Overview about the research of endophytes as biocontrol agents against phytopathogens

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Introduction:

This poster will not show any published experimental research results about different species of endophytes as biocontrol agents (BCAs).
The reality about the success of endophytic BCAs shows a unemotional picture. Many authors of papers write „the analysed endophytic isolate has a potential as biocontrol agent" - but where are further feasible analyses on the way there? Only *few* microbial biocontrol agents are on market or practicable. The main criticism of microbiological control is the lack of consistency. *In vitro* inoculation experiments and inoculations of plants under constant environmental conditions (like greenhouse conditions) can have a positive biocontrol effect but in field this biocontrol agents have no reproducible and constant positive effects mostly. In this case I want to give a little overview about interesting published facts, realistic handling problems, questions and personally comments from me about this topic. This informations could help us to think about more and more about our own work in isolation, screening, mass propagation, formulation and commercialization of *effective* and *competent* endophytes as biocontrol agents. You will find out that some informations are not very new. Otherwise there is not yet a solution of it. So we should discuss together about this really *complex* topic of biocontrol in our COST Action.
When we work about biocontrol agents we should have an imagination of the definition of the word „biocontrol" and „endophytes" at first. The published definitions about it are following:
„Biological control is the *reduction of inoculum density* or disease-producing activities of a pathogen or parasite in its active or dormant state, by one or more organisms, accomplished naturally or through manipulation of the environment, host, or antagonist, or by mass introduction of one or more antagonists."[1] Hereby we have to think carefully about truly functionality of biocontrol agents. Baker & Cook (1974) wrote: „Biological control *rarely eliminates* a pathogen from the site, but rather reduces its numbers or its ability to produce disease; such control may be achieved with little or no reduction in population of the pathogen, or perhaps without preventing infection."[1]
In the light of new distribution strategies of phytopathogens, such as soil-borne phytopathogens (*Verticillium*,..) they can live symptomless in *non-host plants* as endophytes and in host-plants as classical phytopathogens [2]. We should screen very accurate the isolates of endophytes which we use for biocontrol. It could be that the same isolate is an endophyte (as latent pathogen) in the plant species where we isolated it and otherwise a truly pathogen in another plant species. We can not exclude that the endophyte only colonize the plant species where we want to practice biocontrol. The flora which accompany this plant species could be also important for an colonization of endophytic biocontrol agent. It is not easy to discover the truly nature of endophytes in different natural habitats. Here there is also an open discussion about their definition. One possibly definition is the following:
"Endophytes are microorganisms (bacteria, fungi, unicellular eukaryotes), which can live at least part of their life cycle inter- or intracellularly inside of plants usually without inducing pathogenic symptoms. This can include competent, facultative, obligate, opportunistic and passenger endophytes. Endophytes can have several functions and/or may change function during their lifecycle." [3]

Core messages:

There are following important steps for the research about endophytes for biocontrol :

Isolation of putative competent endophytes [4, 5, 6, 7]

→ At first we have to ask us regarding in commercialization of endophytic biocontrol agent: Is there and how strong is the significant economic impact of target phytopathogen on the analysing plant? How strong is the propensity of phytopathogen to become resistant to chemical pesticides?
In order to isolate endophytes which can produce antimicrobial metabolites or can have other useful functions against phytopathogens following points should note:

- Isolate endophytes from plants growing in areas of great biodiversity
- Isolate endophytes from plants where the disease does not occur but should growing in neighborhood of plants which shows symptoms of plant diseases
- Isolate endophytes from appropriate plant parts, also from different developmental stages of plant
- Isolate endophytes under appropriate environmental conditions
- Avoid the use of highly selective isolation media; Around 90% of all microorganisms are not cultivable with classical nutrient media. Here we need more new isolation and cultivation media. One strategy could be to use media with virgin plant extracts because endophytes live and interact with plants
- Maximize the number of endophytic isolates and isolate endophytes from different places of same plant species which are characterized in different ecological conditions, so you have maybe a high diversity in different „ecotypes" of endophytes better adapted in environment.

→ Discovery and development of *endemic* rather than foreign endophytes for biocontrol agents reduce registration costs and increase chances for successful registration
You have to think about following:
Baker and Cook (1974) stated that „ antagonists should be sought in areas where the disease …does not occur, has declined, or cannot develop, despite the presence of a susceptible host…"[1]

Screening tests on different strategies of endophytes to control phytopathogens [8, 9, 10, 16]

In scientific literature we find a lot of informations about *dual culture plate assays* where endophytic isolates are analysed for antagonistic characteristics via microbiostatic or microbicidal metabolites. This tests are not suitable to predict in every case the positive antagonistic effects of endophytes in field. We have to interpretate the results of this *in vitro* test systems with caution.
This *in vitro* system has following disadvantages:
Endophytes can not be screened for competition, avirulence, cross protection and possibly induction of systemic resistance of plants. Abiotic (climate, soil conditions, humidity, pH) and biotic factors (several life stages of target pathogen, antagonistic organisms, soil microflora, microbiome of target plant) in field can not be simulated with this system.

Dual culture plate assays can also produce different results, depending in type of pathogen and antagonist, kind of screening medium, spatial arrangement of microorganisms, incubation period, temperature, production of volatile substances

Some questions came up by me during the reading of publications about this tests: When I isolate the microbial inhibited pathogenic cells from inhibition zone and I plate out it of nutrient medium for this organism. Can the microbial cells grow again in a normal matter? Is there any an unified method for interpretation of inhibition zones??? Is a visual inhibition of phytopathogens really a complete inhibition of growing/propagation of microbial cells?
Some publications show positive effects in inhibition of phytopathogen by endophytic isolate *in vitro* and also a reduction in plant disease in pot culture. Is the antibiotic effect from *in vitro* test also responsible for reduction of disease symptoms of phytopathogens in plant? It could be that the endophytic isolate owns a another strategy to reduce disease symptoms for example via interactions with other microorganisms of the plant. The antibiotic production by BCAs is also influenced by biotic and abiotic factors.

For endophytic biocontrol to succeed, we need to understand the mechanism(s) of action of BCA and the relationship of BCA to the microbiome of plant, the physical environment, and the plant. The aim here is that endophytic BCA is ecologically adapted to the target environment.

Screening tests in future should be more adapted to plants growing in field, *in vitro* tests should be designed with plant extracts or plant tissue. Pot experiments should not be established with surface sterilisated seeds and sterile soil because endophytes could be also influenced by plant- and soil associated microorganisms (like in field). Artificial inoculation experiments do not show the truly behaviour of endophytes which is important for practice (outside in the field). Inoculum density of BCA, population density, population diversity (and their possibly fluctuation) of pathogens and other microorganisms of plant should keep clearly in mind.

Can endophytes have also an impact on quorum sensing of pathogens?

A plant is like a „micro-ecosystem" –maybe a well regulated microflora is a base for ecological fitness and tolerance to phytopathogens. So endophytic BCAs could have an impact on plant microbiome.

Mass propagation, formulation and application of endophytes to plants [11, 12, 13, 14, 15]

Mass propagation (including many cycles of subcultivation) has the aim to induce a high biomass of endophytic strain. In many cases the nutrient media are very rich in components and not really adapted for improvement/remaining of quality of microorganisms. Quality that means genetically stability for productivity, remaining of infectivity and antagonistic behaviour of endophytes in order to be ecological fit. Artificial nutrient-rich media can also induce mutations by bacteria and a reduction of producing important metabolites. We need new recipes of media adapted to the host of endophyte - plant extracts in form of single isolated compounds from plant could be alternative.

An important step for application, storage, commercialization and field use is the formulation which should have following characteristics:
Increased shelf life, not to be phytotoxic to the plants, dissolve well in water and should release the endophytes, tolerate adverse environmental conditions, to be cost effective and give reliable control of plant diseases, to be compatible with other agrochemicals, carriers must be cheap and readily available for formulation development

In the most cases *single endophytic isolates* are applied for biocontrol of phytopathogens to plants but this strategy is mostly not effective enough in comparison to *mixtures* of different endophytic microorganisms. Consequently, a combination of active endophytic microorganisms would increase the genetic diversity of the biocontrol system and may more closely mimic the natural situation in the environment. The population of pathogenic organism presents a certain diversity and a single given strain of an endophytic BCA might not have the same efficiacy on all the pathotypes in the population. Otherwise single isolates of BCA have a relatively narrow spectrum of acitivity compared with synthetic pesticides, and farmers need to control several plant pathogens in the same crop. Endophytic BCAs should be also placed in a systemic approach of integrated production (Integrated pest management).

Commercialization of products [17]

Here a *constant high quality* of BCA product is essential especially for the user. There is no guide like „ Rules for a good quality control" which is needful for many companies which have no temporary and effectively quality control. *In vitro* produced BCA can change their behaviour and/or genetics for biocontrol or contaminations can occur in production system.
It takes a long time – many years – to have a BCA-product which is suitable for using in environment. It should be safe in handling by humans and also in distribution and function in environment. Hereby the registration can be long and cost-intensive. Different EU contries have also different registration processes.
Single strains of following bacterial anf fungal species with endophytic characteristis are on the market: *Bacillus subtilis*, *Agrobacterium radiobacter, Pseudomonas fluorescens, Streptomyces lydicus, Gliocladium virens*, *Trichoderma harzianum*, non-pathogenic *Fusarium oxysporum,* fungal grass endophytes.

Further remarks for the future of biocontrol Agents [18]

Table 4: Some current topics of biocontrol research and development and associated questions:

1. The ecology of plant-associated microbes
   How are pathogens and their antagonists distributed in the environment?
   Under what conditions do biocontrol agents exert their suppressive capacities?
   How do native and introduced populations respond to different management practices?
   What determines successful colonization and expression of biocontrol traits?
   What are the components and dynamics of plant host defense induction?

2. Application of current strains/inoculant strategies
   Can more effective strains or strain variants be found for current applications?
   Will genetic engineering of microbes and plants be useful for enhancing biocontrol?
   How can formulations be used to enhance activities of known biocontrol agents?

3. Discovering novel strains and mechanisms of action
   Can previously uncharacterized microbes act as biological control agents?
   What other genes and gene products are involved in pathogen suppression?
   Which novel strain combinations work more effectively than individual agents?
   Which signal molecules of plant and microbial origin regulate the expression of biocontrol traits by different agents?

4. Practical integration into agricultural systems
   Which production systems can most benefit from biocontrol for disease management?
   Which biocontrol strategies best fit with other IPM system components?
   Can effective biocontrol-cultivar combinations be developed by plant breeders?

→ We need also a new publication policy: It should be more and more possible to publish *negative* experimental results in the research of microbial biocontrol agents in order to understand better their enorm complexicity in the light of nature of organisms and environment and to push up the progress in development of BCAs.

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