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**Isolation, characterization and possible biocontrol  
application of Bdellovibrionaceae (BD) isolated from  
NZ sources**

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## Abstract

Bdellovibrionaceae (BD) are unique, predatory, endoparasitic, Gram-negative bacteria. As the world's smallest living hunter they prey on other Gram-negative bacteria giving them potential as biological control agents. Prior to this study, however, there were no reports of BD in New Zealand. The overall aim of this research was to isolate BD from New Zealand sources, characterise them and investigate their potential role as a biological control agent. The history, characteristics, life cycle and mechanism of predation of this organism are reviewed and the possibility of the industrial applications of BD, are discussed.

In this study, a halophilic species of BD was isolated from fourteen coastal sea water sites around New Zealand. Thirteen isolates were characterised using proven characterisation techniques including general, microscopic and molecular techniques. It was found that the isolates were taxonomically identical or very closely related to each other and belong to the genus *Bacteriovorax*.

The predation pattern of BD isolates was examined against a group of Gram negative bacteria in solid and liquid media. The predation patterns and efficiencies of the different BD isolates were similar, which confirms that the BD isolates are closely related, are selective in their predation, and prey on some Gram-negative bacteria but not all.

The rapid loss of culture viability of BD is well known, but no studies have been reported to date on the survival of pure cultures of BD at different temperatures. The survival rate of BD in dense suspensions at different temperatures without host bacteria was investigated and it was observed that pure BD cultures can be stored with minimal reduction in numbers at temperatures ranging from 4°C to 20°C. However, significant reductions in numbers were observed at -18°C, 30°C and 37°C after 13 to 16 days.

The effects of the 13 New Zealand BD isolates on the growth of a population of *Photobacterium phosphoreum* were examined to select the best isolate for *in vitro* application. All of the isolates tested had considerable reduction effect against *P. phosphoreum*. Some isolates were more

effective than others, despite their taxonomic similarity to each other. The isolate OT2 was selected for further studies based on these results.

The *in vitro* efficacy of BD was assessed against late exponential cultures of a seafood spoilage bacterium, *P. phosphoreum*, originally isolated from Cod fillets from Denmark. Log<sub>10</sub> reductions of *P. phosphoreum* and some other Gram-negative bacteria ranged from 4.5 to 4.8 after 9 h of incubation at 25°C. BD was effective in reducing the numbers of *P. phosphoreum* at pH 5.5 to 8.5 and salinity 0.9 to 4.5% (w/v). A significant interaction was observed between the prey and predator concentrations and nutrient concentration. Prey concentrations were observed to be the most vital factor in predation and the most favourable predation conditions were at a prey concentration of ~8 log<sub>10</sub> colony forming units (CFU)/mL, together with a predator concentration of 3 – 7 log<sub>10</sub> plaque forming units (PFU)/mL and a prey : predator ratio of >5.0. The thresholds of the prey and predator concentrations for predation were observed to be 3.7 log<sub>10</sub> CFU/mL and 3.9 log<sub>10</sub> PFU/mL, respectively. The trials carried out in this study focused on the efficiency of BD on a pure culture of one organism, *P. phosphoreum* and not on mixed cultures of Gram-negative spoilage bacteria, the normal condition observed in saltwater fish. There has been very little research in this field and the results of these trials suggest further investigation into the effect of BD on mixed cultures of Gram-negative spoilage organisms is warranted. Since only one isolate of BD (OT2) was examined against only one spoilage bacterium (*P. phosphoreum*) in liquid medium, the evidence of these findings must be restricted to these particular conditions. Future studies, using a range of BD isolates against a mixture of spoilage and pathogenic organisms in solid medium are warranted.

The biopreservation capability of BD in extending the shelf life of king salmon was evaluated. A significant effect was observed at 20°C but not at 10°C. At 20°C the shelf life was extended through extension of the lag phase of growth of the prey bacteria and a reduction in total numbers attained. Sensory evaluation of the salmon product being tested confirmed that the shelf life was extended. However, at 10°C there was no reduction in prey organisms, which suggested that the strain of BD used is ineffective at refrigeration temperatures.

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