Poster Session 3 – Microbiology

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Detection, characterization and optimization of L-asparaginase production from *Escherichia coli* PTCC 1330

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L-Asparaginase enzyme is a tetramer with a high molecular weight (130 kD). The enzyme is currently produced by *Erwinia chrysanthemi* and *Escherichia coli* for clinical use. It exhibits anti-tumour activity and is used in treatment of childhood acute lymphocytic leukaemia (ALL). However, its use against other forms of leukaemia or solid tumour is limited (remissions are invariably of short duration). This problem necessitates a search for the identification of new enzyme variants. In this study, the detection, isolation and optimization of production of the enzyme by a rapid plate assay and a spectrophotometric method was investigated. Rapid plate assay was used for qualitative detection of the enzyme and for a screening study to obtain the optimum production conditions of the enzyme from a variety of different bacteria. In the assay, *E. coli* and twelve other strains including *Bacillus subtilis*, *Serratia marcescens*, *Staphylococcus aureus*, *Yersinia enterocolitica*, *Enterobacter cloacae*, *Micrococcus luteus*, *Citrobacter freundii*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Listeria monocytogenes* and *Pseudomonas aeruginosa* were used. Bacteria were grown on modified M9 medium (per 1000 mL of water: $Na_2HPO_4.H_2O$, 6.0 g; KH_2PO_4, 3.0g; NaCl, 0.5 g; L-asparagine, 0.5 g; 1 M $MgSO_4.7H_2O$, 2.0 mL; 0.1 M $CaCl_2.2H_2O$, 1.0 mL; 20% glucose stock, 10.0 mL; agar 20.0 g) and Phenol red (2.5% w/v in ethanol, 1 mL L^{-1}) was employed as an indicator. The plates were inoculated with a single colony of a fresh culture of each test organism and incubated at 37°C for 24 h. Formation of a pink colour around the colonies was the basis for enzyme detection (Gulati *et al* 1997). Significant pink colour development was only observed for *B. subtilis*, *E. coli* and *S. marcescens*. Therefore, these strains were studied further using a quantitative test.

Quantitative detection was carried out by a nessleization method (Imada *et al* 1973). In this assay production of the enzyme by *B. subtilis*, *E. coli* and *S. marcescens* was evaluated. Liquid media used consisted of M9-L and six other chemically defined media containing different concentrations of either L-asparagine (0–10 g L^{-1}) or corn steep liquor (0–0 g L^{-1}). Individual cultures were inoculated with each of the specified organisms at a final concentration of 10^6 cfu mL^{-1} and incubated for 48 h (200 rev min^{-1}). The incubation temperature was either at 28, 37 or 45°C. The culture extract was then prepared by centrifugation and filtration of the medium. Release of ammonia by certain amounts of the culture filtrate (as an indicator of asparaginase activity) in the presence of Nessler's reagent (450 nm) was monitored. The maximum L-asparaginase activity was found in cultures of *E. coli* PTTC 1330 in a medium containing L-asparagine 10 g L^{-1} at 37°C. In these conditions the enzyme activity was found to be 0.88 IU mL^{-1}. The bacteria grown under the above conditions should be studied for large-scale production of the enzyme. It may be also necessary to test the anti-tumour activity of the enzyme at some stages of future studies.

Gulati, R., *et al*. (1997) *Lett. Appl. Microbiol.* **24**: 23–26
Imada, A. *et al*. (1973) *J. Gen. Microbiol.* **76**:85–99

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Development of a rapid colorimetric time-kill assay to evaluate the synergy of antibiotic combinations used to treat cystic fibrosis lung infection.

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Chronic bacterial colonisation with *Pseudomonas aeruginosa* and *Burkholderia cepacia* and the resulting intermittent pulmonary exacerbations are the major factors responsible for the morbidity and mortality of cystic fibrosis (CF) patients. Existing treatment of pulmonary infection involves the frequent and prolonged administration of empirically selected antibiotics, which may not be synergistic, therefore resulting in a less than optimal outcome for the patient.

Synergy testing using the time-kill method, which is the most widely used technique to assess bactericidal activity, is labour-intensive and time-consuming to perform. The aim of this study was therefore to develop a rapid colorimetric time-kill assay to determine if there was synergy between ceftazidime and tobramycin, two antibiotics frequently prescribed to treat CF lung infection.

The colorimetric assay developed was based on the reduction of the tetrazolium salt XTT to a water-soluble formazan by the electron transport systems of actively respiring bacteria. Synergy tests for a range of tobramycin and ceftazidime concentrations were performed for a number of clinical *Pseudomonas aeruginosa* and *Burkholderia cepacia* isolates using both conventional plate count and colorimetric time-kill assays. There was good correlation between the colorimetric assay and the conventional plate count method of time kill-analysis (Table 1). The colorimetric time-kill assay is less labour intensive and results are available two hours after sampling.

The use of this rapid method to determine which antibiotic combinations are synergistic against bacteria isolated from patients should optimise treatment of lung infection patients with CF by ensuring that appropriate antibiotics are prescribed.

Table 1 The effect of ceftazidime (Caz) and tobramycin (Tob) on a *Pseudomonas aeruginosa* strain (A4) as measured by a colorimetric assay (XTT) and by conventional viable count determinations (CFU mL^{-1})

	Antibiotic concentration (mg L^{-1})					
T	Antibiotic free		Caz 0.5 Tob 0.1		Caz 5 Tob 1	
	XTT	CFU mL^{-1}	XTT	CFU mL^{-1}	XTT	CFU mL^{-1}
0	0.53	5.8×10^6	0.53	5.8×10^6	0.53	5.8×10^6
2	5.3	3.1×10^7	0.61	2.2×10^6	0.41	1.5×10^6
5	7.25	1.1×10^9	5.9	2.1×10^8	0.39	9.9×10^5
7	7.28	1.8×10^9	6.03	4.9×10^8	0.31	1.4×10^5
24	7.41	3.0×10^9	7.18	9.1×10^8	0.40	1.1×10^6

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Antimicrobial properties of novel polymeric coatings for urinary devices

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Medical device related problems include bacterial adherence and subsequent biofilm formation, which may ultimately lead to infection. One strategy that has been proposed to overcome medical device related infection is the use of microbial anti-adherent coatings on the device surface. In this study a series of polymeric films composed of the biodegradable polyester polycaprolactone (PCL) and the antibiotic rifampicin (Rif) were examined as candidate microbial anti-adherent coatings for urinary medical devices.

Films were prepared by dissolving PCL (10% w/v) and rifampicin (1, 3 and 5% w/w) in dichloromethane with mechanical stirring. Samples (10 mL) of the polymeric solution were cast onto glass petri dishes and the solvent allowed to fully evaporate at room temperature.

Quadruplicate samples of the films (1 cm^2) were challenged with inoculums of the urinary pathogens *E. coli* or *E. faecalis* and incubated for defined periods (4–24 h). Following this, the samples were removed and the percentage of the initial inoculum that had adhered determined following sonication and serial dilution (Table 1). Inhibition of bacterial growth was observed by seeding Müller Hinton Agar plates with *E. coli* or *E. faecalis* and placing a 1 cm^2 sample of each formulation onto the surface of the agar. The plates were incubated for 18 h to

allow bacterial growth to occur, the resultant zone surrounding the material sample that failed to show growth was measured thus giving the zone of inhibition. The materials with an observable zone of inhibition were then placed onto a fresh seeded plate and incubated for 18 h, this process was repeated daily until no observable zone of inhibition occurred. A zone of inhibition study was performed in triplicate for each formulation. The effect of time and rifampicin concentration on adherence and zone of inhibition were statistically evaluated using a two-way analysis of variance ($P < 0.05$ denoted significance).

Table 1 Percentage adherence of *E. faecalis* and *E. coli* to PCL films containing Rif following incubation for 4 h and 24 h

	E. faecalis		E. coli	
% Rif	4 h	24 h	4 h	24 h
0	0.29 ± 0.02	0.22 ± 0.02	0.79 ± 0.02	0.72 ± 0.01
1	0.26 ± 0.02	0.19 ± 0.01	0.75 ± 0.02	0.63 ± 0.02
3	0.23 ± 0.02	0.17 ± 0.02	0.72 ± 0.02	0.41 ± 0.02
5	0.10 ± 0.01	0.07 ± 0.009	0.67 ± 0.02	0.31 ± 0.01

The microbial anti-adherent effect increased with increasing rifampicin concentration. Furthermore, the anti adherence properties of the films were more pronounced following greater exposure periods. Films containing 1% rifampicin showed inhibitory activity against *E. coli* for 2 days and *E. faecalis* for 6 days, whereas films containing 5% rifampicin films showed activity against *E. coli* for 4 days and *E. faecalis* for 8 days. There was a significant increase in the size of the zone of inhibition with increasing rifampicin concentration.

In conclusion, these materials have potential application as medical device coatings, the initial anti adherent properties due to the rifampicin being coupled with the desirable biodegradable/biocompatible properties of PCL.

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Contamination of variously packaged emulsifying wax 3%

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Emulsifying wax 3% is a topical product that is used as a soap substitute by patients suffering from various skin conditions. Baird *et al* (1979) noted that product packaging affected the level of microbial contamination of topical medicines that had been prepared in the hospital pharmacy.

This was a preliminary investigation of the microbial contamination in both unused and used emulsifying wax 3% that had been prepared and dispensed from community pharmacies. The formulations had been packaged in narrow- and wide-necked plastic and glass containers in the unclassified environment of community dispensaries. The extemporaneous preparation of these products added freshly boiled potable water to 3% emulsifying wax B.P. This was mixed until cool.

Six freshly prepared unused product packs were collected from four community pharmacies and 16 used 500-mL containers were collected from patients. The duration of product use by patients varied from one to twelve weeks with storage at ambient temperatures. The total viable count from each container was established by the method detailed in Appendix XVI of the British Pharmacopoeia 2002.

Table 1 Microbial contamination in variously packaged emulsifying wax 3%

Container		Unused product	Used product	
Composition	Design	Microbial count/gram	Microbial count/gram	Principal contaminants
Plastic	Wide neck	7	4.4×10^5	*Staphylococcus* spp. and yeast
	Narrow neck	20	4.67×10^3	*Staphylococcus* spp., yeast and *Penicillium* spp.
Glass	Wide neck	Nil	2.56×10^5	*Staphylococcus*, *Pseudomonas* spp.
	Narrow neck	20	8.46×10^2	Staphylococcus and *Penicillium* spp.

The initial low microbial count in products prepared in an unclassified environment would seem inevitable. However the microbial contamination of used products exceeds acceptable and maximum limits for products similarly prepared in the hospital pharmacy (Davison 1990). In addition, the used products exceed the B.P. (2002) limit for the total viable aerobic count in topical products. While the results presented in Table 1 reflect limited variation between the glass and plastic packaging materials, the effect of the container design on product contamination is indicated. Packaging in narrow neck containers has resulted in reduced microbial contamination in the used products. *Staphylococcus* species are the principal microbial contaminants of human skin (Hill & Marsh 1990). This would coincide with this organism being present as the principal contaminant in all of the tested products and would suggest that limited human contamination occurs during production with either growth occurring thereafter or contamination being more significant during product use. In addition *Pseudomonas* species were also identified in a few of these products. The survival of microbial contaminants in this extemporaneously prepared product would seem to allow this formulation to act as a potential reservoir of infection.

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