SELECTION OF CELL WALL ANTIGENS FOR THE RAPID DETECTION OF BACTERIA BY IMMUNOLOGICAL METHODS

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INTRODUCTION

Among the different methods which could be used for the rapid counting or detection of bacteria in foods, immunochemical techniques are promising, because of their sensitivity, accuracy and rapidity. However, before the setting up of any method, it is necessary to select antigens on which the tests could be based. For gram negative, this selection is relatively easy as different proteins from the outer membrane has now long been proven to be common antigens of a genus or a species. For gram positive, the problem is more difficult as there is not outer membranes and as protein content of the peptidoglycan is frequently unknown. The present paper describes on one hand the selection of different common antigens from the bacteria which generally influence the microbiological quality of meat and on the other hand an ELISA method which could be suitable for a rapid counting of some species.

MATERIALS AND METHODS

Bacterial strains

Most of the bacteria used in this study have been isolated and identified in the laboratory. Only *P. aeruginosa* PAO1 and *Y. enterocolitica* 102 are a gift of the Pasteur Institute from Paris.

Selection of the antigens

Gram negative bacteria
The production and purification of the F protein from *P. fragi* was carried out according to the method of Yoshimura et al. (1983). Purification of the OmpA from *E. coli* K12 was carried out according to the method of Rosenbusch (1974).

Gram positive bacteria
Lactobacillus curvatus 215 was cultivated in 100 ml of MRS broth at 25°C during 24 hours and then centrifuged at 10000g for 15 min. The pellet (1g) was then washed three times in 10 ml of saline and resuspended in 2 ml of 0,1M Tris HCl pH 8,0 containing 0,5% lysozyme (30000 µ/mg SIGMA) and 5 mM EDTA (TEL). After 18 hours at 37°C the cell suspension was extensively dialysed against saline and freezed.
B. thermosphacta Lg D1 was cultivated at 4°C on nutrient agar so that colonies could be easily observed. About one hundred colonies were mixed in saline, washed three times and lysed similarly to *Lactobacillus curvatus*.

Production of polyclonal antibodies against cell wall antigen

Antibodies against all the antigens were raised in rabbits according to the method of Gilleland et al. (1984).

Western blotting

Extracts of proteins were submitted to an electrophoresis according to the method of Laemmli (1970). Western blotting were carried out in a semi-dry apparatus BIOMETRA

(FRG) according to the manufacturer's instructions.

ELISA test for *Enterobacteriaceae* and *Pseudomonas*
Pure cultures of *Enterobacteriaceae* (Nutrient broth) or ground meat samples (*Pseudomonas*) were 1/5 diluted in saline. An aliquot of each sample was used for the counting according to the standard plate count method. DCA medium (DIFCO) was used for the *Enterobacteriaceae*. *Pseudomonas* were enumerated on the medium of Mead (1978). The 1/5 dilutions of the samples were then 1/2 diluted in 0.2M Tris HCl pH 8.0 EDTA 4 mM lysozyme 1%. 200 µl of a protease solution (1000 U/ml) Rapidase, in pH 7.0 buffer (MERCK) were added in each well. The plates were then placed 15 min at 37°C and washed 3 times as previously described. 200 µl of the appropriate serum dilution were then added in each well and the plates were again placed at 37°C for 2 hours. After three washings, 200 µl of conjugate GAR peroxidase (NORDIC) at the 1/2000 dilution were added. The plates were then placed at 37°C for 1 hour, washed as previously and revealed according to Voller et al. (1979).

ELISA for lactobacilli and *B. thermosphacta*
Pure cultures of *Lactobacilli* and *B. thermosphacta* were resuspended at concentrations varying from 10^9 to 10^3 in sterile 1/10 dilutions of the cultures was carried out in double strength TEL buffer. The rest of the test is similar to that used for Gram negative bacteria except that the treatment with the protease is omitted and that the treatment with TEL buffer lasted 3 hours.

RESULTS

Electrophoresis of the final steps of purification of OmpA and prot F shows that homogeneous preparations are obtained.
Electrophoresis of crude cell wall preparations of *B. thermosphacta* and lactobacilli shows many bands which are very similar within a species.

Specificity of the detection

Purified proteins
As shown on the western blot of crude outer membrane from *Enterobacteriaceae* OmpA is always detected whatever the species. The second band of low molecular weight which is observed in some species is due to the activity of some proteases in the sample. A part this problem it is interesting to notice that no cross reactions are observed with outer membranes from *Pseudomonas* or *Acinetobacter* which are gram negative bacteria equally isolated from meat.
Recognition of protein F from *P. fragi* and *Pseudomonas* species in general seems specific as outer membranes of bacteria from the *Pseudomonas* genus is always detected by the antibodies. No cross reactions are observed with the *Enterobacteriaceae* and *Acinetobacter*.

Crude cell walls
Western blots of *L. sake* and *L. curvatus* have shown that two major bands are recognized in *L. curvatus* and one in *L. sake*. It is also interesting to notice that the band of low molecular weight seems specific of *L. curvatus* and the other common to the two species.

All *B. thermosphacta* strains are recognized by the antibodies raised against the LGD1 strains. Two or three proteins are common antigens of the species. One of them of 40 KD is particularly immunogenic.

Direct ELISA to detect *Pseudomonas* in meat samples

As shown on figure 1, counting of *Pseudomonas* in meats is possible whatever their origin. Moreover, 10^5 cells/g are easily detected. Interestingly at this concentration, all the wells which corresponds to the samples where the contamination is superior to 10^5/g appeared yellow or orange, allowing a visual detection of heavily contaminated samples.

ELISA for counting *Lactobacilli* and *B. thermosphacta*

In order to eliminate the influence of 2 different antigens on the detection, only *L. sake* was used for this study. 5 different strains of this species have been taken from the laboratory collection. As shown on the figure 2, detection of all the lactobacilli is perfectly realised by the method proposed in this study. The sensitivity of the detection is about 10^5 cells/ml. The most interesting results is that bacteria are similarly detected whatever the strains used.
For *B. thermosphacta* detection is possible in the same conditions as those described for *L. sake*, however 10^4 cells/ml could be count even if some strains are detected at 10^5/ml.

DISCUSSION

The results presented in this paper show that the rapid counting or detection of bacteria influencing meat quality will be possible by the use of immunological methods.
However, the choice of the method which will be used in the future is entirely to determine. The direct ELISA proposed in this work is obviously not the more suitable. Among the different ELISA proposed by immunologists, competition for the antigen, or sandwich ELISA, appear to be often more sensible and more convenient. Moreover, instead of using enzymes coupled to antibody, it is now well known that IgG coupled with biotin or fluorescent molecules greatly increase the sensitivity of any tests. Apart the sensitivity which is necessary to improve, it seems also important to detect living cells as the detection of antigens does not necessarily means that the producing bacterium is alive. In this study, we always detected living cells, but in foods, particularly in products submitted to more or less decontaminating treatment, many dead cells could be present. This observation is in favour of the selection of epitopes in common antigens which would be very sensible to decontaminating treatment of foods. A part those drawbacks to the use of immunological methods for a rapid counting of bacteria in meats, this work shows that such methods will certainly be of great interest for the screening of meat and meat products of good microbiological qualities.

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Fig. 1
Relationship between the direct ELISA test and the number of *Pseudomonas* 10
different meat samples

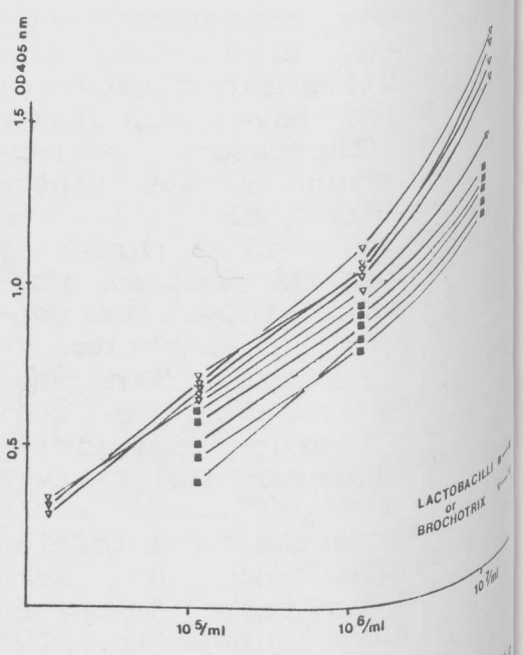

Fig. 2
Counting of *B.thermosphacta* and *Lactobacillus sake* with
ELISA