5:19 # Application of Direct Epifluorescent Filter Technique as a Rapid Method in Microbiological Quality Assurance in the Meat Industry.

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Introduction

Microbiological analyses related to safety, shelf life and hygiene are important aspects of meat production. Since traditional microbiological methods are time consuming the development of rapid methods have aroused considerable interest in the meat industry. The main reasons for this are the advantages related to the speedy release of products, and that rapid results in monitoring critical control points offer the possibility for early corrections during production. Furthermore, rapid results enable the manufacturer to select raw materials according to their microbiological quality.

To-day equipment based on the following principles is available: ATP-measurements, electrical measurements (impedance and conductivity), radiometry, microcalorimetry and direct epifluorescent filter technique (DEFT). So far none of these methods have been used by the Danish meat industry for microbiological quality assurance. It is believed that due to differences in sensitivity, specificity, capacity and rapidity their will be no all-purpose method for the meat industry, but that various methods will apply to specific areas of microbiological quality assurance (MQA).

Epifluorescence techniques have been known and used as rapid methods for some years (Scholefield et al. 1981). Pettipher and Rodriques (1982) have further developed the technique by including a membrane filtration of a pretreated homogenized sample followed by staining of the filter with acridine orange.

It has been the main object of this study to adapt the DEFT-method to MQA in the meat industry, and in the course of the work attempts have been made to select well-defined areas where it was believed that DEFT could be used with advantage.

Materials and Methods

Two categories of meat products were investigated: (a) Raw ground beef with added soya protein and spices. Fresh frozen as well as fresh chilled ground beef were examined. (b) Heat treated sausages. A Bologna type sausage was examined unsliced as well as sliced, and Vienna sausages were examined shortly after production and at the end of declared storage life. All the products were commercially produced, and as far as category (a) is concerned the analyses took place in the factory on samples subjected to routine bacteriological quality control as well.

To meat samples of 10 gram, 90 ml of dilution medium (0,85% NaCl, 0,1% Bacto peptone, pH 7,2) were added, and the samples were homogenized for 2 min. in a Stomacher. After standing for 30 min. for settlement of larger particles 5 ml of the suspension were filtered through a nylon filter (5 µm pore size, 25 mm diam.) mounted in a Swinex holder (Millipore). Filter and holder were sterilized before use (120°C for 15 min.).

2 ml of the filtered suspension were collected in a sterile test tube, and 2 ml 0,5% Triton x-100 and 0,5 ml Bacto-trypsin were added. Both solutions were filtered through a Millipore membrane filter (0,22 µm pore size) before use. The mixture was incubated in a waterbath at 50°C for 10 min. and then filtered through a Nuclepore polycarbonate membrane filter (0,6 µm pore size, 25 mm diam.) mounted on a filter manifold attached to a vacuum pump.

After filtration the membrane was overlayed with 2,5 ml acridine orange (0,025 g acridine orange in 100 ml citrate buffer solution: 35,5 ml 0,1 M citric acid and 100 ml 0,1 M NaOH, pH 6,6).

After standing for 2 min. vacuum was re-applied in order to filter the stain. The filter was washed with 2,5 ml citrate buffer, pH 3,0 (100 ml 0,1 M citric acid and 54 ml 0,1 M NaOH) followed by 2,5 ml iso-propanol to decolourize the filter. The latter procedure was carried out as fast as possible to avoid decolourization of the bacteria. The stained membrane was airdried and mounted in immersion oil on a slide beneath a cover slip.

The mounted membrane filter was then examined by means of an epifluorescence microscope (Olympus BHB fitted with a 75 W mercury high-pressure burner). The number of fluorescent microorganisms were counted at a magnification of 1000 and the number of microorganisms per gram was calculated by multiplying the average number of bacteria counted per microscope field by the microscope factor (MF).

$$MF = \frac{\text{Area of membrane through which sample is filtered } (mm^2)}{\text{Microscope field area } (mm^2) \times \text{sample volume (ml)}}$$

For automatic counting an image analyzer was used. The image analyzer (Micromeasurement 40-10) was connected to a TV-camera, which was mounted on the microscope.

Results and Discussion

Initial examinations of ground beef gave problems due to poor filtration through nylon, as well as polycarbonate filters. These problems were overcome by placing the sample in a gaze pouch ("Tube gaze", No. 78, Fa. Scholl) within the Stomacher bag. In this way food particles disturbing the filtration were separated from the bacterial suspension. To make sure that this procedure did not cause any significant loss of bacteria a comparative study was carried out as shown in table 1. It is seen that no significant difference in bacterial counts were observed by the two methods.

The results from using DEFT in the routine quality control in a meat plant are shown in fig. 1. The results were very similar to the results obtained by the conventional method. A regression analysis was performed and a correlation coefficient of 0,79 was found. However, due to the uniformity of the samples a regression analysis might be somewhat uncertain. Therefore an analysis of variance for two criteria of classification was performed as well. This showed no statistical difference on the 95% level between the two methods. On the 99% level significant differences were found, but numerically these differences were so small that they were considered to be without practical importance.

Since these results were obtainable within one hour the DEFT-method is considered applicable for speedy release of this type of product. However, it shall be emphasized that automatic counting could not be used at this level of microorganisms. This is a major disadvantage since manual counting in a fluorescence microscope is very tiring and time consuming reducing the capacity to 20 samples to be read by one person in a working day.

It is worth mentioning that no measurerable difference in bacterial counts were found between frozen and chilled ground beef. This shows that modern freezing technology to a very little extent affects the flora present in ground beef.

Fig. 2 shows a comparative examination of products where bacterial counts, when determined by traditionel plate counting, varied from 10 to 10^8. The high counts represent Vienna sausages and sliced Bologna type sausages at the end of their storage lives. It is seen that at this high level there is an acceptable agreement between DEFT and counts on PCA. This is probably due to not only the high counts but also to the fact that the proportion of dead cells is very low. It was a general observation that at bacterial levels of 10^7 to 10^8 fluorescent background material did not interfere significantly with the use of automatic counting.

On the contrary a very poor relation exists in products with low counts. These are in fig. 2 represented by Vienna sausages examined shortly after heat treatment, and whole Bologna type sausages heat treated in its casing so that post-contamination was avoided, and growth from surviving bacteria was limited by cold storage. In these products a significant proportion of the bacterial cells will be dead cells.

These experiments show that DEFT cannot differentiate between live and dead cells. In this connection it should also be remembered that the DEFT includes a heat treatment at 50°C for 10 min., which will kill a number of psychrotrophic bacteria present in meat products. It is therefore understandable that for certain products there will be no direct correlation between DEFT and traditional plate counting. However, it should be emphasized that such a correlation is not a prerequisite when using DEFT for certain control purposes. DEFT can give a picture of the total microbiological status of products (dead and live cells) and in this way offer information on the prehistory of the product. Moreover DEFT to a certain extent can be informative on the composition of the microflora and in this way give more information than traditional plate counts.

Products with total counts (live and dead cells) below 10^4 per gram could not be examined by DEFT and problems with sensivity became more severe when using automatic counting. For the present samples fluorescent background material made automatic counting impossible when the bacterial count was below 10^6 per gram.

Finally it should be mentioned that for certain sausages the number of fluorescent starch particles was so high that automatic counting could not be used irrespective of bacterial numbers.

Conclusions

The present study has shown that DEFT can be used for well-defined purposes in MQA in meat plants. As an example Danish meat plants exporting ground beef in most cases have to assure that bacterial counts do not exceed 10^6 per gram. For this product DEFT can be used to supervise that this microbiological criteria is adhered to. The good agreement with conventional methods is very important, because the latter methods are used by the importing countries. The availability of results within one hour offers a speedy release of the product and in this way increases storage capacity.

DEFT cannot differentiate between live and dead cells. Therefore, for many products a direct correlation between DEFT and plate counts does not exist. It is emphasized that such a correlation not necessarily is a prerequisite for using DEFT which give an overall picture of the microbiological status by counting live as well as dead cells. Moreover the epifluorescence microscopy can give important information on the composition of the microflora. The sensitivity of the method is limited to bacterial counts of 10^4 - 10^5 or more per gram.

The greatest problem with the present status of the method concerns the automatic counting which cannot be used for products with bacterial counts below 10^6 per gram. This is generally the situation in meat plants producing ground beef and other products from fresh raw materials. Here the capacity is limited to only 20 samples per person per day since manual counting will have to be used.

This means that DEFT at present will be of limited interest for large scale productions where many bacteriological checks are needed. However, for smaller production units where rapid results are needed DEFT could be used with advantage for examination of raw materials, critical control points and the finished products. Additional DEFT can be a useful tool in trouble shooting.

As for other rapid methods DEFT is still subject to modifications and research. It is hoped that future developments will overcome some of the problems mentioned. Areas of relevant research concerns primarily the development of more specific fluorocromes and improved pre-treatments, which can reduce the disturbing influence from fluorescent background material and allow automatic counting to be used for a wide range of products.

Literature

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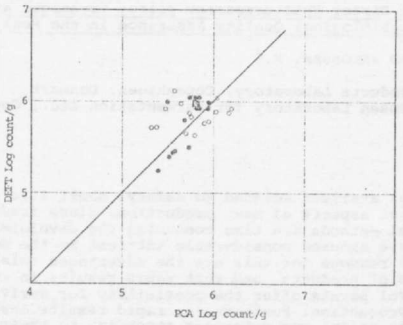
Tabel 1

Sample	Counts per gram A	B
I	2.6×10^5	2.1×10^5
	2.6×10^5	2.2×10^5
II	3.8×10^7	3.1×10^7
	1.9×10^7	3.8×10^7
III	4.5×10^5	4.5×10^5
	3.5×10^5	5.1×10^5
IV	1.3×10^6	4.3×10^5
	1.2×10^6	9.7×10^5
V	2.4×10^9	1.8×10^9
	2.5×10^9	3.6×10^9

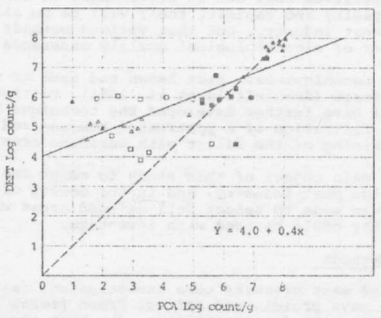
Bacterial counts (PCA, 25 C, 72 h) for ground beef. Duplicate analyses of 5 samples. (A = homogenizing in Stomacher. B = homogenizing in Stomacher with sample placed in a sterile gaze pouch).

Fig.1



Bacterial counts on ground beef with DEFT and PCA (25°C, 72 h). Fresh frozen (o); fresh chilled (●).

Fig. 2



Bacterial counts on meat products with DEFT and PCA (25°C, 72 h). Whole Bologna type sausage (□); sliced Bologna type sausage at the end of storage life (▣); freshly produced Vienna sausage (△); Vienna sausage at the end of storage life (▲).