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Screening criteria for the development of commercial products for biocontrol of plant pathogens

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Abstract: Antagonists for use in commercial biocontrol products have to fulfil many different requirements. Besides being active control agents against the specific targeted plant pathogens, they must be safe and cost effective. The development of new biocontrol products starts with screening programs including hundreds or thousands of candidates. For commercial use, important criteria are market size, efficacy, ecological characteristics, production costs, safety, environmental risks and protection of intellectual property rights. A stepwise screening considering these very different aspects is proposed.

Key words: antagonist screening, biological control, commercialisation

Selection criteria

Programs for screening antagonists for disease control of plant pathogens are often focused on testing antagonistic properties *in vitro*, in bioassays and subsequently in crops. For commercial use, however, antagonists must fulfil many more criteria. Besides the toxicological profile of an antagonist, industries will consider technologies for production and formulation and their costs, genetic stability of the antagonist, market size for the biocontrol product and the possibilities of patent protection for the application (Whitesides *et al*., 1994; Köhl, 2010). A list of criteria for antagonist screening considering such criteria for commercial use has been developed within the EU-project ENDURE. Various criteria are ranked in a stepwise approach to exclude unwanted candidates in early screening steps using inexpensive tests. Consequently, fewer candidates have to be tested in later screening steps when more expensive assessments have to be done.

At the beginning, targeted crops and diseases and the resulting market size for a product is evaluated. Origin of antagonists and isolation techniques are carefully chosen to obtain candidates with the relevant ecological characteristics. In a first rapid throughput screening, simple tests are carried out to exclude candidates which, for example, produce not sufficient inoculum or show no cold-tolerance. After identification at species level, information is collected by data mining in relevant data bases. Species with unwanted toxicological or ecological profiles are excluded, but also patent and marketing aspects are considered. The antagonistic potential of the pre-selected set of candidates is subsequently tested in bio-assays on pathogen-inoculated plants or plant parts. In parallel, mass production of candidates is tested on agar plates or already in small fermenters. Only for a small set of antagonists, tests in crops will follow. Feasibility of up-scaled mass production, formulation and shelf life will

be tested again in parallel for selected candidates. Consequently, only antagonists, which fulfil the major criteria for commercial use will be tested in field experiments using already suitable pilot-formulations.

A case study: Biocontrol of apple scab

Venturia inaequalis can cause apple scab as the major disease in apple production, infecting leaves and fruits during the entire growing period resulting in losses in yield and reduced fruit quality. During summer, the epidemic is driven by conidia produced by the biotrophic pathogen on infected leaves in several infection-sporulation cycles. We initiated a screening program aimed at the selection of antagonists which suppress conidia production of *V. inaequalis* (Köhl *et al*., 2009). Candidate antagonists were isolated from scabbed leaves. Before candidates were tested in time-consuming and expensive bioassays using apple seedlings, a pre-screening was carried out to reduce the number of candidates and to increase the chance that the finally selected isolates will meet requirements for commercial use in practice. Mass production, ecological competence and safety were considered. Isolates of the genera *Aspergillus*, *Penicillium* or *Fusarium* were discarded because various species within these genera have a potential to produce mycotoxins. Remaining fungal isolates were cultured on oat meal agar, comparable to cereal-based media used in industrial solid state fermentation. Isolates which produced less than 10^5 spores per plate were not further evaluated. In the next steps, isolates which grew at 36°C were excluded from further screening. Only cold tolerant isolates, growing at 5°C and drought-tolerant isolates growing at -10MPa were considered for further screening steps. Applying these rapid tests lead to the exclusion of approximately 33% of the isolates. Seedling assays were carried out to assess the antagonism of the pre-selected candidate antagonists against *V. inaequalis*. Young leaves were inoculated with the pathogen, followed by applications of spore suspensions of antagonists. Conidiation of the pathogen was quantified after incubation of seedlings for 9-12 days. A few superior antagonists were selected and subsequently tested under orchard conditions. Isolate *Cladoporium cladosporioides* H39, already applied in a pilot formulation as water dispersible powder (WP), significantly reduced conidia production of *V. inaequalis* during apple scab summer epidemics. The antagonist is currently further investigated in orchard experiments.

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