EFFECT OF SOME FACTORS ON THE PRODUCTION OF ESTERS BY STAPHYLOCOCCI

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BACKGROUND

Strains of *Staphylococcus* added as starter culture in fermented sausage participate to the development of typical flavour (Berdagué *et al.*, 1993; Stahnke, 1995). They produced many aromatic compounds such as esters, these compounds have low sensory threshold values and will impart fruity notes to the sausage flavour (Stahnke, 1994). Most of the esters in sausages are ethyl esters , their production will depend on the presence of ethanol and the different acids as well as on the ester producing strains.

OBJECTIVES

The aim of this work was to characterise the production of esters by different *Staphylococcus* species to select starter on their aromatic potentialities for the development of dry sausages with typical flavour.

MATERIAL AND METHODS

Growth conditions of the strains and preparation of enzyme extracts

The following staphylococci were studied : *Staphylococcus carnosus* (833, 836), *Staphylococcus xylosus* (831, 873, M350) *Staphylococcus warneri* (863), *Staphylococcus saprophyticus* (852).

Resting cells (RC) : After 24 h of growth in PYS media, the cells were harvested by centrifugation at 10000 g for 30 min at 4°C, washed with phosphate buffer 20 mM pH 7.0 and resuspended in this buffer (0.5 g of wet cells/ml) and kept frozen at -20°C.

Extracellular concentrates (EC) : The proteins of the culture supernatants were precipitated with ammonium sulfate. Then the precipitates were dissolved and dialysed in phosphate buffer 20 mM pH 7.0 . The extracellular concentrates were kept frozen at -20°C.

Production of ethyl butyrate

The assay mixture contained 2 ml of substrate (ethanol 25 µmoles/ml and butyrate 12.5 µmoles/ml), 1.5 ml of phophate buffer and 1.0 ml of the different enzyme extracts (RC or EC).
The assays were done at two pH (5.5, 7.0) and at two temperatures (14, 24°C) under shaking during 4 h. To study the effects of the different factors (pH, temperatures , strains and enzymatic extracts) an experimental design was set up.
Samples of 1.3 ml were taken in triplicate and freezed at -20°C before their analysis by SPME-GC. The production of ester was expressed as nmol per g of cells for the RC and nmol per mg of protein for EC.

Analysis by SPME-Gas Chromatography

The esters were extracted by SPME with polydimethylsiloxane coating phase, liquid sampling with NaCl saturated solution and during 15 min. Then they were analysed by GC on capillary carbowax column and quantified by flame ionisation detector (FID, 280°C). The GC oven temperature was programmed as follows : 50°C held 1 min, increased to 220°C at a ratio of 3°C/mm. Helium was used as carrier gas. For thermal desorption the SPME fiber remained in the injector (200°C) for 5 min.

Data analysis

The data from the experimental design were then studied by analysis of variance on STAT-ITCF. Comparisons between means were made according to Newman-Keuls test.

RESULTS

The different species of staphylococci esterified ethanol with different acids from acetic to decanoic in aqueous solutions (data not shown). But as butanoic acid and ethanol were esterified by all the strains and the enzyme extracts, these substrates has been used to study the effect of different factors. The main results are summarized Figures 1, 2 and 3.

Interaction between factors strains-enzymatic extracts

It appeared that the strains *S. warneri* (863) and *S. xylosus* (M350, 873) produced the highest level of ethyl butyrate (Fig 1). At the opposite, *S. carnosus* (833, 836) produced the lowest quantities (Fig 1).
For the strains *S. warneri* (863), *S. xylosus* (831), *S. carnosus* (833, 836), the extracellular enzymes were more active than the resting cells, whereas for the two *S. xylosus* (M350, 873) it was the opposite (Fig 1).

Interaction between factors strains-pH and enzymatic extracts-pH

The production of ester was higher at pH 7.0 than at pH 5.5 for the strains *S. warneri* (863), *S. xylosus* (M350, 873) and *S. saprophyticus* (852) (Fig 2). For the two stains of *S. carnosus* and *S. xylosus* (831) the esterification was similare at the two pH (Fig. 2A).

The pH had no effect on the production of ethyl butanoate by extracellular concentrates whereas it had a drastic effect on the resting cells (Fig. 2B).

Interaction between factors strains-temperatures and enzymatic extracts-temperatures

We can consider two group of strains (Fig. 3A) :

*group 1 included *S. warneri* (863), *S. carnosus* (833, 836) and *S. xylosus* (831) was characterised by a high production of ester at 24°C compared to 14°C.

*group 2 included *S. xylosus* (M350, 873) and *S. saprophyticus* (852) which produced the same quantity of ester at 24°C and 14°C.

The activity of extracellular concentrates was multiplied by 2 when the temperature increased from 14°C to 24°C (Fig. 3B), whereas the activity of the resting cells was not so modified by the temperatures.

CONCLUSION

Resting cells and extracellular enzymes of staphylococci produced esters. The activities of the extracellular enzymes were not affected by the pH (pH 5.5, 7.0) but by the temperatures (14°C, 24°C), whereas for those of the resting cells it was the opposite. So the staphylococci will be able to produce esters during sausages manufacturing depending on the availability of the substrates (acids and alcohol).

REFERENCES

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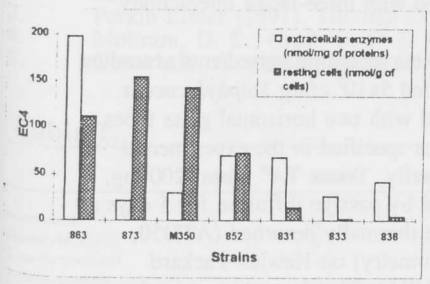

Figure 1 : Effects of factors strains-enzymatic extracts on the production of ester EC4

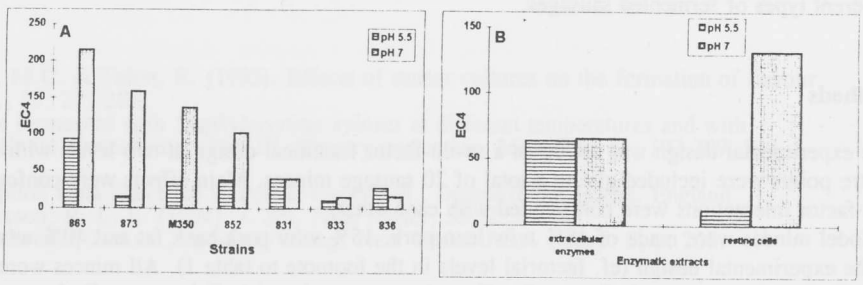

Figure 2 : Effects of factors strains-pH [A] and enzymatic extracts-pH [B] on the production of ester EC4

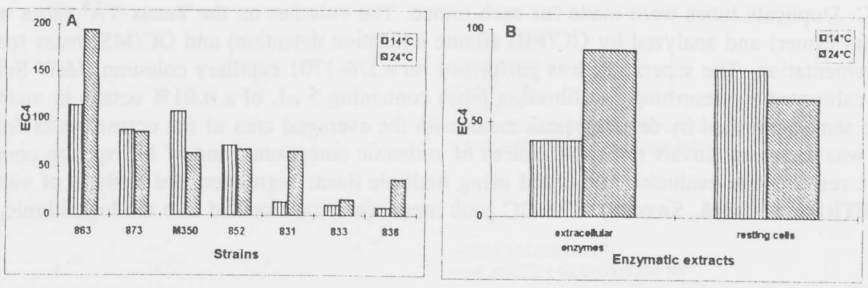

Figure 3 : Effects of factors strains-temperatures [A] and enzymatic extracts-temperatures [B] on the production of ester EC4