Rezensionen

Recension of Stuart Card to: Eckhard Koch, Petra Zink, Cornelia I. Ullrich, Regina G. Kleespies, 2018: Light microscopic studies on the development of *Beauveria bassiana* and other putative endophytes in leaf tissues. Journal für Kulturpflanzen 70 (3), 94-106, DOI: 10.1399/JFK.2018.03.02

Microscopic organisms associate with the majority of plant species found on planet Earth, including those in wild and managed ecosystems. Plant associated microorganisms are no longer viewed purely as agents of disease but are now considered by many researchers as intrinsic plant-partners with fossil evidence indicating their role in early plant transitions from aquatic to terrestrial environments (Remy et al., 1994; Taylor et al., 1995). Furthermore a particular group of microbial species, termed endophytes, are regarded as extremely important plant symbionts that can directly or indirectly improve plant growth, increase yield, reduce disease symptoms caused by plant pathogens, improve plant performance under low water availability and heavy metal pollution, solubilise phosphate and contribute assimilable nitrogen to their hosts and deter herbivorous insects and animals. Some researchers now believe that the low stress tolerance exhibited by many axenic plants is partly due to the absence of mutualistic endophytes (Hallmann et al., 1997). However, it is only recently that these endophytic microorganisms, including their bioactive products, have received deserving attention from the scientific community. Since then, a multitude of manuscripts have been published on this fascinating and intriguing subject. These papers cover the full breadth of scientific endeavours from the discovery of new species and behaviours to investigating fundamental biological processes to the development and commercialisation of endophyte products.

There have been some excellent published articles that discuss the evolution and current use of the term endophyte (Mercado-Blanco, 2015; Sinclair and Cerkauskas, 1996; Wennström, 1994; Wilson, 1995). I strongly encourage new researchers to the subject to read these insightful articles. As biologists we generally like to classify the organisms we study, whether it be to use taxonomy to give the organism a species name or to describe an organism's behaviour. This, of course is very helpful when communicating our findings to our peers and the wider scientific community. But, as for the term endophyte, originally coined in 1866 by the German plant pathologist de Bary to simply mean inside the plant, the danger comes when a term is misused, it is used alone as a label without describing other behavioural traits or the particular part of the lifecycle that was observed or becomes vague and meaningless as more knowledge is gained around the overall scientific concept. For instance if used according to its original definition, the term endophyte incorporates all plant pathogenic and saprophytic fungi as they all, at some point in their lifecycle, penetrate and colonise parts of their host plant's tissue. I fully applaud those authors who use such terms as potential-endophytes or putative-endophytes for their preliminary investigations and then return to their system to undertake a detailed microscopy exploration to observe and further elucidate the intimacy of their plant-microbial interaction of choice. Introducing new scientific language to students or researchers new to a particular field can create confusion. Therefore careful thought needs to be given in the use of scientific terms as these are often more precise and consistent than everyday communication.

Too many published articles exist in the literature describing certain microbial species as endophytic when the methods employed to gain this knowledge, commonly surface disinfection of plant material followed by direct isolation of microorganisms onto artificial media or PCR to confirm presence of the species, were clearly limited and the necessary microscopy-driven investigations overlooked. This may be in part due to the equipment limitations of the laboratory where the work was undertaken and/or due to the researcher who may lack microscopy experience. Whatever the reason, this is unfortunate as with the advent of new, powerful imaging technologies, such as confocal microscopy, researchers are now able to observe and capture three dimensional images of their chosen microorganism in planta like never before in the history of the discipline. In addition, when many of these techniques (such as agronomy, microscopy, traditional microbiology, chemistry, plant physiology and molecular biology) are brought together in unison, this multidisciplinary approach can not only help decide whether the observed microorganism is colonising the plant tissue in question as a mutualistic endophyte, dormant pathogen or saprophyte but also help to explain how the microbe first entered the plant material, how it is disseminated and answer a plethora of other important questions regarding the symbiosis. This approach will also help the researcher understand where the limitations are with regard to the techniques they employ.

The paper by Koch et al., boldly sets out to critically investigate whether a number of key fungal species used in biological control (i.e. *Beauveria bassiana*, *Metarhizium anisopliae* and *Trichoderma harzianum*) can systemically colonise economically important plants, including oil seed rape and maize. These fungal species have previously been termed as endophytes in the literature but does this terminology accurately describe them? The JKI team utilised traditional staining techniques and after clearing they observed fungal structures in planta using light microscopy. Although more sophisticated methods are available, these techniques are still highly valued by those in plant pathology circles and the images, as presented in the paper, is evidence of some very carefully planned experiments. As technology drives much of the biological sciences, we must not forget the importance of some of our more traditional approaches and this manuscript is a great introduction and reminder to students and many current researchers of the importance of microscopy to actually visualize your particular plant-microbe situation. The manuscript also highlights the importance of fully understanding your plant system as the authors utilised their great understanding of plant anatomy to critically assess their observations. This is crucial to the study of those microorganisms highly associated with plants. The authors were able to decipher the exact mechanism of entry into the host (whether directly though the leaf epidermis or via the stomatal opening), assess the extent of leaf colonisation and determine whether fungal colonisation was met with a typical host reaction.

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