# THE β-LACTAMASES OF THE MARINE GENUS *PHOTOBACTERIUM*

A THESIS PRESENTED IN PARTIAL FULFILMENT OF THE
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# ABSTRACT

All naturally occurring isolates of all five species of the marine genus *Photobacterium* have β-lactamase activity. In this study, the β-lactamase from the laboratory strain *P. leiognathi* 206 is fully characterised. The enzyme is constitutive and is released from the cell by osmotic shocking techniques, suggesting a periplasmic location. The enzyme is maximally active at pH 6.2 and has over 90% maximum activity at pH 7.0. The hydrolytic activity of the β-lactamase is independent of zinc ion presence. On the basis of substrate profile and inhibition studies the β-lactamase is classified as a Richmond and Sykes Class II enzyme. Crypticity tests indicated that there is no outer membrane permeability barrier to β-lactam substrates.

Comparative substrate profiles performed with the β-lactamases from three strains of each species of *Photobacterium*, indicate that the enzymes from strains of *P. leiognathi*, *P. angustum* and *P. phosphoreum* are active only on penicillins, whereas those from *P. fischeri* and *P. logei* also hydrolyze cephalosporins. This division is in agreement with an imminent taxonomic change for the latter two species. Analytical iso-electric focusing of the β-lactamases from 45 *Photobacterium* strains resulted in pI values which were not necessarily species specific and there was little correlation between the pI of the β-lactamase and its substrate profile, excepting the enzymes from *P. angustum* and *P. logei*.

Although plasmid DNA is present in many *Photobacterium* strains, conjugative transfer of β-lactamase activity from six different *Photobacterium* donors to a restrictionless *Escherichia coli* mutant was not observed. All attempts to 'cure' the bacteria of β-lactamase activity with five different curing agents, were also unsuccessful. A chromosomal location for the $\beta la^{+}$ gene is postulated.

## DEDICATION

I would like to dedicate this thesis to my parents, Dr and Mrs R.F. Lamb, without whose limitless financial support and unceasing encouragement this work would not have been possible.

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