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ORIGINAL ARTICLE

Factors affecting the attachment of micro-organisms isolated from ultrafiltration and reverse osmosis membranes in dairy processing plants

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Abstract

Aims: To identify the types of micro-organisms involved in the formation of biofilms on dairy ultrafiltration and reverse osmosis membranes and investigate factors affecting the attachment of those isolates.

Methods and Results: Micro-organisms isolated from industrial membranes following standard cleaning were identified using the API culture identification system. Thirteen different isolates representing eight genera were isolated and their ability to attach to surfaces was compared using a microtitre plate assay. Three *Klebsiella* strains attached best, while mixed strains of *Pseudomonas* and *Klebsiella* attached better than individual strains. Whey enhanced the attachment of the isolates. The micro-organisms were characterized according to cell surface hydrophobicity using the microbial adhesion to hydrocarbon (MATH) test, and cell surface charge by measuring the zeta potential. These cell surface characteristics did not show a clear relationship with the attachment of our strains.

Conclusions: A variety of different micro-organisms is associated with dairy ultrafiltration and reverse osmosis membranes after cleaning, suggesting several possible sources of contamination. The cleaning of these membranes may be inadequate. The attachment of the different isolates is highly variable and enhanced in the presence of whey.

Significance and Impact of the Study: Knowledge of persistent microflora colonizing dairy membrane systems will help develop strategies to mitigate biofilm development in this environment, improving hygiene in membrane processing plants.

Introduction

Membrane separation technology is essential in dairy manufacturing plants, including removal of bacteria from skim milk, concentration of casein micelles from skim milk and recovery of serum proteins from whey (Brans *et al.* 2004). Micro-organisms growing as biofilms colonize membrane surfaces, blocking the membrane pores and resulting in reduced flux (Ridgway *et al.* 1983). Frequent cleaning is used to control microbial colonization, but in extreme cases, the membranes have to be replaced. This reduction in operational efficiency during manufacture increases the likelihood of product contamination and subsequent economic loss.

Biofilms have been extensively studied in environmental, clinical and food processing settings. However, only a few studies have assessed biofilm formation on processing membrane surfaces [e.g. the biofilm on membranes processing wastewater (Ghayeni *et al.* 1998; Chang *et al.* 2002)]. We believe that micro-organisms colonizing membrane surfaces in dairy plants can potentially contaminate the final product, slow membrane flux and reduce product yields through proteolysis of milk proteins and lactose fermentation. With the increasing use of membrane processing in dairy manufacture, there is a

need for information on factors involved in the formation of biofilms on dairy membrane surfaces.

Biofilms, which are initiated by the attachment of micro-organisms to a surface, develop when the attached micro-organisms commence growth (Ivnitsky *et al.* 2005). The initial adhesion of micro-organisms to the surfaces is essential for biofilm formation (Dang and Lovell 1999). Given adequate nutrients, time and suitable temperature, the initial sessile microbial population can eventually form a confluent lawn of bacteria on the membrane surface (Ridgway *et al.* 1999).

Spiral wound membranes (Ridgway *et al.* 1983) are the most common configuration for membrane processing in dairy plant because of their competitively priced design. However, this configuration has extreme susceptibility to fouling, owing to the close spacing of the membrane leaves (Cartwright 2003). The retentate and permeate are separated by membrane layers. Retentates are collected from the feed side of the layers, while permeates flow through the membranes and enter a central tube through permeate collection holes.

Physicochemical forces, comprising hydrophobicity and charge, are the only nonspecific interactions between cell and surface (Smith *et al.* 1998) that are involved in cell attachment (Marshall 1991). To reduce fouling, membrane manufacture has focused on increasing the hydrophilicity of membranes and ensuring a negative charge on the membrane surfaces (Chen and Belfort 1999; Espinoza-Gómez and Lin 2001). However, in the dairy industry, membranes with increased hydrophilicity will always be more hydrophobic than the aqueous solutions being treated by membrane filtration. The rationale behind generating negative surface charges on membranes is to prevent negatively charged colloidal particles, such as micro-organisms, adhering to the surface. Hydrophobicity and charge of the microbial cell surface are considered to be important factors in the determination of adherence of the bacteria to surfaces (Klotz 1990; Kumar and Anand 1998; Vacheethasanee *et al.* 1998; Krepsky *et al.* 2003).

In this study, attachment of micro-organisms isolated from dairy plant membranes was investigated, using a microtitre assay plate as a model surface. The effect of cell charge and hydrophobicity, the environment (whey proteins) and interactions between species were investigated to determine key factors involved in microbial attachment to dairy membranes.

Materials and methods

Selection of samples

The ultrafiltration and reverse osmosis membranes were obtained from various managers of dairy manufacturing plants in New Zealand. All membranes had been in routine use in manufacturing plants processing whey or whey permeate. All operated at 15–20°C under turbulent flow at pH 4·6–6·2. Specific details of the shear rate and flux were not provided, though all manufacturers aim to operate the plants according to the membrane manufacturers' guidelines. Membranes had been cleaned, using the standard caustic based clean-in-place system in the plant, before being removed, sealed in plastic bags to retain moisture and sent by courier to our research laboratory.

Examination of the membranes and isolation of micro-organisms

To obtain microbial isolates from the surfaces of polyethersulfone spiral wound reverse osmosis and ultrafiltration membrane samples, the membrane cartridges were cut into sections (30 cm in length) using a sanitized band saw. Small pieces (2 cm × 4 cm) of membrane were cut from the unrolled membrane sections using sterile scissors and observed under the microscope to record the general appearance of fouled zones on the membrane surface. In order to examine the total (viable and non viable) microbial content of the deposits on the membrane more thoroughly, solid deposits were removed using sterile swabs and transferred to microscope slides for Gram-staining and observation.

As the microflora on the membranes had survived cleaning, we assumed that much of the population was firmly attached to the membrane and therefore difficult to remove. To isolate these firmly attached cells, membrane samples were incubated on skim milk agar (SMA) (Merck, Germany) plates by placing either the permeate side or the retentate side directly onto the SMA. For each membrane sample, at least 30 plates were incubated at three different temperatures (25°C, 30°C and 37°C). After incubation, the predominant colony types were streaked onto SMA for subsequent identification using the API culture identification system (BioMerieux, Durham, NC, USA).

Preparation of inocula

Pure cultures of the microbial isolates were grown on SMA for 24 h (bacteria) or 48 h (yeast) and then inoculated into sterile trypticase soy broth (TSB) (BD, Fort Richard Laboratories, Auckland, New Zealand) and incubated for 24 h. After incubation, strains were harvested by centrifugation at 2500 ***g*** for 10 min and then resuspended in sterile PBS (pH 6·5), whey permeate (pH 6·5) or whey (pH 6·5) for the microtitre plate assay, cell surface hydrophobicity and cell surface zeta potential measurements. The optical density (OD) was adjusted to 1·0

at 600 nm using a spectrophotometer (Ultrospec 2000, Pharmacia Biotech Science & Technology Ltd, Cambridge, UK). The number of bacterial cells after adjustment was around $10^7 \sim 10^8$ CFU per ml. For the experiments with combinations of two strains, these were prepared from equal proportions of the two cell suspensions at the same OD_{600} reading of 1·0.

Microtitre plate assay

Sterile 96-well polystyrene tissue culture microtitre plates (Becton Dickinson Labware, NI, USA) surface treated so as to be hydrophilic were used in a standard microtitre plate assay to screen for potential microbial attachment to membrane surfaces (Djordjevic *et al.* 2002). Aliquots (200 μl) of each cell suspension were dispensed into three wells of the microtitre plate. Each plate also contained three wells with 200 μl of PBS, whey permeate or whey as controls. A preliminary screening trial had established that 4 h gave the highest attachment for some strains, ensuring that cells firmly adhered to the surface. The plates were left at ambient temperature for 4 h without agitation. The attachment to the microtitre plate after standing for 4 h in PBS was used as a reference to compare attachment in whey and whey permeate with the same exposure time. Attachment in the presence of individual components of whey was tested at concentrations that reflect the composition of whey: 13% α-lactalbumin, 48% β-lactoglobulin, 18% glycomacropeptides (GMP) and bovine albumin (BA) (0·2 g l^{-1}). The pH of the four components was adjusted to 6·5 – the same as the pH of the whey used in the earlier trial. To determine the attachment of single strains and the effect of microbial interactions on attachment, both single strains and combinations of strains were used as inocula in different experiments. After incubation, the media were removed and attachment was measured as described by Djordjevic *et al.* (2002). Optical density of the crystal violet solution was measured at 595 nm using the ELx808 Ultra Microplate Reader (Bio-Tek Instruments, Inc., VT, USA). An $OD_{595\ nm} > 0{\cdot}1$ was taken as positive for attachment in the microtitre plate assay.

Attachment to the membrane

To confirm the validity of the microtitre plate assay, these attachment results were compared with those obtained using membrane surfaces. Polysulfone membranes with a diameter of 47 mm and a pore size of 0·45 μm (Pall Corporation, Alphatech Systems Ltd, Auckland, New Zealand) were cut into eight pieces using sterile blades, placed in distilled water and autoclaved for 15 min at 121°C. Each piece of the membrane was placed into a 25 ml sterilized glass bottle and immersed in 1 ml of inoculated PBS with $OD_{600\ nm}$ 1·0. They were incubated for 4 h at room temperature without shaking. After incubation, the membranes were washed twice with sterile distilled water. Then 3 ml of sterile distilled water was added. The cells attached to the membranes were extracted by vortexing for 1 min and the numbers of cells released were measured by plating serial dilutions on SMA, followed by incubation at the growing temperatures of the strains (e.g. *Klebsiella B001* at 25°C, *Klebsiella B006* at 37°C and *Bacillus WL004* at 25°C).

Microbial adhesion to hydrocarbon assay

Microbial cell surface hydrophobicity was determined using a modification of the microbial adhesion to hydrocarbon (MATH) test (Rosenberg *et al.* 1980). Samples were prepared as before, with $OD_{600\ nm}$ of 1·0. Xylene (M&B, Dagenham, UK) was used as the hydrophobic target for cells to attach (Flint *et al.* 1997). Each test was performed in triplicate and the results expressed as mean and standard deviation. The hydrophobicity was calculated using the percentage hydrophobicity formula: (Flint *et al.* 1997).

$$Hydrophobicity(\%) = \frac{Ab_{600\,nm}(before\ xylene) - Ab_{600\,nm}(after\ xylene)}{Ab_{600\,nm}(before\ xylene)} \times 100\%$$

In the MATH test, the loss in absorbance in the aqueous phase relative to the initial absorbance value is taken to represent the amount of cells adhering to xylene and this is a reflection of the hydrophobicity of the cell surface. The cell surface hydrophobicity measured in PBS (pH 6·5) was considered to be the base line and compared with attachment in whey permeate. In order to understand how the cell surface hydrophobicity affects the attachment of our strains, cells were incubated in PBS, whey and whey permeate for 4 h at room temperature and the cell surface hydrophobicity and attachment were measured.

Zeta potential

Cell surface zeta potential was measured in PBS and whey permeate using the Malvern Zetasizer Nano NS (Malvern Instruments Ltd, Worcestershire, UK) (Denyer *et al.* 1993). Readings were made in triplicate. The zeta potential measurement relies on light scattering. If whey proteins bind to the surface of the micro-organisms, they will influence the zeta potential. Therefore, the cell surface zeta potential was not measured in whey.

Table 1 Strains isolated from the dairy membrane plants

Strains	Species	Manufacturing plant	Type of plant	From permeate side / retentate side of membrane samples
WL001	*Chryseobacterium indologenes*	A	Ultrafiltration (whey)	Retentate side
WL004	*Bacillus firmus*	A	Ultrafiltration (whey)	Retentate side
WL008	*Lactococcus lactis* ssp *cremoris*	A	Ultrafiltration (whey)	Retentate side
B001	*Klebsiella oxytoca*	A	Ultrafiltration (whey)	Permeate side
B003	*Enterobacter sakazakii*	A	Ultrafiltration (whey)	Permeate side
B006	*Klebsiella oxytoca*	A	Ultrafiltration (whey)	Permeate side
WA001	*Lactobacillus*	B	Ultrafiltration (whey)	Permeate side
WA002	*Bacillus licheniformis*	B	Ultrafiltration (whey)	Retentate side
TR001	*Pseudomonas fluorescens*	C	RO (casein whey permeate)	Retentate side
TR002	*Klebsiella oxytoca*	C	RO (casein whey permeate)	Retentate side
TR004	*Bacillus licheniformis*	C	RO (casein whey permeate)	Retentate side
H1	*Blastoschizomyces capitatus*	C	RO (casein whey permeate)	Retentate side
EL4019	*Klebsiella oxytoca*	D	RO (milk permeate)	Retentate side

Statistical analysis

In all analyses, triplicate tests were done under identical conditions and the results expressed as mean and standard deviation. Multivariate linear regression was used to assess the impacts of hydrophobicity and charge on attachment.

Results

Examination of the membranes and isolation of micro-organisms

Large amounts of solid material were visible macroscopically on the membrane surface. On many membrane surfaces few bacteria were observed microscopically, suggesting that either these membranes were clean or the contamination remaining after cleaning was too low to be detected. However, the scrapings from one RO membrane showed both yeast and bacteria.

Seven of the 13 isolated strains were Gram-negative micro-organisms (Table 1). Strains isolated from the permeate and retentate side of each membrane were different.

Attachment of strains suspended in different media

In PBS, three strains – all *Klebsiella* (*TR002, B001* and *B006*) attached to the microtitre plate wells, while the other 10 strains did not attach. All three strains that attached in the presence of PBS showed increased attachment in whey and whey permeate. Two other strains (*Pseudomonas TR001* and *Chryseobacterium WL001*) did not attach in PBS but did attach in the presence of whey and whey permeate (Fig. 1).

Attachment of mixed strains

When *Pseudomonas TR001* was mixed with *Klebsiella TR002*, *B001* and *B006*, attachment, as indicated by the crystal violet OD_{595}, was greater than that seen for the individual strains, suggesting a synergistic effect (Fig. 2).

Attachment to the membranes

The attachment of *Klebsiella B001* and *B006* and *Bacillus WL004*, representing two strains that attached and one strain that did not attach in the microtitre plate assay were chosen to verify the use of the microtitre plate assay as a screening model for attachment to the polysulfone

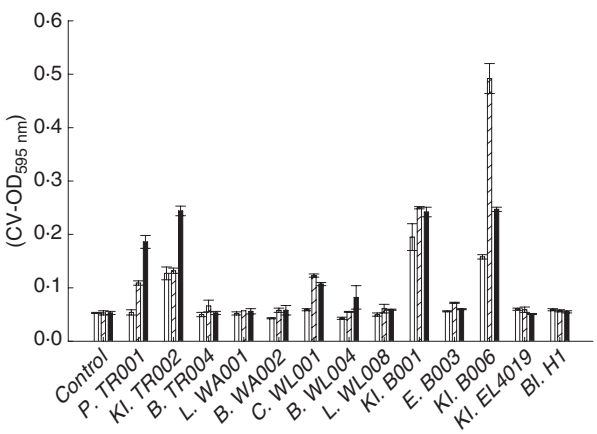

Figure 1 The attachment of strains to microtitre plates in different media in 4 h: (□) PBS, (▨) whey permeate and (■) whey. (Results expressed as mean and standard deviation, which were from triplicates.)

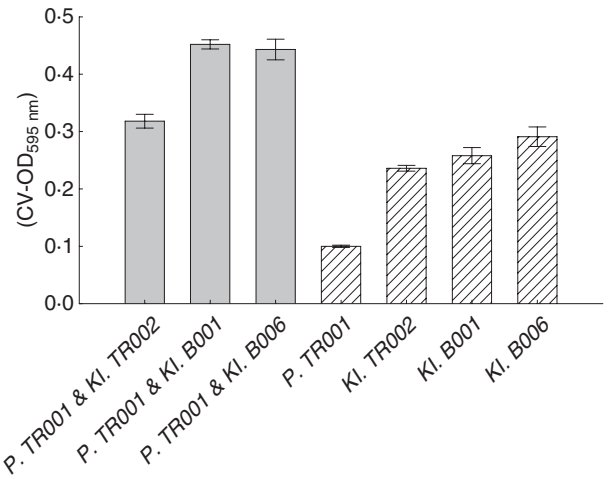

Figure 2 The attachment of mixed strains to microtitre plates in PBS (pH 6·5) after incubation for 4 h. Means were taken from triplicates. The first three bars are *Pseudomonas TR001* mixed with one of *Klebsiella TR002*, *B001* and *B006*. The mixing ratio was 1 : 1 by volume. The striped bars demonstrate the attachment of pure individual strains.

Table 2 Plate counts of the cells attached to the polysulfone membranes

Strains	Plate Counts (CFU ml^{-1})
K. B001	$9{\cdot}45 \times 10^7$
K. B006	$2{\cdot}50 \times 10^7$
B. WL004	$2{\cdot}25 \times 10^5$

Means were taken from duplicates.

Table 3 Attachment ($CV\text{-}OD_{595\ nm}$) of three *Klebsiella* strains in four whey components

	α-lac	β-lac	BA	GMP
TR002	0·005 ± 0·007	0·043 ± 0·005	0·008 ± 0·002	0·011 ± 0·009
B001	0·090 ± 0·009	0·095 ± 0·029	0·008 ± 0·004	0·087 ± 0·037
B006	0·165 ± 0·004	0·124 ± 0·010	0·171 ± 0·010	0·133 ± 0·024

Means and standard deviations from triplicate measurements.

membranes. The numbers of cells that attached to the membrane are shown in Table 2. *Klebsiella B001* and *B006* attached to both the polystyrene microtitre plates and polysulfone membranes, whereas *Bacillus WL004* did not (Fig. 1).

Attachment in components of whey

Previous results showed that the attachment of three strains (*Klebsiella TR002*, *B001* and *B006*) was increased in the presence of whey and whey permeate (Fig. 1). In order to analyse further which component in whey played the major role in attachment, attachment of these three *Klebsiella* strains was measured in each of four whey components using the microtitre plate assay.

None of the four whey components (α-lactalbumin, β-lactoglobulin, bovine albumin and GMP) when used individually appeared to enhance attachment of the three strains that had shown greater attachment in the presence of whey compared with PBS (Table 3).

Cell surface hydrophobicity and attachment

Compared with the hydrophobicity in PBS, the cell surface hydrophobicity in whey permeate was generally higher, while that in whey was generally lower (Fig. 3).

In PBS, three strains (*Klebsiella B006*, *B001* and *TR002*) which had low hydrophobicity (13·7%, 16·8% and 20·7%) showed high attachment (Fig. 4a). However, the other strains with hydrophobicity in the range from 4·1% to 97·5% did not attach. The two strains (*Chryseobacterium WL001* and *Lactobacillus WA001*) with extremely high hydrophobicity (94·3% and 97·5%) did not attach.

Experiments in whey permeate showed little correlation between attachment and hydrophobicity of the cells. Figure 4b shows a slight trend towards increased attachment with increasing hydrophobicity, the exception being strain *B006*, with a hydrophobicity of 2·4%, demonstrating the highest rate of attachment.

In whey, three strains (*Klebsiella B006*, *B001* and *TR002*) that had low hydrophobicity showed high attachment, and *Lactobacillus WA001* that had the highest hydrophobicity did not attach (Fig. 4c).

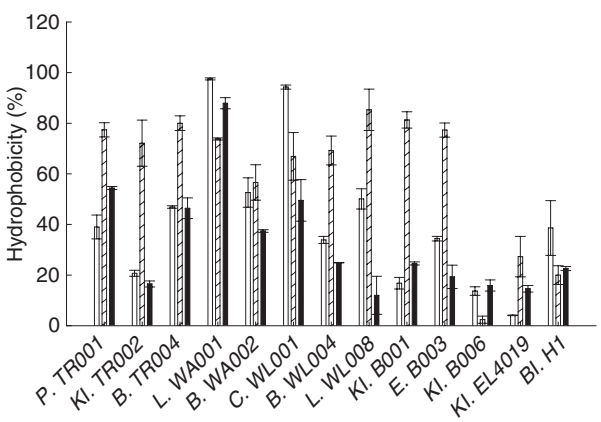

Figure 3 Cell surface hydrophobicity in different media (pH 6·5): (□) PBS, (▨) whey permeate and (■) whey. (Results expressed as mean and standard deviation, which were from duplicates.)

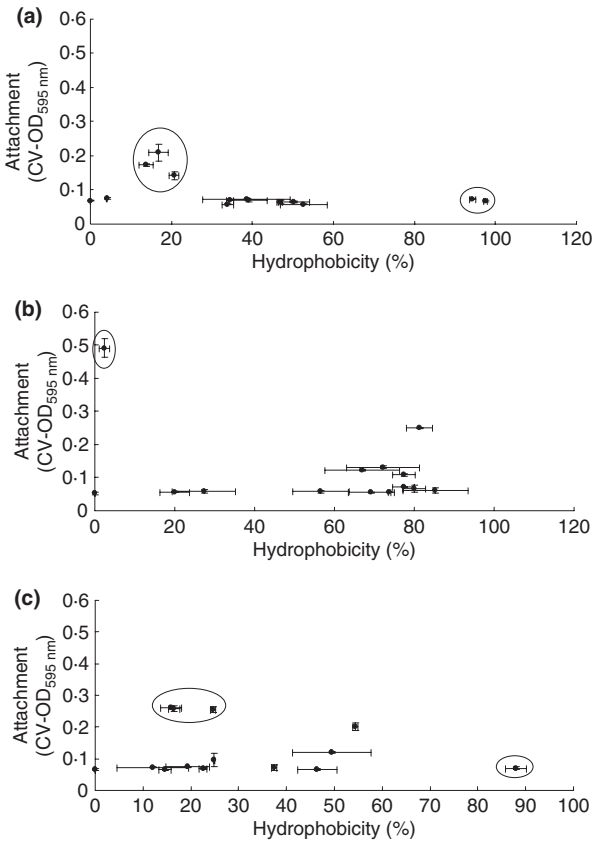

Figure 4 Cell surface hydrophobicity and attachment (a) in PBS (pH 6·5) (b) in whey permeate (pH 6·5) (c) in whey (pH 6·5). (Results of hydrophobicity expressed as mean and standard deviation, which were from duplicates, and those of attachment were from triplicates.)

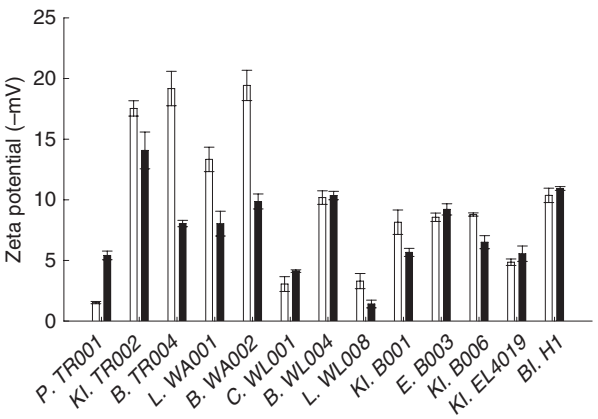

Figure 5 Cell surface charge in (□) PBS (pH 6·5) and in (■) whey permeate (pH 6·5). (Results expressed as mean and standard deviation, which were from triplicates.)

Cell surface charge and attachment

Cell surface charge was determined by measuring cell surface zeta potential. All strains evinced zeta potentials

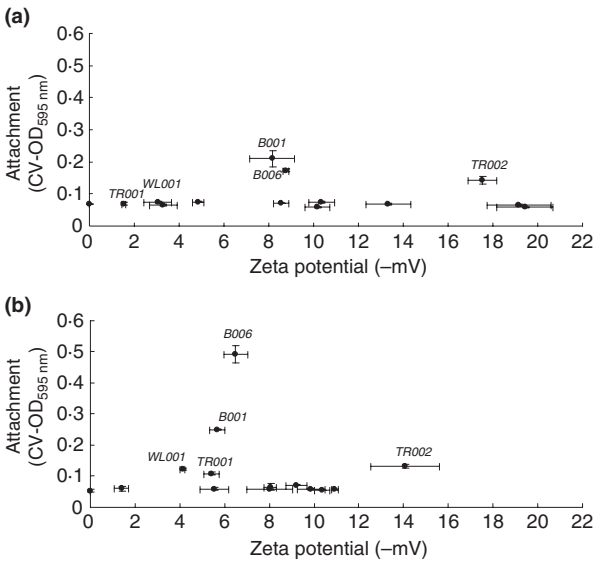

Figure 6 Cell surface charge and attachment (a) in PBS (pH 6·5) (b) in whey permeate (pH 6·5). (Results expressed as mean and standard deviation, which were from triplicates.)

less than −20 mV in PBS (pH 6·5) and whey permeate (pH 6·5) (Fig. 5). In whey permeate, the maximum zeta potential was less than −15 mV, and the zeta potential of seven strains became less negative than in PBS (Fig. 5). Since the membrane surface is also negatively charged, it was assumed that the decrease in zeta potential might increase the attachment of the strains as a result of decreased repulsion between the cells and the membrane surface. The microtitre plate assay confirmed that attachment for three (*Klebsiella TR002*, *B001* and *B006*) of seven strains was higher in whey permeate compared with PBS (Fig. 1). In contrast, *Pseudomonas TR001* and *Chryseobacterium WL001* did not attach in PBS, but attached in whey permeate, where their surface charges were higher than those in PBS (Fig. 6).

Overall, the ability of the isolates to attach showed no clear relationship with their surface charge (Fig. 6).

Discussion

The predominant isolates recovered from these membrane surfaces in dairy plants were Gram-negative bacteria. Whey will normally contain predominantly Gram-positive organisms from the starter population (lactic acid bacteria) (Friedrich and Lenke 2006) or thermo-resistant species such as spore-forming *Bacillus* species (Schreiber 2001). The high proportion of Gram-negative isolates, especially coliforms, found in this study indicates the most likely source of contamination may be the water used for dialysis, or general plant hygiene problems. *Enterobacter sakazakii*

is a common environmental contaminant (Lehner and Stephan 2004) and therefore it is not too surprising that it was found together with other Gram-negative micro-organisms. The presence of this organism is a concern in infant formula (Lehner and Stephan 2004), but none of the manufacturing plants we studied produced infant formula. The different populations found on the permeate side of the membranes may be the result of leaks in the membrane modules or of back-flushing.

In this trial, we assumed that the presence of micro-organisms on cleaned membrane surfaces is a good indication of their resilience in this environment and their potential to form biofilms.

Residual micro-organisms on any surface will start growing and form biofilms when conditions are suitable. On a membrane surface, this has potential to block the pores of the membrane and provide a source of contamination to product passing over the biofilm. In our study, the three *Klebsiella* strains (*TR002, B001* and *B006*) that readily attached were isolated from two different manufacturing plants. The other *Klebsiella* strain (*EL4019*) with poor ability to attach originated from a third manufacturing plant. There is no indication that the isolates with the greatest attachment were specific to any one manufacturing plant.

The microtitre plate assay is a standard assay used to screen micro-organisms for their ability to attach and form biofilm (Djordjevic *et al.* 2002). In order to reduce potential for proteins and bacteria to attach to membrane surfaces, commercial membrane filtration systems are modified to have a hydrophilic, negatively charged surface (Chen and Belfort 1999; Espinoza-Gómez and Lin 2001). Therefore, our model test system to screen isolates for adhesion to membrane surfaces utilized hydrophilic tissue culture microtitre plates.

The microtitre plate assay showed that the three strains with a high ability to attach were Gram-negative bacteria (*Klebsiella TR002*, *B001* and *B006*). Gram-negative bacteria are known to be prolific biofilm formers and this may be due, in part, to their ability to produce polysaccharide slime, associated with the formation of a true biofilm. Biofilms formed by Gram-negative bacteria are more resistant to biocides than those formed by Gram-positive bacteria (Machado *et al.* 2006), hence they are more likely to survive the clean-in-place (CIP) procedures than Gram-positive organisms.

The attachment of various strains measured using the microtitre plate assay compared with attachment to polysulfone membrane verified the use of the microtitre plate assay as a tool to screen for the attachment to polysulfone membrane surfaces.

The increase in the attachment of two mixed strains (*Pseudomonas TR001 with Klebsiella TR002/B001/B006*) compared with the attachment of each individual strain, indicates a synergistic relationship between these strains in the initiation of a biofilm. Biofilms in many environments are multi-species rather than single species (Kawarai *et al.* 2007; Macleod and Stickler 2007). The isolation of the *Pseudomonas* and *Klebsiella* species from one membrane suggests that they are likely components of a biofilm rather than accidental contaminants during sampling. Ten of our 13 strains showed no ability to attach from pure culture, which suggests that either the majority of isolates did not form biofilm but were trapped in the accumulation of protein and biofilm on the membranes, or the required conditions were not present in our experiments (e.g. combination with other micro-organisms or specific environmental conditions required for attachment).

Different media including PBS, whey and whey permeate, all at pH 6·5, were used in the present study. The results in PBS were considered as a base line, while the results in casein whey and whey permeate were taken as reflecting the situation in a dairy environment. Whey and whey permeate were found to increase the attachment of most of the strains. Therefore, further details on the effects of whey components were investigated using three *Klebsiella* strains in the microtitre plate assay. Four whey components: α-lactalbumin, β-lactoglobulin, GMP and BA were used. These experiments did not show which component played a major role in increasing attachment. It can be concluded that all components of whey may enhance bacterial attachment.

Cell surface characteristics, especially hydrophobicity and charge, are generally believed to be the dominant factors that influence the ability of cells to attach (Gilbert *et al.* 1991; Mueller *et al.* 1992; Kumar 1998). To understand how the attachment of our strains was affected by their surface hydrophobicity and charge, these characteristics were measured and correlated with observed attachment. Whey influenced the hydrophobicity of the cell surface, but the degree of change in hydrophobicity varied between different strains. It appeared that the responses to whey permeate and whey were a function of the individual strains. All the strains were negatively charged in PBS and whey permeate (pH 6·5). The charge became less negative in whey permeate. A possible explanation for this is the interaction of ions in the permeate with the cell surface. This enhanced the attachment of the strains to the plate surface, since the plates were also negatively charged (Becton Dickinson Labware, USA). However, in comparing the hydrophobicity and charge with the attachment between different strains, neither cell surface hydrophobicity nor charge showed a clear relationship with the attachment. Multivariate linear regression of attachment on these two factors also failed to show a

significant relationship. It suggests that some other factors may be of equal or greater importance in the attachment of our strains. Other studies have found that the cell surface hydrophobicity or charge did not play a dominant role in determining the extent of attachment (Jamson *et al.* 1995; Flint *et al.* 1997; Vacheethasanee *et al.* 1998).

The MATH test used in this study is a screening tool widely used to compare the hydrophobicity of different bacterial isolates. It does have limitations, as reported by Busscher *et al.* (1995) and a kinetic MATH assay may provide more accurate results.

Studies on the initial adhesion to the surface are important in any programme aimed at biofilm elimination (Dang and Lovell 1999). The results of our study suggest that the environment (whey and whey permeate) and microbial interactions are important in the attachment of microbes to membrane surfaces. Further studies will investigate the biofilm formation on membranes in a flowing system and the factors affecting removal of attached cells and mature biofilm.

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