

## Abstract

Plant growth promoting rhizobacteria (PGPR) colonise plant roots and exert beneficial effects on plant growth and development. The mechanisms of action of these PGPR are not conclusively known, however, there is evidence for the role of indole-3-acetic acid (IAA) and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase production by rhizobacteria in plant growth promotion. In this study, novel-PGPR were isolated from the rhizosphere of native species as well as agricultural crop species, as opposed to other work in this field in which potential PGPR are isolated from the rhizosphere of the target plant species. One hundred and sixty six bacteria were isolated from four rhizosphere soils in Western Australia and 72 isolates were assayed for the production of IAA. In the presence of the auxin precursor L-tryptophan (L-TRP) IAA production ranged from 0-37  $\mu\text{g/ml}$ . Five rhizosphere soils were screened for bacteria capable of utilizing ACC as a sole nitrogen source and 13 isolates were obtained.

To ensure that the isolates were not potentially deleterious to host plants, 14 IAA producing (IAA-PGPR) and all rhizobacteria capable of using ACC as a sole nitrogen source (ACC-PGPR) were tested for their effects on germinating clover and wheat seedlings. Two IAA-PGPR isolates, NCH7 and PMK4, were inhibitory to wheat seedling germination and one ACC-PGPR isolate was inhibitory to clover seedlings. Based on these findings, 6 IAA-PGPR and 4 ACC-PGPR were screened for their effects on germinating wheat seedlings in gnotobiotic growth pouch assays. Prior to these tests, spontaneous rifampicin resistant mutants were generated for 6 isolates. The mutants, or the wild type isolates where rifampicin mutants were not generated, were (re)tested for their ability to produce IAA and utilize ACC. All 10 isolates produced IAA in the presence of L-TRP ranging from 0.11-2.97  $\mu\text{g IAA}/\mu\text{g cellular protein}$  and

7 of the isolates grew on ACC amended medium. Bacterial growth was greatly increased in some isolates in the L-TRP amended media used in the auxin assay, suggesting some of the isolates have a requirement for tryptophan for optimal growth. The largest increases in root lengths in the gnotobiotic growth pouch assays were observed for seed treated with thhe ACC-PGPR, AWMK3 (81% increase). The IAA-PGPR treatments that increased root lengths were PMK4R (76%), WMK10R (66%) and NCH45 (33%). Increases in shoot lengths were recorded for seed treated with isolates WMK10R (42%), AWMK3 (11%), APMK2R (9%) and PMK9 (9%). A reduction in germination was observed in seed treated with some isolates, particularly PMK4R and WMK10R, which reduced germination by 34% and 20%, respectively.

Five of the PGPR isolated in this study were tested in the field on 2 wheat cultivars at 3 locations in Konjonup and Wongan Hills and as a co-inoculant with a commercial rhizobial strain on peas at Kojonup. All the PGPR were delivered in the field using the clay based Alosca™ carrier technology. The increases in yields in response to the inoculation with the PGPR on peas and wheat were small and not significantly different from the controls. However, the yield of wheat was improved by four of the PGPR (NCH45, NCH54, PMK9, WMK10) at the Wongan Hills heavy soil site by 2 to 23% and by NCH54 and PMK9 at the Wongan Hills light soil site by 4% and 3%, respectively compared with the uninoculated controls. On the peas at Kojonup, nodulation was improved with the isolate PMK4 and these plots were visually more vigorous than the other treatments, however this growth was not significant. At harvest, four of the PGPR (NCH45, PMK4, PMK9 WMK10) improved pea yields compared to the Alosca™ control by 6-13%. These results suggest that further testing is warranted.

Improvements to experimental design and sampling have been recommended to allow for the detection of statistically significant small percentage increases if they occur.

The findings in this study demonstrate that novel PGPR can be isolated from non-target as well as target plant species and that the screening of rhizobacteria based on their *in vitro* auxin production and growth promoting effects in growth pouch assays is valid for the selection of effective PGPR.