Endophytes as alternative paclitaxel sources

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Chapter 7

Summary

Concluding remarks & future perspectives

Whether suffering a pathogenic attack, basking in symbiotic comfort, or seemingly symptomless, plants constantly participate in molecular interplay with various classes of microbial organisms. One of the means of interorganismal communication in this dynamic continuum are secondary metabolites. The chemical diversity bearing pharmaceutical potential thus implied reaches beyond the plant kingdom and offers an expended view promising to transform glimpses of reductionist research of the past years to snapshots of an exuberant world of systems biology. Endophytes seem to fit perfectly into this natural 'warehouse', only a small part of which we have been able to tap into so far.

The introductory section of the hereby presented thesis (**chapter 2**) provides an elaborate overview on the current state of knowledge about endophytic organisms – microbes colonizing internal tissues of all plant species, creating a huge biodiversity with yet unknown novel natural products presumed to push forward the frontiers of drug discovery (Staniek *et al*., 2008).

Paclitaxel, the world's first billion dollar anticancer blockbuster, was primarily obtained from *Taxus brevifolia*. While the search for alternative sources of the powerful antineoplastic agent brought an array of reports on paclitaxel producing endophytes, causing quite a controversy over the past two decades, the world's market still relies on yew-derived supply of the valuable diterpene.

The primary objective of the research presented in this thesis is to investigate the potential of endophytes as alternative paclitaxel sources in all its intriguing aspects. On one hand, we strive to dissipate the ambiguity surrounding the very first presumed endophytic paclitaxel producer, *Taxomyces andreanae*, harboured by the original yew synthesizer of the drug (Stierle *et al*., 1993) (**chapter 3**). On the other, prompted by the extraordinary findings of recent years (Hill, 1996; Strobel *et al*., 1997), we describe the isolation and screening of the endophytic flora of an ancient conifer *Wollemia nobilis* in search for other paclitaxel producing specimens (**chapter 6**). In parallel to utilizing chromatographic, spectroscopic and immunoenzymatic detection methods prevalent in literature to confirm taxane presence in endophytic cultures, our research was brought to an alternative level addressing the question of molecular blueprint for paclitaxel production being an inherent genetic trait of the fungal isolates. Our PCR-based, in-depth study of *taxadiene synthase* and *baccatin III phenylpropanoyl transferase* as target genes not only bears clear implications for paclitaxel biosynthetic pathway elucidation

in the endophytes, but also allows to speculate on the origins of fungal taxanes. On one hand, fungi might be an independently evolved system for paclitaxel production. However, the fact that the biosynthesis of this highly functionalized and unique diterpenoid *in planta* involves approximately 20 genes (Walker & Croteau, 2001) makes this rather unlikely. Therefore, it seems plausible that a horizontal transfer of genetic information shaped the evolutionary trajectories of taxonomically unrelated, yet co-existing, species and further influenced the dispersal of endophytic taxane synthesizers in a given ecosystem and, indeed, worldwide (Staniek *et al*., 2009; Staniek *et al*., 2010b).

Moreover, postulating horizontal gene transfer to be a driving force in the evolution of fungal gene clusters – a phenomenon now considered a hallmark characteristic of secondary metabolic biosynthetic pathways (Keller & Hohn, 1997; Keller *et al*., 2005), rises an intriguing question as to whether the genes responsible for paclitaxel formation in *Taxomyces andreanae* and other presumed endophytic taxane producers are indeed grouped in a contiguous cluster. Equipped with this essential genetic information and modern tools to manipulate the biosynthetic machinery, the research on microbial paclitaxel synthesizers could enter a novel combinatorial stage. While several heterologous systems including *Escherichia coli* (Huang *et al*., 2001), *Saccharomyces cerevisiae* (DeJong *et al*., 2006; Engels *et al*., 2008) and *Pichia pastoris* (Schmeer & Jennewein, 2009) were already exploited for expression of plant-derived genes encoding early paclitaxel biosynthetic enzymes, to engineer and co-mobilise a functional gene cluster in a parent producer microorganism affords the advantage of all the regulatory elements being present and functional. This undoubtedly promising approach seems all the more challenging in view of our further findings concerning pure *Taxomyces andreanae* strain. Namely, despite considerable up-scaling endeavours, metabolic profiling of the commercial isolate brought no confirmation of endophytic paclitaxel production in axenic culture conditions. This result suggests that specific plant environment may be required for the induction of paclitaxel biosynthetic genes in the fungal symbiont. Identifying these triggering mechanisms will be of considerable future interest, not only providing further insight into the true nature of the fine-tuned equilibrium of plant-microbe interactions, but also revealing their tremendous therapeutical potential. In fact, a recent report communicates an unprecedented endeavour to re-establish the intriguing co-habitat by proposing a promising co-culture system for *Taxus chinensis* var. *mairei* and its endophyte *Fusarium mairei*

(Li *et al*., 2009). The next challenge lies in the further integration of these approaches to develop a comprehensive picture of how life history traits of both 'players' interact with the environment to shape evolutionary trajectories (Burdon & Thrall, 2009).

Wollemia nobilis, the aforementioned host of endophytic paclitaxel synthesizers, is an exciting object of investigation *per se*. Its discovery in 1994, hailed as the 'botanical find of the century', opened a window into an unimaginably ancient past – a great opportunity of studying the conifer thought to have been extinct for millions of years. **Chapter 4** describes our efforts to obtain callus and cell suspension cultures of Wollemi pine. While ultimately unattained, despite extensive optimization attempts, the aim should not be abandoned as it could prove a milestone in further unravelling of the botanical enigma of 'the living fossil'. In **chapter 5** we turn our attention to the chemistry of *Wollemia nobilis*, proposing a comparative analysis of the essential oil constituents derived form its different organs, namely leaves and twigs. The results obtained allow speculation on the fundamentals of the formation of 'active isoprene units' – basic C_5 terpene building blocks, proceeding via two alternative pathways. While the long known mevalonate (MVA) pathway, localized in the cytosol, provides the isoprene units for sesquiterpene biosynthesis, the plastid-localized methylerythritol phosphate (MEP) pathway, discovered and investigated only in recent years by Rohmer *et al*. (known, therefore, also as the *Rohmer pathway*) is thought to feed the biosynthesis of mono- and diterpenoids. The aforementioned cell-compartmentation seems to be in accord with the postulated direct light interference with the non-mevalonate pathway, and consequently with the obviously prevalent cross-talk between the cytoplasmic and plastidial biosynthetic routes. Hence the significantly higher amounts of sesquiterpenoids observed in the plant organs less photosynthetically active and less exposed to the light than the foliage boasting the abundance of mono- and diterpenes (Staniek *et al*., 2010a).

In summary, the research presented in this thesis investigates the potential of endophytes as yet untapped, prolific source of pharmaceutically relevant natural products, with an unequivocal attention focused on paclitaxel. As the highly desirable search for sustainable and economically feasible sources of this excellent antitumour agent has tempted various authors to draw premature conclusions proclaiming endophytes to be independent taxane bio-factories, we contend that the answer to the issue of paclitaxel supply crisis might lie within the interplay between the plant hosts (often them-

selves unique, like the relictual Wollemi pine) and their microbial inhabitants, under evolutionary and environmental control. While one has to be mindful that the problem we set out to address is several orders of magnitude larger than those with which we are familiar, no one can deny the opportunities that present themselves in the era of modern functional genomics and systems biology.

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