Poster
Endophyten

100 - Production of bioinsecticides with endophytes isolated from a tropical tree: first results

Produktion von Bioinsektiziden mit Endophyten isoliert aus einem tropischen Baum: erste Ergebnisse

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Today a lot of agrochemical and pharmaceutical compounds from plants are obtained via complex extractions in low concentrations. Due to the fact that plants contain endophytes, it can be hypothesized that some of these compounds are directly produced by endophytes. This offers new ways to produce chemical insecticides and antibiotics. An endophyte-containing tree produces insecticidal metabolites and therefore, this tropical tree shows an array of negative effects on insects including ovipositor deterrent, anti-feedant and other inhibitory activities. In a recently granted BMBF project we will examine the endophyte biodiversity in this tree, whether endophytes produce this bioinsecticide directly or associated with the plant metabolism and if so, how this secondary metabolite can be produced in a liquid culture. Based on these findings, an in vitro mass-production process in a 2 L stirred tank reactor will be developed (Figure 1).

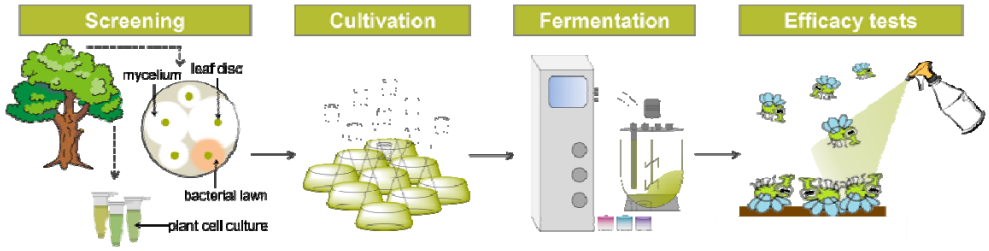

Fig. 1 Isolation of endophytes, cultivation in shake flasks, scale-up of the process to a 2 L stirred tank reactor and efficacy test on insects.

In total, 230 endophytes (91 bacteria and 139 fungi) were isolated from seeds, leaves, stems and roots of 16 tree samples. From 8 different German breedings 107 endophytes were isolated (leaves: 24 bacteria and 9 fungi, stems: 34 bacteria and 2 fungi, roots: 20 bacteria and 18 fungi). Samples from India yielded 65 endophytes (Seeds: 7 bacteria and 25 fungi, leaves: 21 fungi, stems: 6 bacteria and 31 fungi). Only fungi were obtained from Myanmar samples (seeds: 33 fungi).
Then, endophytic fungi and bacteria were cultivated in Sabouraud dextrose and in potato dextrose liquid media for 14 days at 25°C. Terpenoid secondary metabolites that indicate the production of bioinsecticides that so far have been thought to be produced by the tree were detected in the culture broth of one bacterium and three fungi with HPLC-DAD. Furthermore, we will present first data on classification of the endophytes, screening for technical media, identification of metabolites by HPLC-DAD-MS/MS and high sensitive bioassays with insects (*Agrotis segetum*), nematodes (*Caenorhabditis elegans*) and insect cell culture (SF9).

101 - Development of a novel fermentation process for an endophytic *Beauveria bassiana* strain

Entwicklung eines neuartigen Fermentationsverfahrens für ein endophytisches Beauveria bassiana Isolat

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A novel biological plant protection strategy could be the use of the endophytic entomopathogenic fungus *Beauveria bassiana* isolate ATP-02. To use this endophyte as a commercial biocontrol agent, it has to be mass-produced.
B. bassiana was raised in shake flask cultures to produce submerged conidiospores (SCS) which are reported to show a higher shelf life than mycelium and blastospores. In total, 23 technical culture media based on different carbon sources, minerals and technical yeast extracts were screened. Furthermore in mineral media with 5% sugar beet molasses *B. bassiana* produced 0.1 x 10 SCS/g sucrose until 170 h after inoculation (Lohse et al. 2014). By adding 50 g/L NaCl 48 h after inoculation the SCS yield was increased to 1.4 x 10 SCS/g sucrose. The scale-up to a 2 L stirred tank reactor was carried out in mineral media with 5% molasses at 25°C, 200-600 rpm and 1 vvm at pH 5.5. At the beginning of the fermentation the amount of dry biomass increased because the fungus produced mycelium. After 72 h the biomass dry weight decreased due to a critical pO_2 of 4 % which was accompanied by a visible reduction of mycelium. At this time the spore yield started to increase up to 7.6 x 10 spores/g sucrose at the end of the fermentation. However, the biomass consisted of more than 95 % blastospores (Figure 1a). A shift from blastospores to SCS was induced by the addition of NaCl which resulted in an increase of SCS yield to 2.4 x 10 SCS/g sucrose (Figure 1b).

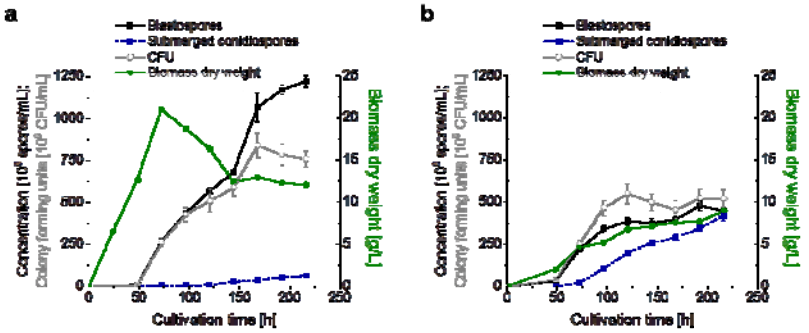

Fig. 1 Cultivation of *B. bassiana* in a 2 L stirred tank reactor. The figures show the mean (±SD) concentrations of blastospores and submerged conidiospores as well as the correlation of spore counts with biomass and mean (±SD) colony forming units. In each case, standard deviations resulted from two technical replicates. (**a**) Without osmotic stress. (**b**) With 50 g/L NaCl after 48 h.

To conclude, the endophytic *B. bassiana* isolate ATP-02 was cultivated to very high spore yields respectively to very high SCS yields without pelleting of the biomass. As other *B. bassiana* isolates can produce several metabolites like oxalate, oosporein and beauvericin, we want to examine whether *B. bassiana* ATP-02 is able to produce industrially relevant metabolites by investigation into the endophyte-plant interaction.

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102 - Biologischer Pflanzenschutz im Rapsanbau: Ein Versuch, den entomopathogenen Pilz *Beauveria bassiana* als Endophyt in Rapspflanzen zu etablieren

Biological control in oilseed rape: An attempt to establish the entomopathogenic fungus Beauveria bassiana as an endophyte in oilseed rape plants

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Mit der rasanten Ausweitung des Anbaus von Raps (*Brassica napus* L.) nehmen auch die Populationen der Schaderreger von Raps zu, im besonderen Rapsglanzkäfer und Rapsstängelbohrer. Das Ziel unserer Untersuchungen war daher, im Rahmen des Biologischen Pflanzenschutzes, den entomopathogenen Pilz *Beauveria bassiana* Naturalis ATCC74040 als Endophyt systemisch in Rapspflanzen zu etablieren. *B. bassiana*-Blastosporen (10 Sp/ml) aus Czapek-Flüssigmedium wurden in Rapsblätter infiltriert. Die Pflanzen wurden bei 80% RH und 20°C unter Langtagbedingungen gehalten. Zwischen 3 Tagen und 4 Wochen wurden Blattproben entnommen und qualitativ fluoreszenzmikroskopisch entweder mit Blankophor oder spezifisch mit polyklonalem Primärantikörper gegen die *B. bassiana*-Gesamtproteinfraktion untersucht (sowohl mit Rohserum als auch gereinigter IgG-Fraktion). Für den spezifischen PCR-Nachweis des verwendeten *B. bassiana*-Stammes wurden diagnostische Primer entworfen und verwendet, die an eine charakteristische Teilsequenz eines selbstspleissenden Gruppe I-Introns des 28S rRNA-kodierenden Gens von *B. bassiana* ATCC74040 binden („gli-Diagnose"). Während die Pilzbesiedlung der Epidermis ausgesprochen kräftig und persistent war, konnten nur vereinzelt Hyphen in Interzellularen des Blattes mikroskopisch nachgewiesen werden. Mittels PCR konnte *B. bassiana* Naturalis in Rapsgewebeproben erfolgreich nachgewiesen werden; ein eindeutiger molekularbiologischer Nachweis systemischen Wachstums innerhalb von Blättern steht noch aus. Mögliche pflanzliche Abwehrmechanismen werden diskutiert.

103 - Die Wirkung von endophytischen entomopathogenen Pilzen auf Phytohormone

The effect of endophytic entomopathogenic fungi on phytohormones

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In previous surveys we proved that successful colonization of an endophytic strain of the entomopathogenic fungus *Metarhizium anisopliae* contributes to a higher dry weight of the inoculated plants. We therefore assessed the amount of several phytohormones (ABA, SA, GA1, GA34, GA3, GA4, IAA, JA, SAG) aimed at understanding differences in the growth of control and inoculated tomato and cotton plants. The experiments were carried out under greenhouse conditions and each experiment consisted of three groups: control plants, seed coated plants with *Metarhizium anisopliae* 150 and seed coated plants with *Metarhizium anisopliae* 153. Samples of newly emerged leaves were then collected at 17.107 BBCH and 15 BBCH for tamato and cotton respectively and processed for a phytohormonal scan using LC-MS. The results demonstrated that tomato and

cotton plants react differently to their colonization with *Metarhizium anisopliae* by up-/down-regulating mainly SA, JA, ABA and IAA, these differences were also detected when comparing the different treatments of each experiment.

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104 - The role of stress-induced signaling proteins in endophyte induced defense responses against root-knot nematodes

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Endophytic fungi can stimulate defense responses in plants, thus making them more resistant towards specific pathogens, like nematodes. Although the exact molecular and biochemical mechanisms underlying this phenomenon is not clear, the use of split root experiments indicate that particular systemically induced defense responses are triggered. The endopytic *Fusarium oxysporum* isolate Fo162 causes a reduction in infection of the root-knot nematode, *Meloidogyne incognita*, in various plant species, including *Arabidopsis thaliana*. Because of this and the extensive molecular knowledge on Arabidopsis together with the availability of a significant number of well-characterized mutants, the role of particular defense signaling pathways in the induction of defense responses triggered by the endophyte can be studied in more detail. We therefore tested the Arabidopsis *oxi1*- and various *mpk*-mutants, which lack or over-express proteins that play a role at various levels in transferring the signal in the stress-induced defense pathway. All tested mutants were still capable of restricting *M. incognita* infection to the same level as the wild type when inoculated with Fo162, thus showing that these signaling proteins are not relevant for inducing nematode defense responses, either because other redundant signaling proteins can compensate for the lack or the particular signaling pathway is irrelevant.

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