Poster Session 3 – Pharmaceutical Microbiology

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Temperature dependent plaque formation in Phage AP205 (HER424) and *Acinetobacter haemolyticus*

A. E. Henein, G. W. Hanlon, J.-Y. Maillard and S. P. Denyer*

School of Pharmacy and Biomolecular Sciences, University of Brighton, Brighton and *The Welsh School of Pharmacy, University of Cardiff, Wales, UK.
E-mail: a.henein@brighton.ac.uk

Acinetobacter species are antibiotic-resistant bacterial pathogens with increasing clinical significance, particularly with respect to infections in high-risk patients such as those with severe burns. Treatment options for these patients are limited and often require highly toxic antibiotics. The need for an alternate approach to therapy in these cases is thus evident (Henein et al 2002). We report here some preliminary investigations into the use of bacteriophages for the treatment of *Acinetobacter* infections of severe burns patients. AP205 (HER424) is a ssRNA phage whose genome has been fully sequenced and has as its host *Acinetobacter haemolyticus* (Klovins et al 2002). Previous work has shown that this phage caused lysis of the host only erratically at 28°C despite the presence of a gene translating into a peptide with lytic function. Purified phage suspensions were enumerated using the overlay technique whereby phage and host cells (100 μm L of each) were mixed with soft agar and poured over the surface of TSA plates. The plates were incubated at a variety of temperatures and plaques counted after overnight incubation. Plates incubated at 20°C, 22°C and 25°C all showed the presence of plaques of heterogeneous size distribution (this heterogeneity was shown not to be due to clonal differences). The number of plaques was significantly lower at 30°C compared with 22°C and 25°C. Furthermore at 30°C the number of plaques was more variable and the appearance less distinct. At 35°C and 37°C no plaques were evident even though the phage was not inactivated at these higher temperatures. Host cells, which were infected at 37°C then washed and subcultured each day over a 3-day period, gave rise to an extremely low frequency of lysis when the temperature was downshifted to 25°C. The host range of AP205 was tested using 9 clinical isolates of *Acinetobacter* species (primarily *A. baumanii/calcoaceticus* complex) and no evidence of lysis was seen even at the permissive temperature of 25°C. The identity of the mediator of the temperature sensitivity is uncertain. However, previous investigators have reported the presence of an open reading frame that does not appear to present in other phages and is putatively involved in translation of the maturation factor.

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Comparative study of haemolytic and surface activities of the biosurfactant produced by *B. subtilis* ATCC 6633 with some synthetic surfactants

G. Dehghan Noudeh, B. S. Fazly Bazzaz and M. Housaindokht

Department of Pharmaceutics, School of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran. E-mail: r_dehghan@hotmail.com

Biosurfactants are amphiphilic in nature and reduce the surface tension of the medium in which they are being produced. These surface-active compounds have applications in industry, agriculture, mining and oil recovery, in addition to functional properties as wetting agents, foaming agents and emulsifiers in pharmaceutical and cosmetic products. These microbial surfactants are interesting because of their biodegradable nature and effectiveness at extremes of temperature, pH and salinity. *Bacillus subtilis* produces a lipopeptide, called surfactin, with exceptional surface activity (Dehghan Noudeh et al 2003). We report here some preliminary findings on the properties of this agent compared with chemically produced surfactants. The production of biosurfactants by a range of *B. subtilis* strains was studied. The results indicate that of the strains studied *B. subtilis* ATCC 6633 was the best biosurfactant producer. *B. subtilis* ATCC 6633 was cultured in nutrient broth and biosurfactant production, as evident from surface tension lowering, started from the first day and continued until 96 h of growth. CD, FTIR and UV spectroscopy (Ishigami et al 1994; Ferre et al. 1997) was carried out to confirm the structure of the biosurfactant and finally its surface activity and erythrocyte haemolytic capacity were compared with those of some synthetic surfactants. A far UV CD spectrum of the biosurfactant showed β-sheet behaviour at 217 nm. The FTIR spectrum showed strong absorption bands of peptides at 3150 cm^{-1} and the absorption region of 1750–1650 cm^{-1} that was due to lactone carbonyl absorption. UV spectra indicated the presence of peptide bonds at 237 nm. Of all the surfactants tested SDS showed the maximum surface tension reducing activity. The haemolytic capacity of surfactants increased with concentration and the concentration of their maximum haemolytic capacity (~100%) was above the relevant critical micelle concentration (Vinardell 1996). According to the data presented in this study, the haemolytic activity of chemical surfactants (SDS, BC, TTAB and HTAB) was greater than that of the biosurfactant produced by *B. subtilis* ATCC 6633. However, the biosurfactant would be considered as a suitable surfactant in drug formulations due to its lower membrane toxicity.

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