Role of bacterial cell-dairy separation membrane interaction on biofilm formation

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Project Objectives

1. To study the role of bacterial cell and dairy separation membrane characteristics on biofilm formation
2. To screen depolymerase produced by lytic phage for their ability to remove biofilm

Description of Work Performed

This study isolated 8 bacterial strains from membranes obtained from the dairy industry and tested them for hydrophobicity, slime formation, and acid and capsule production. The growth curve of individual strains was studied and the OD at 600 nm that gives a log bacterial count of 7.4 was determined for each strain.

A bioreactor was assembled and used for in vitro biofilm formation by individual strain. A combination of 3 strains and 4 different types of membranes obtained from a membrane manufacturer was used in this stage of study to form in vitro biofilm under dynamic conditions. The number of cells in biofilm formed on each of the 4 membranes was counted. There was no noticeable slime production in whey among the three tested strains. However, *Enterococcus faecium* increased the viscosity of fermented whey to a greater extent than did the other two strains. Interestingly, although not forming noticeable slime in fermented whey, *Enterococcus faecium* produced slime when they attached to the membrane surface. This might explain the high ability of this strain to form biofilm compared to *Escherichia coli.* Although there was no difference in fermented whey viscosity, cell hydrophobicity and capsule production between *Micrococcus* sp. and *Escherichia coli*, the ability of the former strain to form biofilm was higher. This is probably due to other factors such as cell surface charge and cell surface structures. While *Enterococcus faecium* seemed to prefer rough surfaces, *Escherichia coli* produced less biofilm on such surfaces. This study shows that membrane surface roughness is one of the important factors influencing biofilm formation on dairy separation membranes but the preferred surface depends on the strain/species. This confirms our hypothesis that cell-membrane surface interactions influence biofilm formation on dairy separation membranes.

Results of Technology or Process Assessed

Objective 2 was not completed due to some unexpected drawbacks. The setup of the bioreactor took much longer time than what we expected. Also, it was a challenge to use whey as a growth medium. Initially pasteurized whey was used. The advantage of using pasteurized whey is that we simulated industrial conditions. However, some indigenous microflora that survived pasteurized could grow and contribute to biofilm formation. This made it difficult to related biofilm formation to only individual isolates. Autoclaving denatured whey proteins and was not a suitable sterilization technique to use. Alternatives such as whey filtration were evaluated. However, this was a laborious process as the amount of whey needed for the bioreactor was large. We tried sterilized tryptic soy broth supplemented with 1% filter sterilized whey protein concentrates. This gave the best results and would be recommended for future research in this area.

Conclusions

There was no noticeable slime production in whey among the three tested strains. However, *Enterococcus faecium* increased the viscosity of fermented whey to a greater extent than the other two strains. Interestingly, although not forming noticeable slime in fermented whey, *Enterococcus faecium* produced lots of slime when they attached to the membrane surface. This might explain the high ability of this strain to form biofilm compared to *Escherichia coli.* Although there was no difference in fermented whey viscosity, cell hydrophobicity and capsule production between *Micrococcus* sp. and *Escherichia coli*, the ability of the former strain to form biofilm was much higher. This is probably due to other factors such as cell surface charge and cell surface structures. In general, biofilm formation as affected by membrane surface was species/strain dependent. This confirms our hypothesis that cell-membrane surface interactions influence biofilm formation on dairy separation membranes.