# IDENTIFICATION OF SOME LACTIC ACID BACTERIA FROM MEAT

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## INTRODUCTION

On meat products lactic acid bacteria may have positive effect (preventing the growth of pathogenic bacteria), or negative effect (greening and souring) so their rapid identification is very important.

The grouping and identification of these lactic acid bacteria has caused some confusion in the past. Now the taxonomy of these microorganisms is not so problematic and some bacilli often called "atypical" were partially characterized. Thus *Lactobacillus curvatus*, *Lactobacillus sake*, strains designated as *"L. sake - L. curvatus"*, *Carnobacterium divergens* (cf. *Lactobacillus divergens*), *Carnobacterium piscicola (cf. Lactobacillus carnis)*, *Lactobacillus viridescens* are the most frequent species found in fresh meat (Holzapfel and Gerber, 1983 ; Shaw and Harding, 1984, 1986 ; Morishita and Shiromuzi, 1986 ; Hastings and Holzapfel, 1987 ; Schillinger and Lücke, 1987 ; Collins et al., 1987).

Nevertheless there is clearly a need to simplify their identification which may be misleading because often based on phenotypic tests in particular carbohydrate fermentation (Champomier et al., 1987 ; Schillinger and Lücke, 1987).

The purpose of the present work is to propose a simple practical identification key with a few characters which permit a quick separation of the main species found in meat. To check the validity of our scheme, strains identified according to this key were tested by DNA-DNA hybridization with type strains.

## MATERIAL AND METHODS

121 strains of lactic acid bacteria had been isolated on MRS or APT agar from fresh meat (beef, pork, lamb) and sausage. The type strains were obtained from DSM or ATCC.

### Biochemical tests

The following tests were performed:

- Gaz production was shown by using the loop test (Sperber and Swan, 1976).
- Growth on Rogosa agar was observed.
- The configuration of lactic acid isomer was detected spectrophotometrically in 24h supernatant by an enzymatic method using L and D lactate dehydrogenases (Boehringer).
- Mesodiaminopimelic acid (mDAP) was detected in whole cell hydrolysates by thin layer chromatography (Bousefield et al., 1985).
- CitrulLine production from arginine (ADH) was measured in Niven's medium as described previously (Montel and Champomier, 1987).

DNA-DNA hybridization was performed at 60°C following an S1 nuclease procedure with trichloroacetic precipitation (Grimont et al., 1980), DNA was labelled by nick translation with $^{3}H$ nucleotides.

## RESULTS (figure 1)

By following the simple identification key indicated above, lactic acid bacteria

were especially assigned to four species *L. sake* (81 strains), *L. curvatus* (22 strains), *C. carnis*(8 strains) strains), *C. divergens* (10 strains). We did not recover other species.

For each strain DNA relatedness results obtained with their DNA and labelled DNA from corresponding type strains are very high (70% to 85% homology). With these data it is obvious that this key permit a rapid separation of strains. In fact *L. curvatus* and *L. sake* (Kandler and Weiss, 1986), two species closely related genetically, are easily distinguishable by only two tests: *L. sake* produces citrulline from arginine at low glucose concentration (<0,05%) and ferment melibiose whereas *L. curvatus* does not. It is not efficient to consider other fermentation pattern because variable reactions are often noticed as demonstrated in previous studies (Champomier et al., 1987).

Two species of *Carnobacterium* are clearly separated from *Lactobacillus* species by the type of peptidoglycane and isomeric form of lactate produced. *Carnobacterium* species contain mesodiaminopimelic acid (mDAP) in their cell walls whereas the type of peptidoglycane for *L. sake* and *L. curvatus* is lys DAsp. Production of L lactate only, but not D lactate by *Carnobacterium* species permit to differentiate these species from other mDAP containing lactobacilli. Moreover it is interesting to notice that only *Carnobacterium* species do not grow on Rogosa agar, medium with a high content of acetate. Nevertheless separation of *C. piscicola* and *C. divergens* remains difficult because relying on fermentation of two carbohydrates.

Our results are in agreement with those of Schillinger and Lücke (1987). We confirm that *L. sake* is the predominant species on beef meat (85% of lactic acid flora).

This key will be applied to the study the ecology of these different species in all kinds of meat and meat products.

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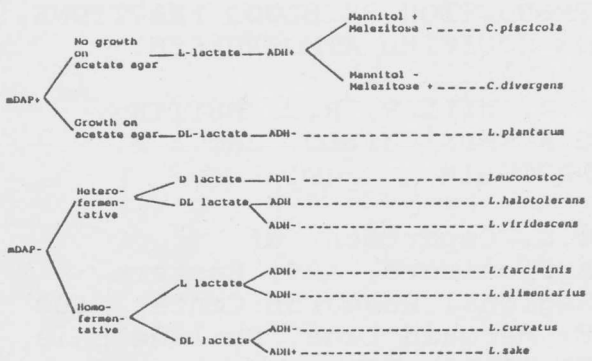


FIGURE 1

SIMPLE IDENTIFICATION KEY FOR LACTIC ACID BACTERIA FROM MEAT