

# The unseen depths of soils - how plant growth promoting microbes may advance commercial forestry practices

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## Abstract

Advances in the broad research fields of plant physiology, microbiology and molecular biology have focused attention on some exciting opportunities to co-ordinate and integrate research on those resources critical to maintaining the productive capacity of forest soils. In the past research has focused on the role of nutrients in determining levels of productivity. However, closer attention should be paid to the role of interactions between soil organic matter, nutrients and microbially produced phytohormones in controlling plant physiological and ecosystem level processes. New insights into rhizosphere function and its impact on tree growth and wood properties may lead to new management strategies. This will sustain the productive capacity of soils and lead to the sustainable production of a range of biomaterials with designer specified properties.

## Plant growth promoting rhizobacteria

Traditionally the relationship between above- and belowground components of forest ecosystems are well recognised because of their inter-dependence in terms of energy and nutrient cycling. There has been considerable attention given to this area bringing a renewed focus to understanding the interactions between plants, soil organisms and soil processes. Recent studies have focused on plant species specific variations in quality and quantity of plant material entering the soil, plant community responses (Selmants *et al.* 2005, Grayston and Prescott 2005) and implications for biodiversity and ecosystem productivity (Williamson *et al.* 2005). Feedbacks commonly regulate these ecosystem level processes and have been widely considered in terms of energy and nutrient flows between aboveground and belowground components (Wardle 2002, Bartelt-Ryser *et al.* 2005).

Plant growth promoting rhizobacteria (PGPR) promote many important ecosystem processes and can include those bacteria and fungi that increase nutrient availability, enhance mycorrhization and produce phytohormones. In forestry, the potential of inoculating tree roots with PGPR has been recognised (Chanway 1997, Reddy *et al.* 1997, Frey-Klett *et al.* 1999). There has been a new focus on investigating the application of plant growth promoting bacteria and fungi to commercial forestry operations (Reddy *et al.* 1997) especially in the areas of enhancing tree growth and survival of tree seedlings (Chanway 1997; Wittenmayer and Merbach 2005, Enebak 2005) though microbially mediated phytohormone production.

## Why are microbially produced phytohormones of particular interest when considering plant development?

Over the past two decades a substantial body of evidence at the physiological as well as at the genetic level has accumulated in regards to the role that soil borne bacteria and fungi have in stimulating plant growth directly through the production of phytohormones including auxins and cytokinins (Patten and Glick 1996, Costacurta and Vanderleyden 1995, Tsavkelova *et al.* 2006). It is well understood that the overall balance of various plant growth controlling substances determine the growth of plants and development of plant properties. This may be in response to environmental signals or from signals mediated by the soil microbial community. There is strong evidence that bacteria and fungi can produce phytohormones that are of the same structural conformation as plants and more importantly that are functionally active in plant tissues. Much of our current understanding of how soil microbes produce plant active hormones has come from the study of pathogenic bacteria and fungi that hyper-synthesize phytohormones after invading the host plant and result in galls and soft tissue rot. In plant cells, auxins [specifically indole-acetic acid (IAA)] are largely formed by *de novo* synthesis from tryptophan via oxidative deamination or decarboxylation reactions. In microorganisms the three known pathways for IAA production are also initiated by the tryptophan precursor and many bacteria possess more than one pathway for IAA production. Interestingly, pathogenic strains tend to produce IAA via a different route than nonpathogenic/symbiont strains (Patten and Glick 2002). Microorganisms can produce a range of cytokinins similar to those produced by plants - including kinetin, zeatin, and isopentyladenine (Tsavkelova *et al.* 2006). The bacterial isopentyltransferase gene (*ipt*) has been well characterised and homologues can be found in eukaryotes, including plants (Kakimoto 2003). Indeed this bacterial gene can be recombinantly expressed in plant tissues (Kunkel *et al.* 1999).

The role that each individual class of phytohormone and combinations of phytohormones have in controlling plant growth has been well studied (Savidge 2000, Kepinski 2006, Weyers and Paterson 2001). Auxins are

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known to be responsible for promoting cell elongation and differentiation of the phloem and xylem tissues (Aloni 2004). Cytokinins on the other hand are known to promote cell division and callus growth in cells that otherwise have become multinuclear (insert refs here). Therefore, the control and supply of cytokinin and auxins to the plant is essential in ensuring the proper regulation of the activity and balance of these phytohormones. Microbial production of auxins and cytokinins are believed to change assimilate partitioning patterns in plants and affect growth patterns in roots, resulting in bigger, more branched roots with greater surface area (Vessey 2003, Patten and Glick 2002; Persello-Cartieaux *et al.* 2003). There are many reasons for why the role of belowground processes in determining whole plant cytokinin/auxin balance requires serious consideration from the perspective of commercial forestry operations.

The auxin indole-3-acetic acid (IAA) is a well known promoter of tracheid production and accumulation of auxins may enhance cambial activity and xylem production. Auxins are furthermore involved in apical dominance, low levels of cytokinin relative to auxins though promotes the allocation of carbon to root growth. Does auxin production by microbes lead to imbalance in the cytokinin/auxin ratio resulting in greater carbon allocation to aboveground plant organs such as branches which results in greater mechanical stresses on trees and wood therein? For example, a mutant of *Hebeloma cylindrosporium* has been identified as an over producer of auxin. The implications of this for the auxin/cytokinin balance and the costs of producing a large number of roots have been discussed by Barker *et al.* (1998). Variations in auxin production by mycorrhizal species associated with radiata pine have previously been identified (Ng *et al.* 1982) but these have not been verified under field conditions or levels of production established for other known mycorrhizal species of radiata.

Cytokinin production by plant pathogenic bacteria fungi and bacteria, such as the *Agrobacterium tumefaciens* strains, is well understood (Brzobohaty *et al.* 1994, Eason *et al.* 1996, Akiyoshi *et al.* 1987) but less attention has been paid to cytokinin production by plant growth promoting rhizobacteria (Garcia de Salamone *et al.* 2001, Eason *et al.* 1996). Such attention is certainly warranted given the accumulated evidence of cytokinin production by a wide range of bacteria and fungi, including mycorrhizal fungi (Jameson and Morris 1989, Nieto and Frankenberger 1989) and these facts should not be down played by assuming that plants are the only producers of cytokinins. Automatically assuming this discounts the importance of soil borne microbes to plant growth especially when there is no direct metabolic need for the microbes to produce cytokinin for its own direct benefit (Persello-Cartieaux *et al.* 2003). Recent work of Arkhipova *et al.* (2005) has shown that cytokinin levels in lettuce roots and shoots remain elevated 8 days after inoculation with a cytokinin producing *Bacillus subtilis* strain. During this time, levels of other hormones including abscisic acid and IAA were also displaced.

Cytokinins are also thought of as important signalling molecules of soil nutritional status especially that of

nitrogen (Wagner and Beck 1993, Sakakibara 2003). Takei *et al.* (2002) have proposed a model outlining the multiple routes for communicating nitrogen availability from roots to shoots through a signal transduction pathway mediated by cytokinin. The importance of nitrogen supply on wood density has been demonstrated (Beets *et al.* 2001) and it seems important to examine the relationship between nitrogen availability and cytokinin signalling because of the consequences for the growth of plants and development of important characteristics such as wood properties through cambial processes. Rapid growth driven by high N supply may cause high levels of cytokinins and therefore more cell division and an imbalance of the cytokinin/auxin balance.

Very few, if any, of the reports detailing the effect of microbial produced phytohormones consider the whole plant implications, most are focussed on the morphology changes in one particular part of the plant. It seems pertinent to ask the question that "if root morphology for example can be altered by these compounds can any other non localised effects be seen". It remains unknown if the input of microbially derived phytohormones could significantly affect other important plant processes such as cambium development. The longer term effects of such a shift in phytohormone balance are of great importance to understand.

#### Taking the research into the field

In forestry, inoculation of seedlings with beneficial microbes has traditionally been restricted to mycorrhizal fungi (Chu-Chou and Grace 1988) however research is now being carried out using PGPR's. Dolmenech *et al.* (2004) showed that inoculation with *Bacillus licheniformis* resulted in enhanced growth of oak seedling, reflected by significant increases in shoot surface area, shoot length and shoot dry weight. Frey-Klett *et al.* (1999) demonstrated that co-inoculating a mycorrhizal helper bacterium together with an ectomycorrhizal fungus could result in an increase of about 40cm in the height of 2 year old Douglas fir seedlings and Chanway *et al.* (2000) found that inoculation of hybrid spruce with a number of Bacilli and Pseudomonad strains increased the dry weight of seedlings by up to 57% of that of the uninoculated seedlings 17 months after inoculation. Furthermore it has been found that mycorrhization of the out planted seedlings is increased when co-inoculated with rhizobacteria (Bending *et al.* 2002, Rincon *et al.* 2004).

Understanding the relationship between the host plant and inoculated bacteria and/or fungus is essential if these associations are to be manipulated to successfully increase plant production. Much of the research to date has been limited to measuring plant physiological responses (height, weight, root morphology, health etc) after the inoculation process. With the increasing availability of sophisticated genomic techniques and more sequence data becoming available (both for the microbial and plant species) the opportunity to measure specific gene response increases.

It is of particular interest to measure the increase in other PGPR responsive genes in the plant tissues as well as genes more traditionally associated with growth/

development, such as the xylem development genes in *P. radiata* (Cato *et al.* 2006). The ability to measure the up (or down) regulation of these other genes would lead to a more holistic approach to understanding how the microbially produced hormones affects plant development. This has been done in a few studies but mainly involves using *Arabidopsis thaliana* as the model plant due to the full genetic sequence being available. Wang *et al.* (2005) were able to show that inoculation of *Arabidopsis thaliana* with a known plant growth promoting Pseudomonad strain could increase plant growth and concomitantly upregulate many of the genes associated with growth and elevated disease resistance.

Little is known about how the soil type, host plant genotype, and environmental conditions affect the ability of the microbial inoculum to colonize and persist on plant roots and the associated changes the inoculum may have on the surrounding soil populations. Advances in molecular biology and plant and microbial science are only now providing the opportunity to explore these interactions.

The future management of belowground resources

It is without doubt that sustainable development of natural resources will require an increased understanding of belowground resources so as to lead to the production of new tools for sustainable plant production (Sen 2003). This will reduce threats to the productive capacity of soils and provide a range of future options for plant based products. PGPR promote many important ecosystem processes including biological control of plant pathogens, nutrient cycling, and seedling establishment, improve soil structure and aid in mycorrhizal development (Barea 1997). Compant *et al.* (2005) believed that PGPR and fungi are associated with most if not all species of plants; however the success of these inoculants to promote growth appears to be related to local edaphic and environmental conditions (Enebak 2005).

Despite the clear evidence that PGPR's are capable of influencing plant development though mechanisms such as phytohormone production, few attempts have been made to exploit these opportunities on a plantation forestry scale. There appears to be a significant knowledge gap relating to the presence and activity of phytohormone producing PGPR and fungi that are present in New Zealand soils. As a result of not doing this, there has been no consideration by the New Zealand forestry sector of the wider applications of such new technologies to the rapidly expanding global plantation estate and the opportunities for business development. This is an area of research that needs attention especially in perennial plants where the results of plant growth and development are so economically valuable.

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