Clarification of meat suspensions using column filtration and rapid quantification of viable microbial cells using flow cytometry

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Summary and Implications

A simple column filtration was developed to clarify meat homogenate suitable enough to quantify and assess the viability of bacterial population using flow cytometry. The removal of debris from homogenized meat suspensions was achieved by passing through different column materials such as sand particles, glass beads, dowex resin, and cell debris remover (CDR). Total plate counts on tryptic soy agar and lactic acid bacteria counts on MRS agar were done on the clarified suspensions. Absorbance of the clarified suspensions was measured at 600 nm. Among the material compared, sand and glass beads gave more than 90% recovery of bacterial cells. The absorption spectra of the filtered suspensions were considerably less and the best results were obtained with CDR followed by dowex resin.

BacLight viability kit was successfully used to stain the bacterial cells in the meat suspensions. Injured sub populations of *E. coli* in pure culture were also studied using this stain.

Introduction

Techniques for rapid assessment of microbial viability is critical in many areas of microbiology including food industry, public health, and dairy industry. Conventional culture methods for determining total viable counts on meat and meat products take at least one day to produce a result (Shaw et al., 1987). A quick assessment of the microbiological condition is also required during processing and distribution of the finished products. Among the various rapid methods available, direct microscopic enumeration using direct epifluorescent filter technique (DEFT) appears advantageous. It has the potential to generate results within 30 minutes and give a good estimate at the same time (Pettipher and Rodrigues, 1981). A pretreatment method involving enzymatic digestion to remove food debris and clarify food suspensions was also developed (Jaeggi et al. 1989).

Materials and Methods

Meat Samples: Ground pork (frozen) was purchased from the Meat Lab at Iowa State University and thawed overnight in a refrigerator. Thawed samples were incubated aerobically at 4°C. Aerobic plate counts on tryptic soy agar (Difco) were done once a day. Thirty ml. of 1-10 dilution of meat homogenate was used for clarification.

Flow Cytometry: A flow cytometer (Coulter EPICS XL-MCL) available in the Cell and Hybridoma Facility at Iowa State University was used in this study. The flow cytometer was equipped with a FL1 - green filter of 525 nm. BP (505 to 545 nm.) and a FL4 - red filter of 675 nm. BP (660 to 700 nm.). The excitation of the dye was at 488 nm. All the samples were counted up to 20,000 events.

Staining Protocol: BacLight Viability Kit (Molecular Probes, Inc.,) was used to stain the bacterial cells. Reagents A and B were added at a concentration of 1μl./ml. each. The samples were incubated in dark for 15 minutes.

Stress Conditions: *E. coli* cells were heat treated at 65°C for 5 minutes (sub-lethal dose) and 30 minutes (lethal dose). Acid treatment of the cells was done in minimal media acidified to a pH of 3.0 using lactic acid. Sub-lethal doses included incubating the cells for 20 minutes and 60 minutes respectively.

Evaluation of Column Materials: The following column materials were evaluated for their suitability in clarification of meat suspensions:

(1) Sand, white quartz (-50 +70 mesh)-(Sand I)
(2) Glass beads (106μ and finer; acid-washed).
(3) Dowex 1 (Ionic form: chloride-strongly basic anion exchanger).
(4) Cell debris remover (CDR) (Modified cellulose).
(5) Sand II (100 mesh and finer).

Sand (I), glass beads and dowex 1 were purchased from Sigma, USA and CDR from Whatman, UK. Sand II was prepared by grinding Sand I in a pestle and mortar. It was sieved using a 100 mesh sieve and then acid washed three times followed by three washings with deionized water. It was then dried at 110°C for 12 hour.

Each of the above material was evaluated individually in a Bio-Spin disposable chromatography column (Bio-Rad Laboratories, CA). Two filter paper discs (Whatman 41) were cut to the size of the support material and were placed above and below the support material. The columns were then loaded with the column materials and autoclaved. One ml. of meat suspension (1-10 dilution), after letting it stand for 10 minutes. was loaded onto the column and filtered using vacuum. Absorption of the filtered meat suspensions was measured at 600 nm. using spectrophotometer (Spectronic 601, Milton Roy). Aerobic plate counts on TSA and MRS agar were also done on filtered meat suspensions. Sand II was used as

the column material for use in flow cytometry analyses. The sample preparation protocol is shown in diagram 1.

Results and Discussions

Evaluation of Column Materials: Among the different column materials tried, sand I, glass beads, and sand II were found suitable for use as filtration material. The percent recovery was above 90% for both TSA and MRS counts (Figures 1 and 2). The degree of clarification was studied by measuring the absorption at 600nm. There was a three fold reduction in absorption using all the materials except sand I (Figure 3). The main reason for this is the size of sand particles. The size of the particles influences both the degree of clarification and percentage recovery of the cells. Though dowex 1 and CDR gave good clarification, the recovery of cells was poor.

Flow Cytometric Analysis of Clarified Meat Suspensions: Based on the above results, sand II was chosen for clarification of meat suspensions to be used in flow cytometry studies. The percent recovery of cells are presented in Figure 4. Figure 5 shows the direct analysis of sample spiked with *E. coli* and a day 0 sample. It is evident from the figure that both bacteria and meat debris take up the stain but bacteria show up in a different region. The stain is a DNA stain and thus could stain the meat tissue debris in the clarified meat samples. The growing bacterial populations were visible starting day 4 when the plate count showed a 5 log population (Figure 6).

Flow Cytometric Analysis of Injured *E. coli* Cells: *E. coli* cells were treated with a lethal and sublethal dose of heat and two levels of sub-lethal doses of acid. In both the cases sub-lethal doses resulted in a sub population of injured bacterial cells. This population was different from the dead cells and live cells (Figures 7 and 8). The live cells stain green and the dead cells stain red. The sub-lethally injured cells show both the colors due to varying degrees of membrane permeabilization. Thus it is evident from these results that flow cytometric analysis afford us a quick assessment of the injured population of bacteria. This could have several applications in the food industry and could provide new ways of studying the physiology of injured bacterial cells.

Conclusions

(1) We have developed a simple column filtration method for clarification of meat samples.

(2) The degree of clarification was objectively quantified by a simple spectrophotometric analysis.

(3) The clarified meat suspensions were found suitable to be analyzed directly in a flow cytometer.

(4) It is possible to quantify the viable population of bacterial cells directly and rapidly from the meat samples.

Acknowledgments

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References

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Diagram 1

Sample Clarification

10g. ground pork and 90 ml. BPW
↓
Homogenized in a Stomacher (2 minutes)
↓
Transferred 30 ml. into centrifuge tube (Initial)
↓
10 ml. loaded onto a column (Bio Rad) with sand (100 mesh)
filtered under vacuum (After filtration)
↓
Column washed with three 10 ml. volumes of PBS
↓
Centrifuged (12,000 rpm.; 10 minutes)
↓
Pooled the filtrate
↓
Centrifuged (12, 000 rpm.; 10 minutes)
↓
Pellet dissolved in 1 ml. of PBS
↓
Centrifuged (1000 rpm.; 2 minutes)
↓
Supernatant (After Centrifugation)
↓
Flow Cytometry