DEVELOPMENT OF A NEW DETECTION METHOD FOR YERSINIA ENTEROCOLITICA IN MEAT

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SUMMARY

Pigs are an important reservoir of pathogenic Yersinia enterocolitica. This makes it important to have rapid and sensitive detection methods to determine the possible contamination of meat and meat products. In this paper the techniques ImmunoMagnetic Separation (IMS) and conductance measurement are described. The conductance assay offers good prospects to become a rapid and sensitive screening method for Y. enterocolitica, but needs further optimization. Application of IMS leads to purification and concentration of Y. enterocolitica from the competitive microflora in minced meat. Both techniques can be used, possibly in combination with other techniques, to achieve a reliable detection method for Y. enterocolitica in meat and meat products.

Introduction

Pigs are an important reservoir of pathogenic Y. enterocolitica (De Boer et al., 1991). For the detection of this pathogen it is important to have rapid and sensitive detection methods. Because traditional techniques reveal results after 4-6 days, there is a growing demand for a more rapid detection of pathogenic strains of Y. enterocolitica.
The intention of our study is to develop a new detection method for Y. enterocolitica based on two or more of the following detection techniques: ImmunoMagnetic Separation (IMS), conductance measurement, Polymerase-Chain-Reaction (PCR) and ImmunoFluorescence (IF). The technique of IMS has already proven to be successful in the detection of pathogenic micro-organisms, like Salmonella spp. (Vermunt et al., 1992) and Listeria monocytogenes (Fluit et al., 1993). In this paper the IMS technique and a conductance assay are described for Y. enterocolitica O:3.

Materials and methods

Conductance measurement: 0.2 ml of diluted pure cultures of micro-organisms were transferred into 3 ml of Campden Yersinia Impedimetric Medium (CYIM, (Walker, 1989)) in Malthus tubes. For minced meat 3 ml of a homogenized suspension (5 g in 20 ml CYIM) was added to 0.2 ml peptone-saline solution. The tubes were connected to a Malthus 2000 system (Malthus Instruments) and incubated at 25°C or 30°C. Conductance responses were measured every 18 minutes.
Immunomagnetic separation: Magnetic beads from Dynal (Norway), precoated with a secondary antibody, were coated at RIKILT-DLO with a monoclonal antibody (Progen) or a polyclonal antibody (Accurate) against Y. enterocolitica O:3. Coating was carried out overnight at room temperature and the suspension was continuously shaken. Pure cultures of bacterial strains were grown overnight at 25°C in Trypton-Soy-Broth (TSB). Diluted cultures were incubated for 20 min or 1 hour at room temperature with immunomagnetic particles (IMP). This suspension was continuously shaken. A magnetic particle concentrator (MPC-E or MPC-M, Dynal) was used to separate the IMP from the cell suspension. Five washing steps in Phosphate Buffered Saline (PBS) supplemented with 0.1% caseïn (PBS-0.1% caseïn) were carried out. The IMP were suspended in the original volume of PBS-0.1% caseïn. Recovery of Y. enterocolitica cells was measured by plating on Nutriënt Agar (incubation 1-2 days 25°C).
Tonsils: From every tonsil 10 g was homogenized with 90 ml peptone-saline solution. For the traditional technique 1 ml of this homogenate was added to Irgasan, Ticarcillin and potassium Chlorate (ITC) broth. After

incubation for 2 days at 24°C 0.5 ml was mixed with 4.5 ml of 0.5% potassium hydroxide solution for 3 sec and streaked on Cefsulodin, Irgasan and Novobiocin (CIN) agar plates. After incubation for 2 days at 30°C suspect colonies were corfirmed by detection of urease, phenylalanine deaminase, motility at 25°C and 37°C and agglutination with antisera (de Boer et al., 1989). For conductance measurement 0.2 ml of the incubated ITC culture is added to 3.0 ml CYIM, supplemented with 10 mg/l malachite green, 1 mg/l ticarcillin and 1 g/l potassium chlorate. The Malthus tubes were incubated at 30°C.

Results and discussion

Conductance measurement

For the detection of Y. enterocolitica in pasteurized milk, an impedimetric medium (CYIM) was described by Walker (1989). In this paper the development of a suitable growth medium for the detection of Y. enterocolitica in meat by conductance measurement is described.

In the CYIM ureum is present as elective agent. Y. enterocolitica converts this to ammonium, which gives a relatively great conductance change. Several micro-organisms which are part of the common microflora of raw meat, like Citrobacter freundii, Enterobacter agglomerans, Aeromonas hydrophila, Serratia marcescens and Pseudomonas fluorescens do not give such a characteristic conductance change (fig. 1). Naturally contaminated minced meat samples, in which Y. enterocolitica was absent, also showed an increasing conductance curve (fig. 1). Minced meat itself was not responsible for this response, because samples, sterilized by irradiation showed flat conductance curves. Apparently competitive micro-organisms from the minced meat caused the increasing conductance signals. It was also obvious that the conductance curve of Y. enterocolitica was influenced by some of the competitive micro-organisms. To inhibit the growth of the competitive micro-organisms, 3 other selective agents were added to the CYIM: malachite green (10 mg/l), ticarcillin (1 mg/l) and potassium chlorate (1 g/l) and the incubation temperature was increased from 25°C to 30°C. Although competitive micro-organisms present in the minced meat were still able to give an increasing conductance, this alteration resulted in an improved sensitivity for the detection of Y. enterocolitica in spiked samples, because it was possible to detect Y. enterocolitica in the presence of a higher concentration of competitive micro-organisms. Testing of various samples of spiked minced meat showed that a great difference occurred between the detectable ratio of Y. enterocolitica and competitive micro-organisms (range 1:1 - 1:300). The use of a pre-enrichment before conductance measurement did not show consistent results either, but in general the sensitivity was improved with a factor 10. Both effects are probably due to interactions between the microflora from the minced meat and Y. enterocolitica.

For 22 samples of tonsils similar results compared to the traditional technique were obtained: 19 samples were positive with both techniques, from the 3 samples which showed a negative result with the traditional technique one sample showed a conductance curve, characteristic for Y. enterocolitica, due to the presence of Pasteurella spp. (a Yersinia related micro-organism). This illustrates the importance of confirmation of a positive result after screening with the conductance assay.

Immunomagnetic Separation

Application of IMS to pure cultures gave high recoveries: 10^7 sheep-anti-rabbit beads/ml coated with 65 µg polyclonal antibody/mg beads and incubated for 1 hour at room temperature with pure cultures of Y. enterocolitica O:3 led to a recovery of 80.8 $\pm$ 10.1 % (n=5) of Y. enterocolitica O:3. The use of 10^7 sheep-anti-mouse beads/ml coated with 50 µg monoclonal antibody/mg beads showed similar results. Y. enterocolitica incubated with uncoated beads were only recovered for 0.0 - 0.2 %.

In order to reduce the recovery of competitive micro-organisms, the protocol of IMS is optimized by lowering the incubation time of IMP (optimal concentration 10^7 /ml) with the samples from 1 hour to 20 min and by addition of 0.05% Tween 20 to the washing solution. Results of 2 spiked samples minced meat showed that the recoveries of Y. enterocolitica decreased when minced meat was present in IMS (Table 1). However, the ratio between Y. enterocolitica and the competitive micro-organisms was still improved (for sample A and B with 74 and 167 respectively).

Conclusions

The technique of conductance measurement offers good prospects to become a rapid and sensitive screening method for Y. enterocolitica in meat and meat products. Because competitive micro-organisms still interfere in the conductance assay, optimization of the assay needs further attention. If this is successful and more suppression of the growth of the competitive micro-organisms is obtained, the sensitivity of the conductance assay will be improved.
Application of IMS to minced meat improves the ratio between Y. enterocolitica and the competitive micro-organisms. This purification and concentration of Y. enterocolitica leads to a higher sensitivity of detection-, confirmation- and identification-techniques.
Together with a PCR-assay, which is being developed with the PCR-fingerprint technique (Giesendorf et al., 1993), or ImmunoFluorescence (IF) these techniques will be combined to achieve a reliable detection method for Y. enterocolitica in meat and meat products (fig. 2). In this method a corfirmation or identification (by PCR, IF or DNA-hybridization) is performed on all samples which show a positive result after screening by conductance assay or by PCR-assay.

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