SCREENING OF STAPHYLOCOCCI ON THEIR ABILITY TO PRODUCE KETONES

Fadda S., Leroy-Sétrin S., Diot A., Talon R.
INRA Clermont Ferrand - Theix, Station de Recherches sur la Viande, 63122 Saint-Genès Champanelle, France

Background

Staphylococcus carnosus and *Staphylococcus xylosus* are used as starters in sausage manufacture. Inoculated in sausages, they increased the level of some volatile compounds such as ketones and secondary alcohols which contribute to the cured aroma of the product [1]. However, the production of methyl ketones by bacteria is not documented although it is widely accepted that methyl ketones are formed from incomplete β–oxidation of medium chain fatty acids in filamentous fungi [2]. In fungi, the oxidation is thought to stop with the release of β–ketoacyl-CoA esters which are deacylated into β–ketoacids by a thioesterase. The corresponding β–ketoacid is then decarboxylated to the methyl ketone. We have already shon the presence of β–oxidation and β–decarvboxylase activities in one strain of *S. carnosus* [3, 4].

Objectives

The aims of this work was to identify the β–oxidation pathway and the presence of a β–decarboxylase activity in diverse species and strains of staphylococci of technological interest.

Methods

Strains

14 strains of *Staphylococcus carnosus*, 10 strains of *Staphylococcus xylosus* and 3 strains of *Staphylococcus equorum* were grown in complex media (CM) [3] for 24 h at 30°C and under stirring (150 rpm). CM was supplemented with ethyl laurate (3.5 mM) or ethylbutyryl acetate (2mM) to stimulate the β–oxidation pathway synthesis and the β–decarboxylase activity, respectively. Cells were collected by centrifugation and kept freezed.

β–oxidation assays

β–oxidation was assayed by measuring the 3-hydroxyacyl-CoA dehydrogenase activity (EC 1.1.1.35) on cell free extracts (CFE). CFE were obtained from cells resuspended in HEPES buffer (20 mM, pH 7.5) containing 1 mM EDTA and 1 mM PMSF and disrupted by repeated vortexing of the cells with glass beads as described by Engelvin et al. [3]. The assay mixture contained 72 nmoles of coenzyme A, 1.7 µmoles of NAD, 72 nmoles of palmitoyl-CoA, 280 µg of CFE protein in a final volume of 1 ml K_2HPO_4/KH_2PO_4 buffer (300 mM, pH 8.0). The reduction of NAD at 30°C was measured by its absorption at 340 nm against control sample without substrate. β–oxidation activity was expressed as nmoles of NAD reduced/mg of protein/min. [ε_{NAD} (340) = 6220 M^{-1} cm^{-1}].

β–decarboxylase assays

The assay was based on the measurement of 2-pentanone formation following the incubation of the resting cells with ethylbutyryl acetate. The incubation mixture contained 400 µl of resting cells (0.2 g of wet weight cells), ethylbutyryl acetate (4 mM) as substrate, esterase (2.7 units) in a 1 ml final volume K_2HPO_4-KH_2PO_4 buffer (100 mM, pH 7.0). Either the bacteria or the substrate were omitted from the controls. The reaction mixture was incubated with shaking in a 2 ml Eppendorf tube at 30 °C for 1 h. The reaction was stopped by removal of the cells by centrifugation (15000×*g*). The supernatants containing 2-pentanone were kept at -20°C until enzymic quantification by a NADPH-dependent alcohol dehydrogenase (ADH) (EC 1.1.1.2) [4]. The reaction mixture, in Tris-HCl buffer (100 mM, pH 7.8) made up to a final volume of 1 ml, contained the following reagents: 200 µl of the supernatant, 100 µl of NADPH (100 mM) and 21 µl of ADH solution (0.94 units ml^{-1}). The NADPH oxidation was followed continuously at 334 nm at 40°C for 10 min. A calibration curve of 2-pentanone was made from 0 to 500 µM. The results were expressed as µmoles of 2-pentanone cell g^{-1} h^{-1}.

Results and discussion

β-*oxidation*

The presence of a β–oxidation cycle in different strains of staphylococci was demonstrated for the first time in this paper (Table 1) and confirmed former results on the *S. carnosus* 833 strain [3]. The enzymatic activity of of 3-hydroxyacyl-CoA dehydrogenase was very high for the *S. xylosus* strains. For *S equorum* two of the three strains had a high β–oxidation activity. For *S. carnosus*, 4 strains had a high activity. For *S. carnosus* 833 strain, we have already shown that intermediates of fatty acid oxidation were released [3] suggesting that β–oxidation is involved in the synthesis of methyl-ketone. For the *S. xylosus* and *S equorum* strains, the β–oxidation system should be characterized to see if intermediates are released.

β-*decarboxylase activity*

All the strains of staphylococci were able to produce 2-pentanone from ethylbutyryl acetate (Table 1). The highest β-decarboxylase activity was measured for the *S. carnosus* strains and varied from 3.33 to 13.08 µmol cell g^{-1} h^{-1} according the strains. The lowest activity was found for the *S. xylosus* strains with a maximum of activity of 2.00 µmol cell g^{-1} h^{-1} for one strain. The 3 strains of *S. equorum* had an intermediate activity around 2.00 µmol cell g^{-1} h^{-1}.

Conclusion

From these preliminary results, it seems that even if *S. xylosus* and *S. equorum* strains had a high β-oxidation activity, the limiting step to produce methyl ketones will be their very low decarboxylase activity. On the contrary, the *S. carnosus* strains had a high decarboxylase activity. These results could explain why Montel et al [1] found higher level of ketones in sausage models inoculated by *S. carnosus* than *S. xylosus*.

Pertinent literature

[1]Montel, M.C., Reitz, J., Talon, R., Berdagué, J.L. and Rousset-Akrim, S. (1996) Biochemical activities of *Micrococcaceae* and their effects on the aromatic profiles and odours of a dry sausage model. Food Microbiol. 13, 489-499.
[2]Hwang, D.H., Lee, Y.J. and Kinsella, J.E. (1976) β-Ketoacyl decarboxylase activity in spores and mycelium of *Penicillium roqueforti*. Int.

J. Biochem. 7, 165-171.
[3] Engelvin, G., Feron, G., Perrin, C., Mollé, D. and Talon, R. (2000) Identification of β-oxidation and thioesterase activities in *Staphylococcus carnosus* 833 strain. FEMS Microbiol. Lett. 190, 115-120.
[4] Fadda, S., Lebert, A. and Talon, R. Development of an enzymic method to quantify methyl ketones from bacterial origin. J. Agric. Food Chem. (in press)

Acknowledgements Christian Hansen A/S provided financial assistance for this work.

Table 1: β-oxydation and β-decarboxylase activities of staphylococci

Strains	β-oxidation (nmol $mg^{-1}min^{-1}$)	β-decarboxylase (µmol cell g^{-1} h^{-1})
Staphylococcus carnosus		
20501 (DSM: German collection)	22.39	12.95 ± 0.07
833 (CIT: collection INRA, Theix)	5.75	13.08 ± 0.11
836 (CIT)	4.59	11.50 ± 1.06
M427 (CIT)	2.30	7.65 ± 0.35
TM300 (UT: University of Tuebingen)	28.71	8.78 ± 0.88
M379 (NRIC, Japan collection)	2.30	3.33 ± 1.31
M429 (UB: University of Bath)	22.97	7.53 ± 0.18
M431 (UB)	25.83	5.33 ± 0.46
M433 (UB)	11.49	7.90 ± 0.71
S1 CH	5.63	3.58± 0.11
S2 CH	ND	7.75 ± 0.00
S3 CH	ND	3.83 ± 0.95
S4 CH	ND	6.20 ± 0.99
S5 CH	ND	4.50 ± 0.14
Staphylococcus xylosus		
20266 (DSM: German collection)	63.13	0.88 ± 0.18
M203 (CIT)	17.23	0.65 ± 0.35
M505 (CIT)	74.64	1.20 ± 0.07
C2a (UT)	45.93	1.30 ± 0.07
S15 CH	63.16	2.00 ± 0.35
S16 CH	54.55	1.30 ± 0.42
S17 CH	27.28	1.40 ± 0.00
S18 CH	51.68	0.93 ± 0.46
S19 CH	43.06	1.30 ± 0.42
S20 CH	63.16	0.38 ± 0.18
Staphylococcus equorum		
20674 (DSM)	17.23	2.08 ± 0.11
S25 CH	40.19	2.08 ± 0.46
S27 CH	43.06	1.25 ± 0.35

ND : not done