the *cylA* determinant. The presence of *gelE* was detected only in two strains – *E. faecalis* 3 (Figure 4) and *E. faecium* 42.

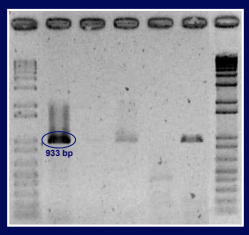

Figure 1. PCR amplification products of the virulence *esp* gene in the two *E. faecalis* strains (Lanes 4 and 6). Lanes 1 and 7: PCR Marker, Lane 2 – Positive control

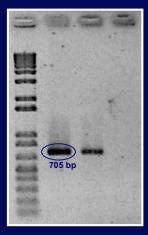

Figure 2. PCR amplification products of the virulence *efaA*$_{fs}$ gene in the *E. faecalis* strain 1 (Lanes 3). Lane 1: PCR Marker, Lane 2 – Positive control

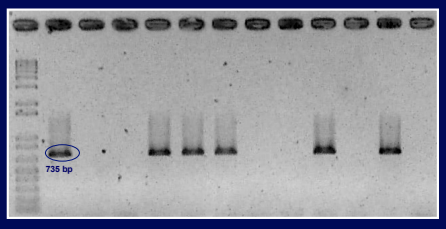

Figure 3. PCR amplification products of the virulence *efaA*$_{fm}$ gene in the *E. faecium* strains 45, 50, 51, 57 and 61 (Lanes 5, 6, 7, 10 and 12). Lane 1: PCR Marker, Lane 2 – Positive control

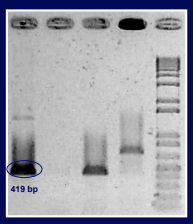

Figure 4. PCR amplification products of the virulence *gelE* gene in the *E. faecalis* strain 3 (Lane 3). Lane 5: PCR Marker, Lane 1 – Positive control

CONCLUSION

Our results indicated that the incidence of virulence determinants was very low, so most of enterococci isolates tested may potentially be used as starters. Only two isolates belonging to *E. faecalis* species harboured virulence.

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