Improving industry through nature

Dr Dan Funck Jensen gives *International Innovation* an overview on his work with biological control agents (BCAs) to better understand fungal interactions and reduce the use of chemical pesticides

For those who are unfamiliar, what are biological control agents (BCAs) and how do they contribute to biological control of plant diseases?

In biological control of plant diseases the idea is to apply living microorganisms called biocontrol agents or BCAs to protect plant crops against diseases. The BCAs – mainly either fungi or bacteria - are doing their job either by interacting directly with the pathogens in soil or at plant surfaces or they trigger the plants to become resistant to attack by the pathogens. In this way we are aiming at sustainable crop production with reduced input of chemical pesticides. In our case we are working with a fungus named *Clonostachys rosea* – the species is naturally occurring in most soils worldwide – our strain of the fungus called strain IK726 is from a barley field in Denmark found in the mid 1990s.

Can you provide us with a brief overview of the FunSecProt project? What are your aims and what do you hope to achieve?

FunSecProt is short for our Danish-Swedish research project: "Secreted proteins in fungal parasitic interactions for feed, food and non-food industry". Besides biocontrol, we are working with enzyme discovery. Working with enzymes produced in fungal to fungal interactions, we envisaged that we might come about new secreted enzymes from fungi parasitising their host which can be exploited in industrial processes. Most industrial applications are targeted towards modifications and conversion of plant and animal material. Industrially relevant discoveries of enzymes can therefore be expected from the pool of proteins expressed when microbes interact with their plant or animal host.

Until now gene discovery research based on gene sequencing has mainly been concerned with single organisms, but our new approach for gene discovery has fungi interacting with their host in focus. Our project gives the excellent possibility of serving two purposes in one: expanding discovery into new areas for industrial exploitation and improving our understanding of fungal interaction biology.

How will industries like the food and agro companies benefit from your research projects?

We embrace both applied and more basic research and first of all it relates to exploiting biological control of plant diseases. We have allied ourselves with industrial partners for large scale production and we are aiming at approving our IK726 for use as a BCA in the EU. Our work with secreted enzymes may reveal new or more efficient enzymes which can be exploited in industrial processes. For this we collaborate in FunSecProt with Novozymes A/S – the world's biggest enzyme producer. They have special bioinformatics pipelines for revealing if an enzyme can be useful in various industrial processes.

Further to this, what are some of the commercial considerations you must take into account when developing an effective BCA?

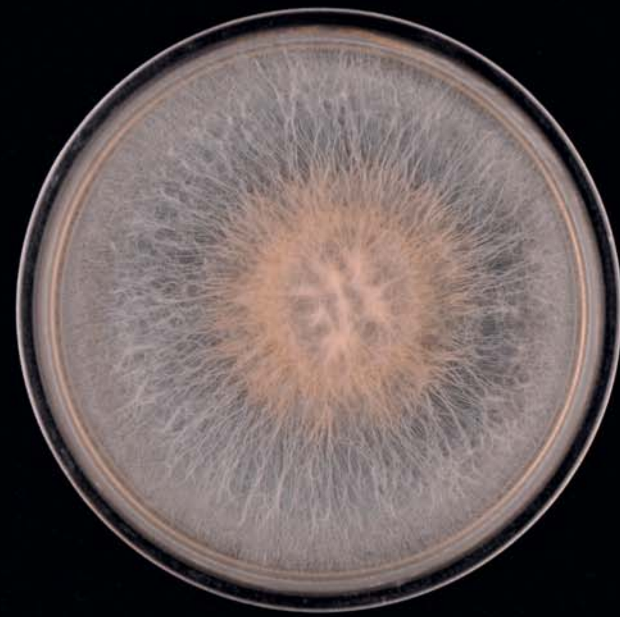
There are technical considerations like how to produce, formulate and apply the BCA to the crop. One important point which we have solved in small scale is how to obtain a product of a living BCA which can be stored for several months before used. This

is being addressed at the moment for large scale production of IK726. Then there is the requirement for registration of the BCA in the EU before it is to be used commercially. This implies a thorough risk assessment of the organism and how it is used as well as how it is performing as a biocontrol agent in the crops it is meant for.

In addition the company will consider the market size – it might be too expensive to develop a BCA for one disease in a crop that is not grown extensively, although a BCA which is effective against more diseases in different crops or which is for use in high value crops might have a better chance for a reasonable market size. Biocontrol is considered a "green technology" which if possible should replace or reduce the use of chemical pesticides. Public acceptance of this is also important when introducing this new technology.

Are there any other aspects you would like to highlight?

A Center for Biological Control (CBC) funded by The Swedish Ministry for Rural Affairs has been established at SLU in 2011. CBC will cover both biocontrol of plant diseases, insect pests and later also biocontrol in aquaculture. The tasks are in addition to securing the scientific competences also to facilitate the dialogue - both nationally and internationally - between various public institutions, the agroindustry and end users like farmers and consumers. My SLU team is now affiliated to CBC.



Triggering nature's defences

The **BIOCONTROL** programme at SLU benefits from collaborating with the Danish FunSecProt where enzymes in various fungal-host interactions are studied. This helps the SLU group to provide an alternative method to control plant diseases

IN AGRICULTURE, PLANT disease is a serious threat that can lead to loss of yield and poor product quality. Due to this, the use of pesticides grew over time as more farmers searched for ways of eradicating this problem. However, as early as the 1980s, experts began to realise that pesticides could have adverse effects on the environment, food quality and our safety. With this in mind, research was undertaken to find new, less harmful methods that could reach the same levels of disease reduction.

Plant disease is caused by pathogens. Serious pathogens are found in fungi, bacteria, viruses and nematodes. The majority of microorganisms are beneficial to plants or important for various processes in soil, while a few are able to cause plant disease. Beneficial microorganisms aid plants with the uptake of nutrients, promoting plant growth and protecting against plant disease.

Biological control agents (BCAs) help prevent plant disease by either interacting with the pathogen directly at the plant's surface or by triggering the threatened plant's natural defence system against the pathogen. In spite of understanding this, only a few BCAs have been commercialised, with the main deterrents frequently cited as legislative aspects and the costs involved in registering a BCA.

> Learning how biocontrol agents function, where they function and how and where they interact can aid us greatly in combating plant disease

Learning how BCAs function, where they function and how and when they interact can aid us greatly in combating plant disease. This can lead to more sustainable crop production with reduced or no input of chemical pesticides. Realising that, Dr Dan Jensen initiated a research programme in the 1990s on biological control of plant diseases when he worked at University of Copenhagen (KU-Life) in Denmark. These investigations in Denmark led to Jensen's vested interest in BCAs and fungal interactions and his move to SLU in 2008 opened up for new opportunities for following his interests. "The Department for Forest Mycology and Plant Pathology at SLU probably hosts the biggest group of researchers worldwide working on mycology, fungal ecology and fungi related to plant pathology," Jensen explains. "It has been a stimulating challenge to form a new research strategy within plant pathology and biological control of plant diseases. I did that in close collaboration with associate professor Magnus Karlsson who has been in my new SLU-team from the start in 2008."

TAKING THE FIRST STEPS

During biological studies, a fungal strain of *Clonostachys rosea* (IK726) was proven as a potential BCA against *Fusarium culmorum*, which

FunSecProt

STUDYING THE ROLE OF SECRETED ENZYMES IN FUNGAL HOST INTERACTIONS

OBJECTIVES

- To discover interesting genes and their products for commercial exploitation within the food, feed and non-food industry
- To contribute to improved biological understanding of fungal parasitic interactions leading to plant protection measures with reduced use of pesticides.

The vision is to contribute to more sustainable production, improved product qualities and improved use of raw materials.

KEY COLLABORATORS

For full details of all project partners and collaborators, please see:
www.aztcservices.com/fosu/contact.asp

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DAN FUNCK JENSEN has been a professor in Plant Pathology at SLU since April 2008 following a decision made by the NL-faculty to strengthen research in plant pathology with focus on agricultural crops. Over this time he has helped to build up a new research team of five researchers and four PhD students. In this, complementing methodological competences have been gathered, creating a platform for exploiting technologies with filamentous fungi.

causes disease in wheat and barley. This discovery was made initially in greenhouses and then later proven further through the use of small and larger plots of land. The team at KU-Life has been looking into the strain's uses against other diseases, finding it effective under field conditions against pathogen species of *Fusarium, Pythium, Botrytis, Alternaria, Tilletia* and *Bipolaris*. The KU-team has investigated isolation, screening, selection, production, formulation and delivery and, studied biocontrol interactions by using DNA reporter technologies (such as GFP and DsRed), gene knockouts and fluorescent microscopy.

Found in a barley field in Denmark, *Clonostachys rosea* exists naturally in soil around the world. Among 400 other potential BCAs, it seemed the best candidate to develop into a BCA from its successful biocontrol of various plant diseases on plants like wheat, cabbage, strawberry, tomato and barley. Jensen's team at KU-Life has already developed an IK726 prototype with a longer 'shelf life', insisting that any exploitation of IK726 for biocontrol purposes will be addressed in collaboration with end-users of the agriculture industry.

Since Jensen moved to SLU he and Karlsson have set up the *Chlonostachys rosea* genome project to concentrate on understanding the underlying mechanisms in interactions between IK726, fungal pathogens and host plants. They are studying this at the gene and cellular level using the methods known as next generation sequencing.

Jensen's SLU- team has found supporting evidence to show that the IK726 strain interacts directly with the plant pathogen by secreting enzymes which degrade the pathogen's cell walls. This degradation allows IK726 to exploit the pathogen as a nutrient. The team has also found the strain to excrete a range of enzymes protecting it from a soil's environment or helping a plant against its pathogen threat.

JOINING FORCES

In 2008 Jensen's SLU team and his former KU-team became part of a large Danish programme entitled 'Secreted proteins in fungal parasitic interactions for feed, food and non-food industry' (FunSecProt). Dan Jensen is the coordinator of FunSecProt and the role of Jensen's SLU team is to look at the biocontrol interaction between IK726 and pathogenic fungi and reveal the secreted proteins involved in the interaction. They also have another task in the project which is to study pathogenic interactions between pea and Phytophthora pisi, which is a new pathogen attacking pea plants. Jensen's group, in particular, is interested in root rot that occurs in a plant due to pathogens.

Also contributing to the FunSecProt project is Lund University where work on an ectomycorrhizal fungus- a fungus living in symbiosis with birch trees - is conducted. Two partners at University of Copenhagen (KU-Life) are also involved - one focusing on plant pathogen interactions and one on fungi interacting with insect pests and social insects. Both the Swedish and the Danish partners at KU-Life are working towards understanding how a fungus interacts with its host. They work alongside Danish partners at Aalborg University, Solum A/S and Novozymes A/S who have their main focus on gene discovery for industrial exploitation.

FunSecProt allows Jensen's team to improve current knowledge of fungal interaction biology such as the basic ecology of the BCA and its role with pathogens. Jensen's side of the FunSecProt project incorporates members from various expertises, including fungal genetics, gene evolution and next generation sequencing. This mixture of knowledge and talent has aided them in setting up the relevant experiments that will give more valid results regarding the biocontrol interaction in a natural environment.

Initially funded by the Danish Council for Strategic Research and later also by Biotech Denmark, the FunSecProt continue until 2013. Jensen feels that the project is successful in meeting the needs of its many stakeholders. "We are now planning a PhD student and postdoctorate training course for our research groups in October where we will focus on the advanced methods we are using in fungal gene expression and we will have a larger workshop organised in Uppsala in 2013 which will be open for more participants," Jensen explains.

The FunSecProt project has certainly influenced their main foci at SLU. Jensen has also obtained new funding from The Swedish Research Council Formas and other national funding resources in Sweden and the team is partner to a new Nordic research project and an EU funded project. They are also heavily funded by the SLU.

Being part of a larger team such as FunSecProt has not only served to help Jensen and his team achieve their goal of reducing plant disease in a safer and more sustainable way. It has also ensured they continue to learn about their own field. The more the team keeps informed of bioinformatics and new molecular technologies (such as next generation sequencing), the more they find themselves learning about the biology of biocontrol interactions.