Biofilmed biofertilisers: Novel inoculants for efficient nutrient use in plants

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Abstract

Microbial communities attached to surfaces, or biofilms, are found in many environments, including the soil. This chapter describes the potential applications of developed biofilms as biofertilisers in crop production. Formation of fungal–bacterial biofilms (FBBs) by bacterial colonisation on biotic fungal surfaces gives the biofilms enhanced metabolic activities compared to monocultures. Incorporation of a nitrogen (N_2)-fixing rhizobial strain to FBBs to form fungal–rhizobial biofilms (FRBs) has been shown to improve potential biofilm applications in N-deficient settings and in the production of biofilmed inocula for biofertilisers and biocontrol in plants. A developed biofilmed inoculant of the FRB significantly increased N_2 fixation in soybean by ca. 30% compared to a conventional inoculant of rhizobium alone (monoculture inocula). The FBB and FRB increased biomass in early growth of rice by ca. 25% compared to the conventional inocula. Root colonisation of wheat by FRBs forming 'pseudonodules' was also observed recently. The FRBs increased N and phosphorus (P) availabilities when inoculated directly to the soil. They also improved P biosolubilisation from rock phosphate. The FBBs of beneficial endophytes produced higher acidity and plant growth promoting hormones than their mono- or mixed cultures with no biofilm formation. This indicates that the highest microbial effect may not be achieved by plant inoculation with the conventional inocula of effective microbes, but only by biofilmed inocula.

Introduction

Certain microbes can attach to biotic or abiotic surfaces and differentiate to form complex, multicellular communities called biofilms. A biofilm consists of microbial cells (algal, fungal, bacterial and/or other microbial) plus an extracellular biopolymer (known as an extracellular polymeric substance (EPS)) produced by the cells which provides structure and protection to the community. These communities can be found in medical, industrial and natural environments. They can also be engineered in vitro for various biotechnological applications (Seneviratne 2003; Seneviratne et al. 2007). Microbes undergo profound changes during their transition from planktonic (free swimming) organisms to cells that are part of a complex, surface-attached biofilm. Genes and regulatory circuits important for initial cell–surface interactions, biofilm maturation, and the return of biofilm microorganisms to a planktonic mode of growth have been identified (O'Toole et al. 2000). Biofilms have a unique pattern of gene expression that is different from their non-biofilm-forming stages (Vilain and Brözel 2006).

The distinctiveness of the action of beneficial biofilms developed in vitro has already shown potential for bringing numerous favourable effects to microbial biotechnological applications (Seneviratne et al. 2007). Their use in agricultural and environmental

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settings, enzyme technology, drug discovery studies and green energy research are being investigated. In this chapter the adoption of biofilms as biofertilisers is discussed with the aid of preliminary demonstrations which showed their promising applications as 'next generation biofertilisers' in the future.

Microbial biofilms in the soil

There are three major types of biofilms that can occur in the soil—bacterial (including *Actinomycetes*), fungal and FBBs. The bacterial and fungal biofilms are formed on abiotic surfaces in the soil. In FBBs fungi act as the biotic surface to which the bacteria adhere (Figure 1). In the case of non-filamentous fungi, both bacteria and fungi can act as the biotic surface. Formation of FBBs by bacterial colonisation on biotic fungal surfaces gives the biofilm enhanced metabolic activities compared to monocultures, and perhaps also to multispecies bacterial or fungal biofilms on abiotic surfaces (Seneviratne et al. 2007). Such microbial associations between bacteria and mycorrhizal fungi have been observed to occur naturally in the soil (Artursson and Jansson 2003), promoting mycorrhizal symbiosis (Frey-Klett et al. 2007). Biofilms attached to the plant roots of some crops help in the cycling of nutrients as well as the biocontrol of pests and diseases, resulting in improved agricultural productivity (Seneviratne 2003). However, because the density of biofilms in the soil is not adequate to give maximum beneficial effects, biofilms developed in vitro should be applied as inocula to increase plant growth.

Biofilmed biofertilisers

With the first in-vitro development and observation of interactions between common non-mycorrhizal soil fungi and rhizobia, forming FRBs (Seneviratne and Jayasinghearachchi 2003), a series of studies was conducted to demonstrate their potential applications for various purposes. It was observed that FRBs fixed nitrogen (N_2) biologically, as revealed by nitrogenase activity and N accumulation, in contrast to when a rhizobial strain was grown as a monoculture (Jayasinghearachchi and Seneviratne 2004a). The rhizobial strain used here was *Bradyrhizobium elkanii* SEMIA 5019, a soybean-nodulating strain with a high N_2-fixing capability.

Application of a developed biofilmed inoculant of the FRB can significantly increase N_2 fixation in soybean (by ca. 30%) compared to a rhizobium-only (conventional) inoculant (Jayasinghearachchi and Seneviratne 2004b). Another recent study showed that the contribution of developed biofilmed inocula in enhanced release of organic acids and plant growth promoting (PGP) substances led to a ca. 25% increase in plant dry weight in early growth of rice compared to conventional monocultured inocula (M.L.M.A.W. Weerasekara, unpublished). Co-inoculation of PGP rhizobacteria and arbuscular mycorrhizal fungi in rainfed wheat fields produced the highest protein contents of grains compared to their monocultures (Roesti et al. 2006). Initial observations of FRB formation on wheat roots by a biofilmed inoculant were made recently (Figure 2; G. Seneviratne et al., unpublished).

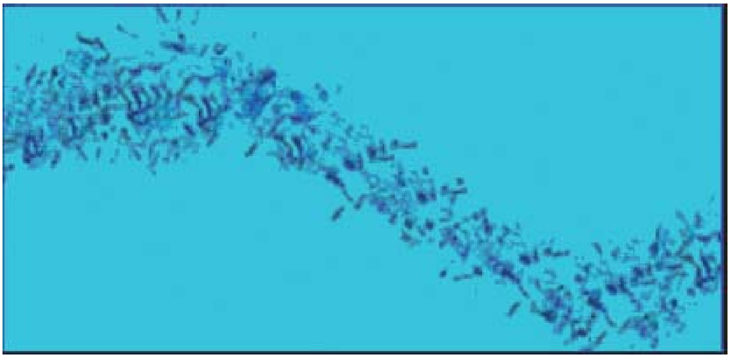

Figure 1. A phase-contrast microscopic view of a fungal filament attached by bacterial cells forming a fungal-bacterial biofilm (FBB). Magnification: 2,000×.

FRBs may act as ‘pseudonodules’, fixing N_2 biologically on the roots of non-legumes. In a review by Bashan (1998) on microbial inocula in agriculture, mixed inoculation with arbuscular-mycorrhizal fungi and diazotrophic bacteria has been reported to generate synergistic interactions. Possible consequences of this synergism include significant increase in growth and in the P content of the plants, enhanced mycorrhizal infection and an improvement in the uptake of nutrients such as P, N, zinc, copper and iron. These inocula stimulate plant growth through a range of mechanisms that improve nutrient acquisition and inhibition of fungal plant pathogens (Biró et al. 2000; Artursson et al. 2006; Toljander et al. 2006). When applied directly to the soil, FRBs increased N and P availabilities by ca. 2-fold and 15-fold, respectively, and showed a high nitrogenase activity, even under a very high soil NO_3^- concentration, compared to the monocultures (Seneviratne and Jayasinghearachchi 2005).

Figure 2. A fungal–rhizobial biofilm (FRB) on a wheat root. The FRB may act as a ‘pseudonodule’, fixing N_2 biologically on the roots of this non-legume.

The biofilmed inocula can be effectively used in biosolubilisation of rock phosphate. This was demonstrated by developing biofilms from *Penicillium* spp., *Pleurotus ostreatus* and *Xanthoparmelia mexicana*, a lichen fungus which increased the P solubilisation up to ca. 230% compared to the fungus-only cultures (Jayasinghearachchi and Seneviratne 2006a; Seneviratne and Indrasena 2006). Furthermore, the biofilmed inocula can also be used for successful establishment of introduced beneficial micro-organisms in plants for biocontrol of diseases etc. This was confirmed in vitro by a *Pleurotus ostreatus* – *Pseudomonas fluorescens* biofilm that increased endophytic colonisation of tomato by *P. fluorescens*, a biocontrolling agent, by over 1,000% compared to inoculation with *P. fluorescens* alone (Jayasinghearachchi and Seneviratne 2006b). As such, diverse forms of the biofilmed inocula may satisfy the future demand of augmented crop productivity with increased N_2 fixation, nutrient uptake and biocontrol of diseases.

FBBs of beneficial endophytes were observed to produce higher acidity and PGP hormones than their mono- or mixed cultures with no biofilm formation (Bandara et al. 2006). The higher acidity is generally important for pathogen suppression. Further, there was a significant negative relationship between pH and the production of indoleacetic-acid-like substances (IAAS) in liquid culture media of FBBs, but not in mixed cultures with no biofilm formation of a large collection of microbes (Figure 3). Thus, when biofilms are formed, high acidity reflects high IAAS production. As such, the highest microbial effect may not be achieved by the conventional practice of plant inoculation with monocultures or mixed cultures of effective microbes, but rather by biofilmed inocula.

In conventional inoculant technology of microbial monocultures, a major problem yet to be addressed is the poor survival of introduced micro-organisms in the soil due to various environmental stress factors. Biofilmed inocula were observed to help their rhizobia survive at high salinity (400 mM NaCl) and tannin concentrations (0.4 mM tannic acid) by 10^5-fold and 12-fold, respectively, compared to rhizobial monocultures (Seneviratne et al. 2007). Their higher tolerance than the monocultures for low pH, chromium and predation by earthworms was also noted. It has been reported that the formation of microcolonies and the production of toxins are effective mechanisms that may allow bacterial biofilms (e.g. *Pseudomonas aeruginosa*) to resist protozoan grazing and so persist in the environment (Matz et al. 2004). Similar observations of Burmolle et al. (2006) revealed that, in multispecies biofilms, the synergistic interactions cause an enhancement of biofilm formation and increased resistance to antimicrobial agents. Bacterial cells are protected from antimicrobial agents in biofilms through the formation of persister cells—a highly protected state adopted by a small fraction of the outermost cells of a biofilm (Roberts and Stewart 2005).

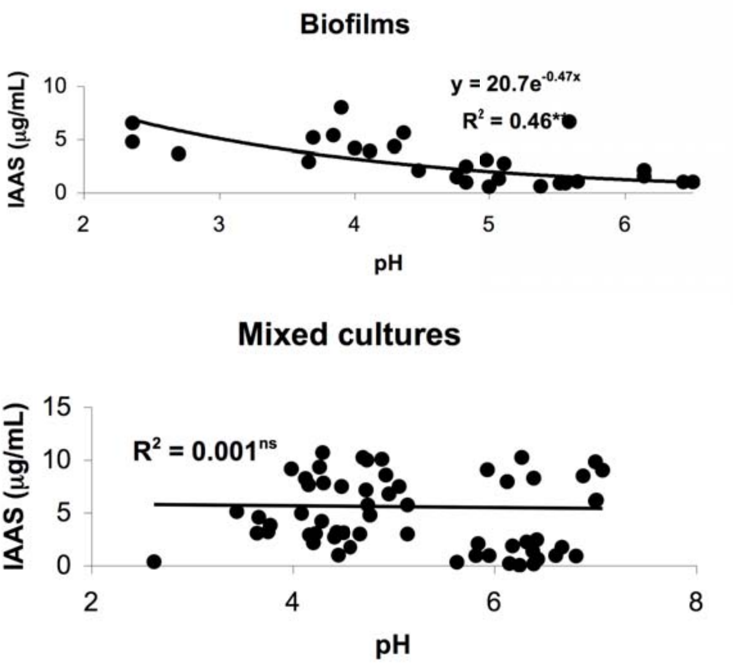

Figure 3. Relationships between pH and indoleacetic-acid-like substances (IAAS) production in liquid culture media by fungal–bacterial biofilms or mixed cultures with no biofilm formation of a large collection of microbes.

Conclusions and future research needs

Studies reported here have shown that FBBs/ FRBs are more effective in their biological performance than mono- or mixed microbial cultures, and perhaps also multispecies bacterial or fungal biofilms on abiotic surfaces. The soil application of FRBs as biofilmed inocula appears to be important if soil fertility is to be sustained in nutrient-depleted lands, and survival of rhizobia is to be improved in the soil in the absence of their hosts. However, applications of this biotechnology are scarce because it is still understudied. Selection of combinations of microbes for highest efficiency, simultaneous biofertilising and biocontrolling activities is a key in future research in this technology. Thus, more research should be done under laboratory and field conditions in order to optimise biofilmed inocula for various crops.

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Future perspectives for biofertilisers: An emerging industry needing a scientific approach

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Abstract

The occurrence of peak oil supply and the resulting increase in urea prices provide ideal socioeconomic conditions for the adoption of new technology able to improve the efficiency of nitrogen utilisation by crops like rice and wheat. The application of biofertilisers containing catalytic amounts of inoculant micro-organisms, such as the product BioGro studied in this project, represents one possible means of meeting this demand. There are a number of caveats that must be attached to any proposal to promote the application of inoculant biofertilisers on a large scale. Several major economic and technological hazards have been identified during this ACIAR project that threaten success unless specific actions are taken to minimise their risk. The commercial profitability of biofertiliser production has been limited more by the rate of adoption of the technology by farmers than by the technological capacity to provide good quality mother cultures. Reasons for the slow rate of adoption include lack of financial credit and doubts concerning the efficacy of the biofertiliser.

By contrast, chemical fertilisers in Vietnam are promoted by sales companies in liaison with government extension agents, often using company loans repayable at harvest. Successful introduction of biofertilisers will require similar credit arrangements during a concerted and sustained period of demonstration and extension, innovative commercial production and technological support to ensure quality control for the overall process. One model showing promise would be introduction of the technology with consistent expansion of its use based on the franchising of local commercial producers, who would be supplied with effective cultures and protocols for production and sales. This process would also be subjected to rigorous quality control to ensure efficacy of the products and protection of human and environmental health.

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Introduction

To grow rice productively, Vietnamese farmers have typically needed to apply at least 100 kilograms of fertiliser nitrogen (N) per hectare to harvest each crop. Until recently, such fertilisers have been inexpensive and their cost to farmers was often subsidised. But it has been clear for some time that such practices using excessive inputs of urea were not sustainable for both economic and environmental reasons, and programs such as the 'Three Reductions' for seed, fertilisers and pesticides were implemented with incomplete success in the Mekong Delta (Tran 2008).

In the past few years the price of N fertilisers such as urea has trebled as oil prices continue to reach record highs. Yet no more than 40 kg N/ha is recovered in the rice crop and the remainder is directly lost to the environment as ammonia or as nitrate in groundwater, or is evolved as the greenhouse gas, nitrous oxide. More than 30 million Vietnamese people involved in rice production are now being affected economically by this problem of increasing input costs (Figure 1).

Better management with split applications of fertilisers can have some beneficial effects on improving the efficiency of use of applied N, but there is a trade-off in increased labour needs as farm workers are being attracted away from the land to other manufacturing industries. Any new technology that could improve the efficiency of use of fertilisers, reducing the total input costs and improving environmental health, would significantly increase the wellbeing of the rural poor in these areas.

Innovation

The research output of this project has shown that this problem of high cost and waste of N can be lessened if steps are taken to improve the efficiency of N use by rice plants. More efficient uptake of N can be achieved by ensuring that specific micro-organisms are present in the root zone of rice plants (Kennedy 2008). The strains of microbes present in the biofertiliser known as BioGro were isolated from rice paddies near Hanoi by Professor Nguyen Thanh Hien of Hanoi College of Science, Vietnam National University (Nguyen 2008). These microbes were selected for properties such as plant growth promotion by hormone production, the capacity to fix atmospheric nitrogen (N_2) and an ability to mobilise phosphorus from insoluble forms. These properties together allow rice growing with lower inputs of fer-

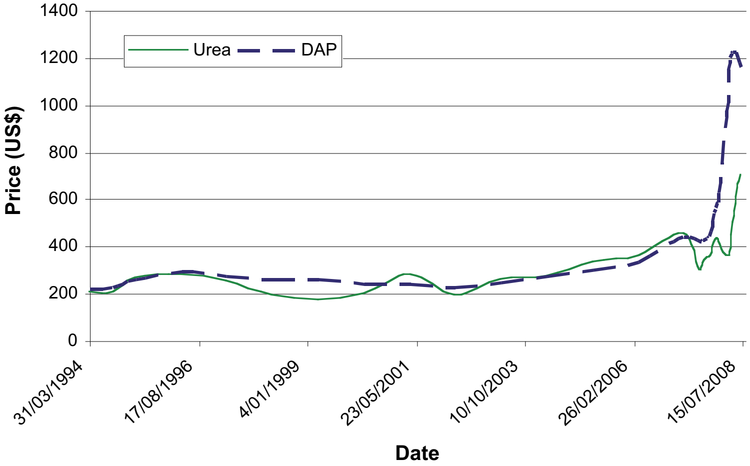

Figure 1. Price of urea and diammonium phosphate (DAP) fertilisers (US$), using annual data from the United States Department of Agriculture (04/1994 – 04/2007); <http://www.ers.usda.gov/Data/FertilizerUse/> and monthly data from The Market (04/2007 – 06/2008); <http://www.fertilizerworks.com/fertreport/>

tilisers such as urea and superphosphate. All the microbial strains except the yeast have been identified to species level by our group of colleagues at the University of Sydney, and their biological safety and effectiveness in BioGro have been established by a large number of laboratory tests. These micro-organisms were introduced to rice plants by inoculation of seedlings or of rice paddies at a rate of about 1 g (a million million (10^{12}) cells) per ha. This pioneering technology has been verified as effective by the research results of this ACIAR project in Vietnam.

More than 20 field experiments were performed in northern and southern Vietnam during the project, proving the high rate of effectiveness of BioGro (Pham et al. 2008a; Tran et al. 2008). For example, Phan and Tran (2008) clearly show that rice can be grown in Tay Ninh province with only half as much urea-N supply, some 50 kg/ha less, as that needed to give maximum yields. Under some conditions inoculation with BioGro can even lead to extra rice yields of the order of 10–15% as well as the reduction of input costs for urea-N. These results support earlier field trials in northern Vietnam that demonstrated similar fertiliser reductions and yield increases (Nguyen et al. 2003). Understanding the agronomic conditions that support such general increases in yield requires further research. It should be noted that the inocula contain negligible quantities of plant nutrients, even when the carrier materials used to deliver them are taken into account. Instead, by biologically multiplying their numbers in the rice plant rhizosphere, the catalytic effect of the microbes on nutrient mobilisation and uptake by rice plants becomes enormously amplified.

Although the technology itself is innovative, this project was also innovative in several other ways. While the use of inoculant biofertilisers has been proposed before, the active micro-organisms in most available products are not disclosed and little or no quality control of the product is assured, leading to unreliable results. BioGro now involves a unique production system characterised by a high degree of quality control with defined operating protocols and ongoing validation of effectiveness. Information on the microbial strains in the product is freely available and scientifically proven. Any microbial strains posing a likelihood of risk to human, animal, plant or environmental health have been eliminated. Furthermore, a number of different production–supply systems were investigated in this project in order to overcome the supply and demand variability that often impedes new technology uptake (Marsh and Nguyen 2008; Tran et al. 2008). Currently, no integrated production systems that include built-in quality control are known to exist for any biofertiliser product to be used on non-legume crops. A production model capable of being commercially franchised is now proposed to speed up the rate of adoption of this inoculant biofertiliser technology.

Reducing risks

Economic risks

The economic risks in this technology are related to profitable production of BioGro by the biofertiliser manufacturer, the feasibility of profitable sales to farmers and the profit gain by farmers that dictates their capacity to purchase the product on a sustainable basis (Tran et al. 2008). In general, these economic risks are not unique to this application of technology, but there are some special features that need consideration. In order to minimise economic risks, the following strategy is envisaged: the supply chain arrangements associated with BioGro production and the economic risks will be considered as a single commercial package, requiring defined equipment items and special protocols to be developed together with a set of standard quality controls for monitoring the success of the process.

These proceedings contain recommendations regarding commercial production models. The concept that this biofertiliser production package could be franchised is advanced here as a step towards sustainable commercialisation, based on the field results in Vietnam and elsewhere during the past 10 years (Figure 2). If supported by sufficient training of extension personnel and commercial producers, with a parent company providing advice and quality control, many of the risks to the introduction of the technology could be reduced to acceptable levels.

The capacity of farmers to afford even the reduced costs of using biofertiliser is also of concern, and it is possible that systems providing farmers with access to micro-credit for short-term loans during the crop production cycle may need to be promoted. Non-government organisations (NGOs) such as World Vision have previously provided cash loans to farmers involved in collaborative projects with the Institute of Agricultural Sciences (IAS) in the central coast region of Vietnam.

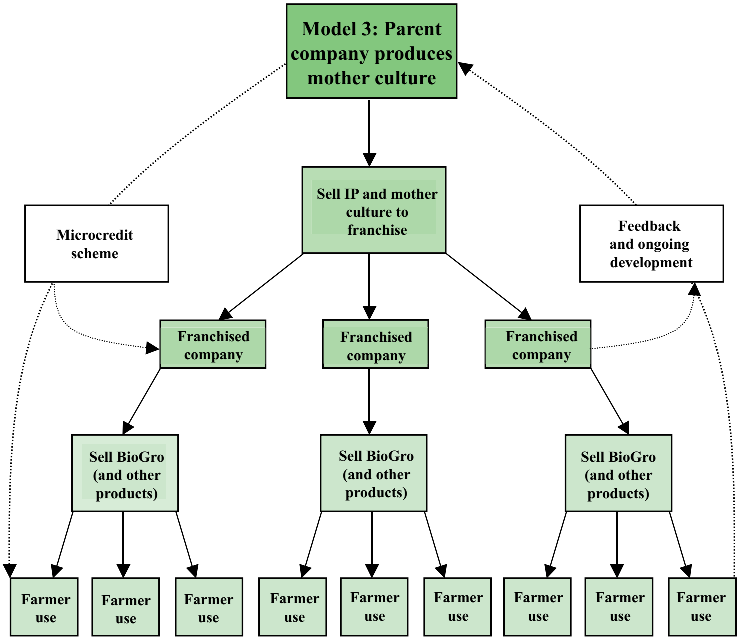

Figure 2. Proposed production–supply chain for sustainable uptake of inoculant biofertiliser technology

Technological risks

The technological risks identified relate to the safety of the inoculants, the manufacture of effective inoculants and their successful agronomic application. The definition of a biofertiliser, with which BioGro complies, is given in Vietnamese legislation as: 'addressing all safety matters related to human, animal, plant and environmental health' (Pham et al. 2008b). Risks in manufacture should be strongly mitigated by the quality control manual (QCM) developed as an output of the ACIAR project terminating in 2008. The QCM will contain details of methods developed during the project (Kecskés et al. 2008) that allow identification and quantification of the beneficial microbial strains in BioGro. The manual will also contain methods for guaranteeing and demonstrating the effectiveness of the agronomic application of the inoculant biofertiliser products on farms. A detailed description of methods for conducting and analysing farmer field trials as part of the process of extension will also be included. These clearly demonstrate the benefits for farmers from BioGro, expressed as increases in rice yields with lower fertiliser inputs.

Human safety

Several strains that raised questions regarding human health (Nguyen et al. 2003) were excluded because of such doubts. These strains, such as *Citrobacter freundii* and *Klebsiella pneumoniae,* were originally employed in BioGro because of their strong plant growth promoting (PGP) effects. They were used successfully for several years without reported adverse effects, and the degree of actual risk was probably extremely low given that these strains were isolated from rice soils in the first place. Similar reasons prevented the application of *Burkholderia vietnamiensis,* also shown to be an effective PGP organism for rice (Van et al. 2000), as a commercial inoculant in North America. However, it is essential for the good name of these biofertiliser products that all strains used be properly identified as done here (Kecskés et al. 2008; Deaker et al. 2008) and excluded if of even slightly doubtful human safety. All strains now employed in BioGro meet this criterion.

Sustainable growth potential

The major challenge regarding sustainability and growth will be the effectiveness of commercialisation of biofertilizer production. The current best model of production based on the ACIAR project is one with mother or starter cultures purchased by biofertiliser producers from the Biofertilizer Action Research Centre (BARC), who can then sell the product multiplied at least 100-fold in quantity to farmers using readily available carrier materials such as peat, high-organic matter soils or other composted materials. Biofertiliser effectively replaces N fertiliser, which can then be applied at a reduced rate without sacrificing yield.

Prior experience in the north of Vietnam indicates that enterprising producers will come forward, but sustaining production will depend on generating a sustainable market demand. Given the rapidly increasing price of N fertiliser, farmers will now be more willing than ever to consider this product instead of urea. Globally, given the urgent need to reduce the production of greenhouse gases such as nitrous oxide (400 times as significant as carbon dioxide in warming effect per molecule), the environmental benefits of less factory production and transport of urea fertiliser justifies the application of locally produced biofertiliser. Indeed, the technology may ultimately attract greenhouse reduction credits.

Dr Cong and Professor Hien at the final workshop in Hanoi in October 2007, discussing a critical point about BioGro's action

It is predicted that a large extension project will allow operation on a scale adequate to enable the technology to reach a 'take-off' stage, where production of biofertiliser will be considered profitable in a sustainable fashion. The key to financing the operation will be to expand production at such a rate that adequate new cash flow will be generated as profits are made. There is a clear role for micro-credit to be made available to farmers to cover input costs, with repayment possible at harvest. One of the production models for an earlier version of BioGro in the north of Vietnam involved an NGO providing cash to establish a factory and maintain the supply of biofertiliser (Marsh and Nguyen 2008). But this model had no scope for expansion and could only continue with significant production while the NGO provided cash inputs.

Currently, the IAS team led by Phan Thi Cong works in close collaboration with World Vision Vietnam, who have assisted with training and decision support for farmers to improve soil fertility in the central coast region. Throughout this collaboration, World Vision has provided loans and cash to poor farmers. A more dynamic model that includes franchising, favoured by the prospect of rapidly rising prices of chemical fertilisers continuing into the future, can take advantage of a greater sense of entrepreneurship in southern Vietnam.

For training programs to promote the BioGro technology, both the IAS and Mekong Delta Developing Research Institute (MDI) have a mandate to extend technology of all kinds to farmers in southern Vietnam. They are well placed to carry out such promotion. The IAS and MDI also have significant microbiological expertise in their institutions and will be able to provide support to BioGro manufacturers for both production methods and quality control of their products. Vu Thuy Nga of the National Institute of Soils and Fertilizers (Hanoi), associated with BARC, and Tran Thi Kim Cuc of IAS can fulfil specific roles related to quality control procedures. Two months' special training at the University of Sydney in early 2009 has already been funded under the AusAID Leadership Fellowship Award scheme.

While an extension project can only realistically target a cohort of several thousands of rice farmers, the scope for further expansion of the application of BioGro or of competitor products of similar effectiveness is virtually unlimited. Given that approximately 30 million Vietnamese people are involved in growing rice, there is extraordinary scope for the technology in Vietnam. Although it can be expected that many rice growers will take up other occupations as Vietnam's wealth increases, this need not diminish the use of these biological products as the technology could easily be adapted to more automated systems of rice production. Moreover, if the production–supply model is demonstrated to be successful, it could logically be applied to a range of scientifically reliable and transparent microbial products for other agricultural industries, both nationally and internationally.

Financial viability

To ensure the financial viability of biofertiliser application, it is essential that a supply chain for profitable production and application of inoculant biofertilisers on farms be established. The current global conditions of increasing fertiliser prices linked closely to the increasing price of oil provide a very favourable environment for success of this technology. Indeed, for poor farmers there is now an imperative need for alternatives to more expensive fertilisers such as urea and superphosphate, and more efficient use of urea-N will be most valuable.

A significant uptake of the technology in the Mekong Delta and the central southern coastal region, together with proof that it is likely to be effective over a period of 2–5 years, will provide a compelling demonstration of this approach. If rice production can be maintained or even increased, a flow-on effect would be anticipated to other areas of Vietnam and to other rice-producing countries. In these circumstances the potential scale for growth is very large.

Taken province by province with a 50% uptake rate, the number of beneficiaries is potentially in the millions, even if remaining within Vietnamese borders. In these conditions the franchising concept for biofertiliser production may be particularly effective as a means of more rapidly spreading the technology. If capital to manufacture the inoculants can be obtained locally for moderate levels of production, it is anticipated that economic viability might be enhanced by the sense of community involvement and the local cash flow generated. The increasing availability of micro-credit would also be important in allowing farmers to make outlays for biofertiliser in a similar way as they have done when financed by chemical fertiliser companies in the past.

Possible income generated by the production and sale of biofertilisers can be estimated from known production costs of BioGro and sell-on costs to farmers at US$150/t (estimated at 25% of the cost of equivalent chemical-N fertiliser, i.e. currently US$600 for urea). Considering an anticipated service area containing 10,000 ha of rice production using 100 kg/ha of BioGro, production and sale of 100 t of BioGro per season per franchise is estimated. This is equivalent to a gross income of US$15,000 per season, with a nominal net profit of US$1,500 per season per franchise after transport costs, worker salaries and production and sale costs. Such a minimum income may be sufficient to ensure franchise uptake and sustainable production to supply farmers' needs.

Current estimates of the financial benefit to individual farmers project an average annual profit increase of more than 10% with uptake of this technology (Marsh 2008). These figures are based on average reduction in use of N-fertiliser afforded by the technology, and are likely to increase with oil price increase.

Agronomic viability

Financial viability will also depend on constant improvements to the agronomic performance of the technology. Currently, BioGro contains four microbial strains (three bacteria and a yeast), and has normally been applied in significant quantities in carrier (ca. 200 kg/ha) at similar rates to fertilisers. This is partly as insurance to maintain cell numbers against the need for a shelf life of at least 1 month for the

Project team and review panel at Ba Vi factory in Ha Tay province near Hanoi, October 2007 product before application. Application of a large quantity of carrier also ensures more even distribution of the microbes and may be significant for adequate pricing. However, since only 1 gram of microbial cells is actually needed per hectare as inoculant, there is now an opportunity to substantially reduce the cost of BioGro and the quantity of carrier if delivery of sufficient numbers of microbes can be verified, that is if cell numbers can be increased. This is most likely if the use of biofertilisers increases and more experience is gained in obtaining optimal results.

Conclusion

The prospects that inoculant biofertilisers can be applied on a significant scale are greater than ever before. The main drivers for success will be the increasing price of chemical fertilisers and the need to minimise the production of greenhouse gases such as carbon dioxide and nitrous oxide. Achieving such an ‘evergreen revolution’ will still be extremely challenging but there are many indicators that it can now succeed.

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