Peter Gilbert and **Alex H Rickard** discuss the arcane subject of bacteal aggregation into biofilms

Biofilms...

where Angels fear to tread

WORKING LUNCHES at the *Dog and Duck* will never be the same again. Once the place to retreat, to escape inane questioning by undergraduates, and to ponder the meaning of life, it has now become a hotbed of philosophical debate. The topic, are biological events driven by chance or by necessity? Sadly, rather than discuss the juxtaposition of Darwinian and Lamarkian thinking, the biological events at the centre of this controversy relates to the propensity of bacterial cells to stick to one another (Rickard *et al.*, 2003). To those of us who work with pure cultures this property is an annoying feature of the late stationary phase. For many of our favourite laboratory work-horses such “clumping” undermines the co-incidence between viable counts and the number of colony forming units and has the potential to infer an apparent resistance to disinfection. We therefore endeavour to select strains that are deficient in the clumping trait (easily done with repeated passage in laboratory media), or we spin and shake, to wash-off the natural bits, and resuspend the now denuded cells in isotonic salts.

To other, more enlightened, microbiologists the ability to self-aggregate is a highly evolved mechanism by which bacterial cells may couple and pass on heritable traits. For the imaginative, aggregation reflects a primitive drive towards multicellularity and the formation of microbial tissues. Regardless, aggregation between physiologically distinct partners allows cooperative communities to be established in environments as diverse as the gut, mouth, river sediments and industrial pipework, even, perish the thought, those channelling beer through the *Dog and Duck*.

In spite of a general preoccupation with single cells in planktonic phase there is a wealth of literature available that deals with the particular properties of microbial aggregates and of their applications in applied microbiology. Thus, flocculation of yeast cells is an essential element of the brewing industry, and the formation of activated sludge vital to sewage treatment. The latter is truly polymicrobial with fastidious, obligate anaerobes lying protected within castellated aggregates formed by strongly aerobic partners. These flocks possess a collective metabolism that greatly surpasses that of the individual component species.

Within most ecosystems the greatest bioactivity happens, not in suspension but with organisms firmly associated with interfaces. When polymicrobial aggregates form at interfaces they are often referred to as biofilms and, by virtue of this epithet, highly fundable.

A whole new vocabulary has emerged to describe the various nuances of stickiness relating to biofilm formation. With the emergence of each new term, tenuous assumptions made about their determinative nature and causality become more dogmatic. Autoaggregation (attachment of one species to its clonal decendents) is distinguished from coaggregation (attachment of different species to one another), yet the ability to autoaggregate precludes the laboratory assessment of coaggregation. Where aggregation is between benthic (sedentary) and planktonic cells (floaters) then it is referred to as coadhesion, as is aggregation in disperse, planktonic, phase followed by attachment to the surface. Distinctions made between coadhesion and the attachment of multispecies flocs, however, leads to the assignment of temporal sequences to biofilm formation and the identification of primary and secondary colonisers. The surface appendages involved in intercellular adhesion are either polysaccharides or proteins, and the former class are described as receptors whilst the latter are described as adhesins. The use of such terms respectively imposes passive and active elements to the coaggregative relationship, and implies selective advantage gained by individual partner organisms. Are such interactions simply the result of chance, after all the cell envelope must interact with a complex extra-cellular milieu, that would include inert surfaces and dissolved materials such as sugars and peptides, or are they a necessary part of multicellular behaviour and subject to the laws of natural selection?

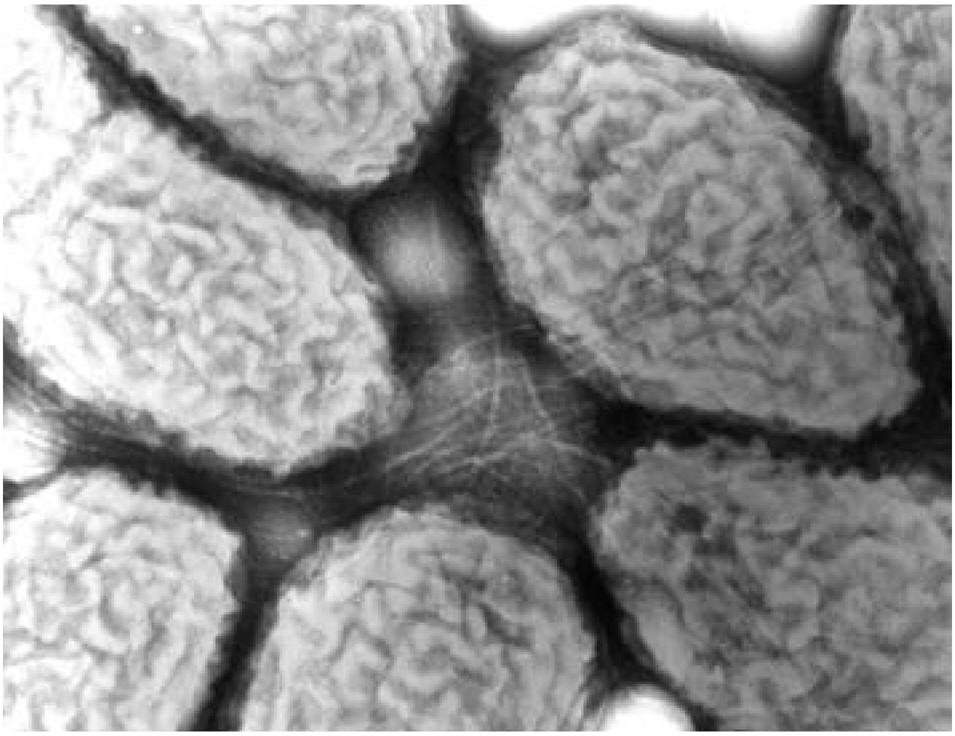


fig 1. *Vision of autoaggragation: Sphingomonas natatoria grown in pure culture and demonstrating the propensity to autoaggregate in rosettes through surface associated fimbriae clearly visible within the central region of the micrograph*

Photograph courtesy of P S Handley and A H Rickard

Since it has been demonstrated that coaggregation is a common phenomenon within a wide variety of multi-species biofilm communities, then its ecological significance deserves assessment. The key assumption must be that the strength and specificity of the interactions will be subject to natural selection forces and will reflect the degree of benefit, or harm, conferred upon bacterial partnerships by coaggregation. Those bacteria that benefit from living within a coaggregated community will survive and proliferate, better than the corresponding single, non-coaggregated cells. Thus, where organisms in a partnership possess complimentary metabolisms that enable them to collectively utilize available substrates more efficiently than any competing single organism, then coaggregation will not only maintain a close proximity of cells, and exclude competition, but it will also allow the partnership to proliferate and to co-emigrate to new situations. Where such mutualism is not demonstrated then local competition for nutrients will place the partners at a disadvantage. Mucin is a complex substrate for which complete catabolism requires the mutualistic action of several different oral species possessing complimentary yet overlapping enzymic activities. Thus, Palmer et al. (2003) showed that *Streptococcus oralis* and *Actinomyces naeslundii* form nutritionally beneficial coaggregates that, in the human mouth, facilitate growth where neither organism was capable of growing independently. This supports the earlier in-vitro observations of Bradshaw *et al.*, (1994), that such metabolic co-operation results in the liberation of additional nutrients, and that this may help to maintain the characteristic diversity of biofilm communities found in many natural habitats. Clearly, in such relationships the close proximity of participating organisms, brought about by coaggregation, maximizes the efficiency of the consortium. Caldwell *et al.*, (1997) have taken this concept several stages further and argue that polymicrobial consortia should be considered as evolving units in their own right, selected for on the basis of their combined functional efficiency. This proliferation hypothesis transcends Darwinian evolution by condoning mutualistic partnerships rather than encouraging competition and survival of the fittest. In such a fashion it has been argued that the eukaryotic cell, with its prokaryotic mitochondrial ancestry, epitomizes the partnership as a successful evolutionary unit.

Five years ago coaggregation of bacteria was mainly the preserve of the oral microbiologist. Coaggregation offered the potential to explain the exquisite patterns of differentiation and maturation found within supragingival plaque; primary and secondary colonizers were identified that together with polygamous adherent species such as *Fusobacter nucleatum*, could be used to draw up temporally-interactive

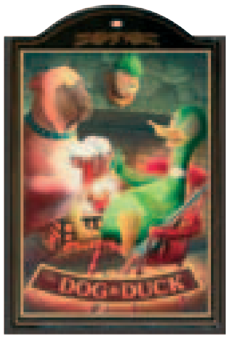
fig 2. *Coaggregated multispecies biofilm formed on a surface glass immersed in liquid culture medium following inoculation with Spingomonas natatoria and Micrococcus luteus. Only the Sphingomonas is capable of autoaggregation, albeit weak (see fig 1). Coaggregation therefore leads to the formation of structured mosaics of biofilm at the colonised surface*

Photograph courtesy of P S Handley and A H Rickard

plaque networks (Kolenbrander, 2000). Such networks strongly implicate coaggregation as the moderating-process most closely involved with plaque development. Today the concept has been found to be equally persuasive in providing explanations for the presence of stable communities within freshwater ecosystems, and within human and animal gastrointestinal and urinary genital tracts (Rickard *et al.*, 2003a). Whilst there are many similarities between coaggregation of dental plaque bacteria and those of other ecosystems there appears to be one major difference. Whereas oral microorganisms are generally constitutive expressers of the aggregative phenotype, fresh-water isolates expressed this optimally only during the stationary phase of growth, exponentially growing cells being incapable of coaggregation. The ability to switch the coaggregation phenotype 'on and off' could indicate some form of environmental control of expression of adhesins and/or receptors through starvation and stress. This leads to a 'Mechano-kit' model of microbial communities where each component species evolves separately but that multispecies aggregates appear temporarily in response to the prevailing nutrients (Wimpenny, 2000).

The proliferating units disperse once the advantages of communal life disappear. Reproductive success then depends upon the ability of individual species to both make and break partnerships. Presumably, in the mouth, loss of adhesion would ultimately result in oral bacteria being swallowed and digested thereby driving a strong selection pressure towards the constitutive expression of a coaggregative phenotype. In the immortal words of Tom Lehrer, *'Fish have got to swim and birds have got to fly, but they don't last long when they try'*.

In a simple experiment involving a fish tank and a re-circulating high-pressure water pump it was noticed that the most luxuriant and extant biofilm was formed where the hydrodynamic shear forces were greatest (Rickard *et al.*, 2003b). This was consistent with the hypothesis that biofilms formed under high shear are subject to a selection pressure that favours coaggregation partnerships. In fast-flowing rivers or the mouth, then if this did not happen the organisms would be washed away from their optimum ecological niche. Further experiments were conducted using a Concentric Cylinder Reactor to control hydrodynamic shear force, and biofilms established over three months using direct, potable water feeds. These showed, not only that the species diversity of the communities was inversely related to hydrodynamic shear force, but also that under high shear aggregative networks were optimized.



For the reasons outlined above specific coaggregation processes are likely to have an important ecological role as an integral process in the development and maintenance of mixed biofilm communities. The evidence available so far strongly suggests that this is true for dental plaque and probably for freshwater biofilm communities. Whilst only time will tell how widespread coaggregation is among other multi-species ecosystems, it may turn out to be a very widespread and truly ancient phenomenon.

Our thanks to SfAM for providing financial support (Student into Work Scheme) to Ms Amy Stead, a final year microbiology student, enabling her to work with us last summer. This allowed her to follow up ideas on the role of coaggregation in freshwater ecosystems using the Concentric Cylinder Reactor. Our thanks also go to the Presidents Fund, without the help of which one of us (AHR) would have been unable to present the findings to the 104th Annual General Meeting of the American Society for Microbiology in New Orleans. Our musings on life, the universe and everything can temporarily relocate from the *Dog and Duck* to warmer surrounds.

Peter Gilbert and Alex H Rickard
School of Pharmacy
University of Manchester

References

■ Bradshaw, D J., Homer, KA., Marsh, P D and Beighton, D (1994) Metabolic cooperation in oral microbial communities during growth on mucin. *Microbiology.* **140**, 3407-3412.

■ Caldwell, D E., Wolfaardt, G M., Korber, D R and Lawrence, J R. (1997) Do biofilm communities transcend Darwinism. *Advances in Microbial Ecology* **15**, 105-191.

■ Kolenbrander P E. (2000) Oral microbial communities: biofilms, interactions, and genetic systems. *Annual Reviews in Microbiology* **54**, 413-437.

■ Palmer, R J. Jr, Gordon, S M., Cisar, J O. and Kolenbrander, P E. (2003). Coaggregation-mediated interactions of streptococci and actinomyces detected in initial human dental plaque. *Journal of Bacteriology* **185**, 3400-3409.

■ Rickard, A H., Gilbert, P., High, N J., Kolenbrander, P E and Handley, P S. (2003a) Bacterial Coaggregation: An Integral Process in Multi-Species Biofilm Development. *Trends in Microbiology* **11**, 94-100

■ Rickard, A H., McBain, A .J., Ledder, R., Handley, P S and Gilbert, P. (2003b) Coaggregation between freshwater bacteria within biofilm and planktonic communities. *FEMS Microbiology Letters* **220**, 133-140.

■ Wimpenny, J. (2000) An overview of biofilms as functional communities, pp. 1-24, In, *'Community Interactions and Biofilms'*. Allison, DG., Gilbert, P., Lappin-Scott, H M and Wilson, M. Eds., Society for General Microbiology Publications, Reading.