



**The Enhancement of Wheat Growth after Inoculation
with Free-Living Nitrogen-fixing Bacteria**

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Summary

A wide range of microorganisms within the soil environment is capable of fixing atmospheric N_2 . Diazotrophic or N_2 -fixing microorganisms are generally classified as symbionts or as free-living associative organisms. Many of the free-living diazotrophic bacteria associate closely in the rhizosphere of Gramineae and have been studied for their potential for improving plant growth.

Reduction in application of nitrogenous fertilisers has both economic and environmental benefits and the development of N_2 -fixing inoculants for wheat to compensate for any lower N fertiliser application has been promoted in various countries. Selection of inoculants suitable for both host and geographic location has been the aim of many studies, because use of inoculants suitable in one region is not always successful in another. Problems encountered with the use of introduced inoculants include host specificity, persistence and competition from local species already adapted to the environment. Selection of local species for use as plant growth-promoting organisms may provide a better alternative than the introduction of an already developed commercial inoculant.

The focus of this work is the selection of bacteria for use as inoculants, to assist in early establishment of wheat in environments where winter rainfall is variable. This early establishment evokes the concept of early development of root growth. The beneficial effects on plants of mixed N_2 -fixing bacteria from 3 local soils (Kapunda, Avon and Waite) were compared to identify soils from which to isolate potential inoculants. Criteria for selecting soils included evidence for associative N_2 fixation within the potentially N_2 -fixing populations and stimulation of wheat roots. One of the soils (Kapunda) was selected for further study.

Experiments to identify dominant bacterial species within the mixed N_2 -fixing assemblage were carried out using fatty acid profiles from fatty acid methyl ester analysis (GC-FAME). The community fatty acid profile from bacteria from the Kapunda soil source showed more diverse fatty acids compared with the profiles from

Avon and Waite soils. *Stenotrophomonas maltophilia*, a potential plant growth-promoting bacterium, contained fatty acids similar to dominant fatty acids within the community profile from the Kapunda soil source. The bacterium was selected as one of three used as potential inoculants for wheat. Molecular tools were used to characterise and group bacteria isolated from the soil into sub-groups of the Proteobacteria, identify isolates carrying N₂ fixation (*nif*) genes and distinguish between them using DNA fingerprinting techniques. The resulting information enabled the selection of reduced numbers of isolates for further testing without unnecessary duplication.

After preliminary tests, 3 bacterial isolates were selected to be co-inoculated onto wheat. The isolates were identified as *Pantoea agglomerans*, *Stenotrophomonas maltophilia* and *Enterobacter taylorae* (isolated from within the wheat plant). Inoculation tests were carried out with wheat growth in low nutrient sand with ammonium nitrate as a supplement. Results indicated that raised levels of plant N in the inoculated treatments could be attributed to N₂ fixation by the bacteria. The potential for these bacteria to increase wheat growth and yield now requires further testing under field conditions.

Declaration

I declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any University and to the best of my knowledge and belief contains no material previously published or written by another person, except where due reference is made in the text.

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Conference Articles

S. Wiebkin, K. Ophel Keller, I. Singleton, M. Ryder and P. Mele. 1998. Selection of a source of potential free-living nitrogen-fixing inoculants for wheat. *1st International Conference of Federation of Asia-Pacific Microbiology Societies*, 135, Singapore.

S. Wiebkin, K. Ophel Keller, I. Singleton, M. Ryder and P. Mele. 1998. Selection of a source of free-living nitrogen-fixing inoculants for wheat. *8th International Symp. Microb. Ecology*, 349. Halifax, Canada.

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