Challenges in commercializing a phosphate-solubilizing microorganism: *Penicillium bilaiae*, a case history

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Received 17 December 2002. Accepted in revised form 2 January 2003

Key words: Phosphate-solubilizing microorganisms, fungi, commercializing, market, inoculant commercialization, JumpStart, *Penicillium bilaiae*, phosphate solubilization

Abstract

The commercialization of a phosphate inoculant is a challenging process. The active ingredient of the phosphate inoculant *JumpStart®* (*P. bilaiae*) was isolated in 1982. Although the concept of P solubilization was proven, much additional research was required. Full-scale, cost-effective manufacturing, packaging and QA systems; easy-to-use, shelf-stable formulations needed to be developed. Extensive field research to confirm efficacy and comprehensive data on compatibility with seed-applied pesticides were required. In addition, we needed to develop and refine the product positioning and branding to ensure we were delivering value to the farmer. Development continues to be an on-going process with the use of the product on new crops, improved production methods and formulations, new applications, and continuing market research to monitor changing farmer needs.

Introduction

Commercializing any product is a difficult process. Commercializing a biologically based product presents additional challenges, and commercializing a biological product aimed at enhancing phosphorus nutrition is even more difficult. This paper describes the scientific, technical, and marketing challenges involved in commercializing and marketing *JumpStart®* (a phosphate inoculant based on the phosphate-solubilizing fungus *Penicillium bilaiae).*

Kucey (1983) isolated *Penicillium bilaiae* from Canadian prairie soils in 1983. He demonstrated that the organism could solubilize phosphate (P) on agar plates and in liquid culture (Kucey, 1983). He also demonstrated that inoculating soil with the fungus could increase the growth and P uptake in wheat and beans in greenhouse and field trials. Philom Bios acquired the rights to commercialize this product in 1986. Agriculture and Agri-Food Canada (AAFC) obtained a patent (Canadian patents 1,308,270 and 1,308,566) and a royalty agreement was signed between Philom Bios and AAFC. This agreement was crafted such that the royalty funds would go back into research rather than into general government funds. Philom Bios has paid AAFC $1.7 million from 1990 to 2002.

The concept of *P. bilaiae* as a phosphate inoculant had been demonstrated (Asea et al. 1988; Kucey, 1983, 1987, 1988). However, a tremendous amount of work was required to make this into a commercial product. Extensive field trials were conducted to prove that the organism was effective over a wide range of soil, environmental conditions, and crop types to support the registration of the product. We had to develop a production method, a commercially acceptable formulation, a quality assurance plan, and information on application strategies. Initial

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E. Velázquez and C. Rodríguez-Barrueco (eds.),
First International Meeting on Microbial Phosphate Solubilization, 215–222

knowledge of the behavior and mode of action was limited.

We had to develop a marketing plan. The research needed to bring the product to market was expensive and so we had to find financing. It could not all be completed at Philom Bios and so we found cooperators to assist with the work. All of these operations were interconnected and the process required coordination and teamwork.

The phosphate inoculant was registered for use on wheat in 1990 and is now registered on most major crops in Western Canada. From a small number of hectares inoculated in the first year, some one million hectares were inoculated in 2002. These advances have resulted from an increasing commitment to research and a constant willingness to develop cooperative projects with researchers across Canada.

This paper will discuss the challenges involved in developing and marketing a commercially viable phosphate inoculant.

Field research

Inoculant organisms are affected by many soil and environmental factors and must be thoroughly tested under conditions that will exist where they will be used.

Pre-commercialization

Field trials had been done on two crops (wheat and beans) at one site (Kucey, 1988). The inoculated bran media had been applied in furrow (by hand) in these studies.

Commercialization

Philom Bios did not have the resources to carry out all the field tests needed for registration. Philom Bios therefore joined with Dow Elanco Canada (DEC) to carry out field tests across the Canadian Prairies. The Saskatchewan Wheat Pool (SWP) also conducted independent trials to ensure an unbiased source on information for the registration package. As an in-furrow application using bran material was impractical for large scale farming operations, a seed treatment was used. Thirty-eight trials were established across the three Canadian Prairie Provinces (Manitoba, Saskatchewan and Alberta) in 1987 and 1988. All trials were arranged in a split plot experimental design. The control (untreated) and *P. bilaiae* treatments were compared over four rates of P_2O_5 (0, 10, 20, 30 kg P_2O_5 ha^{-1}). *P. bilaiae* increased yield and P uptake in wheat (Table 1) at the lower rates of P application.

Subsequent field-work proved the fungus could also increase P uptake and yield in pea, lentil, bean, canola, and alfalfa.

On-going

The field program continues to increase as the market expands and sales move into the Northern U.S. states. In addition, as each new crop or new formulation is added field trials are conducted to ensure inoculant effectiveness.

Production and formulation

A good quality inoculant must be able to survive storage, desiccation after inoculation onto the seed, and natural competition in the rhizosphere (Maurise et al., 2001). Formulation development is a complex process and is still more of an art than a science (Daigle and Connick, 1990).

Pre-commercialization

Kucey (1988) used a straw substrate to produce spores for greenhouse and field trials. This was effective for the small trials but impractical on a commercial scale. The production process was cumbersome and only a limited amount of material could be produced. Twenty-three pyrex dishes were needed to produce the inoculum for

Table 1. Effect of *P. bilaiae* on the yield of wheat. Multiple year field summary of 38 locations

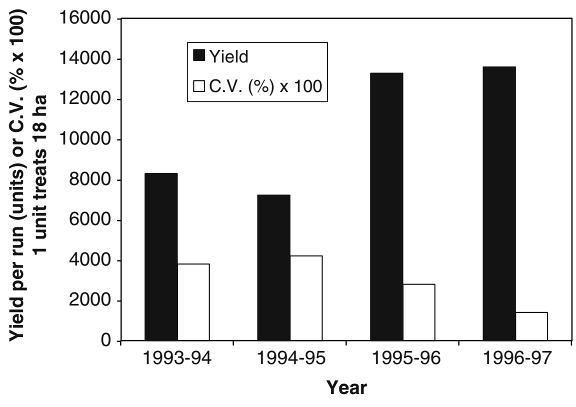
Phosphate applied	Mean yield (kg ha^{-1})			
	Untreated	*P. bilaiae*	Difference	Statistical significance
0	2771	2827	56	0.01
10	2909	2958	49	0.04
20	2930	2962	32	0.15
30	2962	2948	(14)	0.54

From Hnatowich et al. 1990.

five research sites (approximately 1.0 ha). The material was applied by hand as it could not be applied through a commercial seeder.

Commercialization

A liquid fermentation method was developed which produced sufficient spores to inoculate 25,000 ha of wheat per batch. The spores were collected and processed into a dry powder, which had to be kept frozen to maintain viability for an effective inoculant. This frozen powder, *PB50*®, was introduced to the market place in 1990. The economics were prohibitive however, as up to 80% of the viability was lost during the drying process. The development of a frozen liquid formulation, *Provide*®, solved the problem and raised the number of hectares treated to 126,000 per batch. The batch-to-batch variability of the production process was large. Attempts to adjust the titre by changing the conditions in response to a low titre in one batch resulted in an overall decrease in average yield in the fermentor and an increase in variability. Implementation of statistical process control procedures that monitored yields but did not change conditions until detailed laboratory research showed it was warranted, resulted in an increase in yield (increase in treated ha to 235,000 per batch) and a



Year	Yield	C.V. (%) x 100
1993-94	8290	3800
1994-95	7222	4200
1995-96	13268	2800
1996-97	13583	1400

Figure 1. Improvements in production after adoption of Statistical Process control practices, in 1994–95.

decrease in variability (Figure 1, Philom Bios unpublished data, 1999). The stability of a formulation on seed is also an important criterion when assessing the acceptability of a formulation. An improvement in the formulation allowed for the introduction of a room temperature stable powder, *JumpStart*®. The move from the *Provide*® frozen liquid formulation to the dry room temperature stable *JumpStart*®, increased the half-life of the fungus on seed from 10 to 35 days.

On-going research

The search for improved formulations is ongoing. The current formulation has a half-life of 4 weeks at 28 °C. In the Canadian prairies the product will rarely be subjected to long periods above 25 °C so this is an acceptable shelf-life. As we move into warmer climates, increased stability at higher temperatures is a commercial imperative.

Quality assurance

Once an inoculant is developed for any crop, or area, there must be strict adherence to quality standards (Hedge et al., 1999). Substandard materials restrict the popularity and acceptability of the product (Hedge et al., 1999).

Pre-commercialization

Before commercialization, the *P. bilaiae* spores had been produced and applied with the rate based on the amount of dry material added per meter of furrow (Kucey, 1983). This did not allow for the development of a quality control procedure as the quality and the amount of fungus in a gram of substrate varied from batch to batch.

Commercialization

The first step in the development of a quality assurance program was to set parameters for the product. In Canada, rhizobium products are regulated by the Fertilizers Act and the number of bacteria per seed is pre-determined based on seed size (Olsen et al., 1994). As the phosphate inoculant was a new product, Philom Bios assisted

Ottawa in establishing a standard (the minimum number of spores per seed required for efficacy) by submitting field data. Once this level was determined, a quality assurance system could be developed. As the quality (cfu per g) varies from batch to batch, each batch must be evaluated separately. Enough samples must be taken from each batch to ensure that a statistically valid number could be obtained. We chose a dilution plating method and cfu count as the basis for our quality assurance system because a large number of samples could be assayed without expensive analytical tools. This assay is, however, a tedious process that becomes more cumbersome as production volume increases. The number of agar plates for the quality assurance program for the phosphate inoculant at Philom Bios has increased from 3000 in 1992 to 46,000 in 2002.

On-going

The increase in production is increasing our need to develop a fast reliable method to assess the number of viable spores in our product to replace the cfu plate assay.

Application

The inoculant use must conform to standard application practices used on-farm. An inoculant can be applied either as a seed treatment or as in-furrow application. Seed inoculation is the most commonly used method of inoculation. However, seed-applied pesticides are also commonly used and many seed treatment chemicals contain fungicides that reduce the survival of *P. bilaiae* on seed. We therefore must be concerned about the ability of the inoculant organism to survive on fungicide treated seed long enough to be effective in the field.

Pre-commercialization

Fungicide use on wheat was not universally practiced in the 1980's. All of the early trials were conducted with untreated seed.

Commercialization

The use of seed treatment fungicides increased in the 1990s. Farmers needed these materials to protect their crop from increased disease pressure and could not omit the fungicide in order to use the phosphate inoculant. We therefore developed a system to test the compatibility of *P. bilaiae* with commonly used seed applied chemicals. The chemical and the inoculant are applied to seed and the population of the *P. bilaiae* on the seed is determined. The seed is stored at room temperature and the population of *P. bilaiae* is monitored for up to 4 weeks. A regression line is determined using SigmaPlot software. The intersection of this regression line with the minimum number of organisms required on seed at planting determines the planting window (the minimum time allowed between inoculating and seeding for that seed treatment–inoculant combination). The application methods used to apply the two materials mimic the application methods a farmer would use. The fungicide and the inoculant may be mixed together in a slurry (tank-mix), applied to seed at the same time through separate hoses (simultaneous), or the chemical may be applied to the seed first and allowed to dry before the inoculant is added (sequential). Although the tank-mix is usually the most damaging to the fungus, farmers prefer this method, as it is quick and easy (Table 2).

Generally, the planting window is the longest when the two materials are applied sequentially (Table 2). Each chemical formulation must be analyzed separately as it is often the formulation ingredients, rather than the active ingredient that affects the fungus. A change in the formulation of either the chemical or the inoculant will alter the planting window.

Table 2. Planting windows for use of *JumpStart*® with commonly used fungicides on wheat

Seed treatment*	Planting window (days)		
	TankMix	Simultaneous	Sequential
Bare Seed	15	15	15
Baytan® 30	10	10	10
DB Green	Do not use	2	7
Proseed®	Do not use	10	10
Vitavax® Single	Do not use	10	10
Vitaflo® 280	Do not use	1	4

Baytan 30, Vitaflo 280 and Vitavax Single Solution, are registered trademarks of Uniroyal Chemical Ltd.
DB Green is a registered trademark of Agsco Ltd.
Proseed is a registered trademark of Zeneca Agro.

P. bilaiae must also remain alive throughout any application process. Some air-seeders are equipped with tanks that allow seed to be inoculated as it is sown. It can take 6–8 h to empty one tank. We know that the fungus population in slurry does not drop ($P < 0.05$) in 8 h so we are confident that the number of viable spores is still adequate for effective inoculation during the entire planting operation. We tested each inoculant formulation in a range of commercial air-seeders to ensure that the material would not clog the hoses or screens or lose viability during application.

On-going

We must continue to test our materials with new chemicals or formulations. Our current compatibility tests look at loss in viability on seed due to chemicals but do not look directly at efficacy. We plan to add a greenhouse or field-screening component to these tests. Seed coating companies are constantly looking at polymers to improve seed flow in seeders, protect rhizobial inoculants from environmental stresses, and manipulate seed germination. We constantly evaluate these materials to determine if they will reduce or increase the survival of the *P. bilaiae* on seed.

Registration

Inoculants in Canada are registered as fertilizer supplements under the Fertilizers Act administered by the Canadian Food Inspection Agency (CFIA). This process requires proof that the organism is safe and that it will perform according to the claim on the label. All new inoculants must go through this process and any claim on the label (including pesticide compatibility) must be reviewed by the CFIA.

Pre-commercialization

The product could not be marketed and sold until it was registered. The product was first registered for use on wheat in 1990 under the name *PB50®*.

Commercialization

Every new crop must be registered and so field and compatibility data was submitted and reviewed before canola, pea lentil and alfalfa were added to the label in 1992, 1993 and 1996, respectively. The amount of data Philom Bios submits to the CFIA is increasing rapidly as new crops, and chemical compatibility information are added to the label and new formulations are developed. This is beginning to present a problem as the large volume of material that has to be reviewed creates a backlog in the system and delays the introduction of new applications. The market introduction of a granular formulation was delayed by one year due to delays in the registration system.

On-going

Philom Bios continues to work with the CFIA to try to streamline the registration process. We ensure that they approve of the format of our reports and the statistical analysis we use before we send in large submissions.

Mode of action and behavior

The more we understand about the mode of action and behavior of the *P. bilaiae* the more we are able to manipulate it so we can maximize the effectiveness of the inoculant.

Asea et al. (1988) used the ^{32}P-dilution method and found that greenhouse-grown wheat inoculated with *P. bilaiae* obtained 18% of its P from sources unavailable to non-inoculated plants. Often this work requires equipment and areas of expertise that are not available at Philom Bios. We therefore collaborated with university and government scientists to provide this information.

Pre-commercialization

Philom Bios, DEC, and SWP conducted field studies to gather data to support registration and monitored P uptake as well as yield (Gleddie et al., 1991) to show that the fungus increased the phosphate nutrition of the plants. This information was backed up by greenhouse experiments with ^{32}P using wheat, and flax conducted by Chambers and Yeomans (1990) of the University of Manitoba. They found that plants inoculated with *P. bilaiae* increased tissue P concentration, primarily through increased soil P contributions (as opposed to fertilizer P).

Commercialization

Researchers continue to discover that the effects of our phosphate solubilizing inoculant are more complex than a simple solubilization of P. Recent work at the University of Manitoba has shown that root growth (Vessey and Heisinger, 2001) and root hair development are increased by *P. bilaiae* (Gulden and Vessey 2000). *P.bilaiae* may increase the absorptive capacity of roots, which may lead to increased P, other nutrients, or even water uptake (Vessey and Heisinger, 2001).

We need to study the ecology of the fungus *in situ* if we are to fully understand the behavior and the limitations of the inoculant. A polymerase chain reaction (PCR) assay developed by O'Gorman et al. (1998) demonstrated that *P. bilaiae* was able to effectively colonize the roots of six plant species in non-sterile soil.

On-going

We will continue exploration of factors that affect the ability of *P. bilaiae* to colonize roots, solubilize P, and increase yield. We will continue to access expertise at university and government labs to help us to develop and use techniques that will help with these investigations.

Marketing

It is extremely important to have a coordinated marketing plan with clear descriptions of the benefits of the inoculant.

Pre-commercialization

This was especially important for our phosphate-inoculant, as it was a new concept in the marketplace. This concept had to be clearly linked to the research plan. Research results were continually used to clarify and refine the benefit statement and, just as importantly, to define the limitations of the use of the inoculant. Growers had to be educated on the value of phosphate to crop growth and on the value of inoculants. We had limited internal resources and marketing expertise, so a marketing partnership with DowElanco Canada was particularly beneficial.

Commercialization

The benefit statement has been constantly refined as we discover more about *P. bilaiae*. As we learned more about the inoculant and the need to fully support the product with timely information, we discovered we needed to be closer to our customers. Each farmer is different, and his or her needs must be addressed separately. In 1996 we assumed direct responsibility for marketing our products, and developed a one-to-one marketing strategy. This strategy allows us to provide individual customers with specific information about the product, its benefits, and how to use it to optimum value. At the same time this approach ensures a rapid feedback system. Questions or concerns of farmers that require more research can therefore be incorporated into the research plan. A thorough response to customer concerns and questions is crucial to the successful commercialization of an inoculant.

It is also important to continually demonstrate the value of the inoculant. Every year Philom Bios puts in Inoculant Performance Trials (IPT). These are large-scale (5–50 ha) on-farm trials that directly compare inoculated and non-inoculated portions of fields. Over 300 trials have been done in the last 10 years demonstrating an average yield increase of 7% in crop yield due to inoculation. The success of this approach can be seen in market surveys conducted every year. In 1990, most farmers had not heard of a phosphate inoculant. By 2001, *JumpStart*® was the most recognized inoculant product across the Canadian Prairies with 72% of farmers aware of its use.

Financing

The commercialization of a new inoculant is not a trivial matter and as such it requires significant funding. This need for research funding does not end with the commercialization of the product. It must continue, as there is always a requirement for product support and improvement. The discovery and initial testing of *P. bilaiae* took about 6 person years. The pre-commercialization stage required 32 person years, and since then, Philom Bios has invested over 120 person years to continue to improve the inoculant by adding new crops, new formulations, and continual support to the use of *JumpStart*®. These figures do not include the time and money used by external researchers. We are fortunate to have received assistance in funding some of the work from AAFC Matching Investment Initiatives (MII) and NRC Industrial Research Assistance Program (IRAP) grants.

Pre-commercialization

During this phase Philom Bios did not have any products or revenue and financing had to be sought based on a clear description of the potential value of the inoculant to the Western Canadian market. The value of the product to growers had to be clearly understood. This was made more difficult as the value of phosphate itself was poorly understood by Prairie farmers. Inoculants were not recognized as important crop inputs, and new biological technologies were perceived as ''snake-oil''. Financing was obtained, however, and development advanced.

Commercialization

There is a constant need for ongoing research investment to drive market expansion to new crops and new regions, to develop new formulations, and to assess new equipment and chemical seed treatments as they become available to farmers.

On-going

Over the past 10 years we have built a successful business by maintaining a tight strategic focus and practicing a responsive business model. We focus solely on developing, manufacturing, and marketing high-end inoculants, and we believe we do this better than anyone else. The business model is a seamless link from Research to Manufacturing to Marketing, overlaid with Corporate systems. Superior R&D generates product improvements and competitive advantages, and process improvements which enable Manufacturing to achieve cost leadership with rewarding gross margins. Disciplined Marketing has grown the business consistently over the past 5 years, steadily increased market share, and built the highest brand strength of all inoculants in our marketplace. It is not enough to have superior R&D; it must be combined with high market share and economies of scale to deliver better products to establish sustained market leadership. Market share only matters if it generates high returns through cost leadership in manufacturing.

This focused business approach is creating value for our customers and our shareholders. We have been financially self-sustaining since 1991. The Company is essentially debt-free, is demonstrating appealing returns on capital, is profitable, and boasts positive retained earnings. We intend to continue to increase delivery of high-end inoculants to more farmers in an expanding market in the years ahead.

Conclusion

The development of a commercial phosphate inoculant has been a challenging and rewarding process. The procedures had to be developed (and mistakes made) as we went as there was no ''users manual'' to lead us through the process. The challenges do not end with the commercialization process but continue to arise as we improve our product and processes, keep pace with new developments in agriculture and expand our market.

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