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Identification of the main esterase involved in lipolysis by *Propionibacterium freudenreichii*

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Rationale

Free fatty acids are important flavor compounds in cheese, where they bring pungent, rancid, cheese, and fruity notes [1]. In Swiss cheese, lipolysis mainly occurs during the growth of *Propionibacterium freudenreichii*, a specie used as a ripening culture, which is responsible for almost the whole release of free fatty acids during the ripening.

Aim

To identify the most probable lipolytic esterase(s) involved in cheese lipolysis by *P. freudenreichii* CIRM1-BIA1, we focused on secreted and surface-exposed esterases, because Swiss cheese lipolysis mainly occurs during *P. freudenreichii* growth (without lysis).

Strategy

- ❖ Targeted genes: a previously identified secreted lipolytic esterase, PF#279, and a putative esterase predicted from the genome sequence of strain CIRM-BIA1 to be anchored to the cell wall, PF#774 [3].
- ❖ Inactivation and over-expression of both targeted genes in the wild type (WT) strain CIRM-BIA1, separately
- ❖ Validation of the lipolytic activity of WT and mutant strains during their growth in the presence of a milk fat emulsion

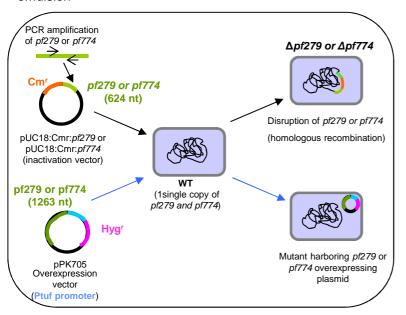


Fig 1: Construction of the recombinant strains $\Delta pf279$, $\Delta pf774$, and strains over-expressing pf279 or pf774

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Results

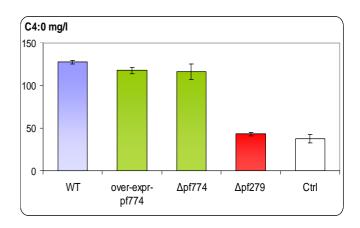


Fig 2: Extent of lipolysis during growth in the presence of an emulsion of milk fat. Strains were cultured in yeast extract lactate broth containing milk fat emulsion (~30% final fat concentration) at 30°C for 60 h. Butanoic acid (C4:0) was determined in the supernatant of culture medium by CG-FID. Ctrl: Control medium without inoculation.

- ❖ The inactivation of *pf279* resulted in a very low residual lipolytic activity (>90% less C4:0 than WT)
- ❖ Inactivating or over-expressing *pf774* had no impact on lipolysis level
- ❖ We previously demonstrated that the overexpression of *pf*279 resulted in 5-8 fold greater lipolytic activity [3]

Conclusion

This study shows that only the secreted lipolytic esterase PF#279 is involved in milk fat hydrolysis in *P. freudenreichii* CIRM-BIA1.

This enzyme is likely a key component in Swiss cheese lipolysis.

References: [1] Dherbécourt et al. (2010) J. Agric. Food Chem. 58: 11732-11739; [2] Dherbécourt et al. (2008) Microb. Cell Fact. 7, 16; [3] Dherbécourt et al. (2010) Appl. Environ. Microbiol. 76:1181-1188.







