Investigations on secondary metabolites of lichens and lichen mycobionts in culture: production and biological activity

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Lichen fungi produce a great variety of secondary metabolites, including aliphatic, cycloaliphatic, aromatic and terpenic substances. Lichen secondary metabolites, especially polymalonyl derived polyketides, have been screened for manifold biological activities, e.g. antiviral, antibiotic, antitumor and antiherbivor issues (HUNECK 1999, 2001; DAYAN & ROMAGNI 2001; MÜLLER 2001). They can also inhibit growth of higher plants. Culture experiments in recent years have shown that optimised culture conditions result in the formation of fats and fatty acids instead of secondary compounds. A sophisticated interaction of environmental factors, present nutrients and morphological complexity of the lichen influence the induction of secondary metabolite formation and therefore the production of particular lichen substances in culture. Mycobionts of various lichen species (e.g. *Heterodea muelleri, Lecanora rupicola, Xanthoria elegans, Cladonia leporina)* were isolated and cultured by means of single spore (AHMADJIAN 1973) and tissue culture (YAMAMOTO 1990) methods. Then, well grown subcultures were exposed to stress factors, e.g. shortwave UV and cold temperature. Lichen thalli and cultured mycobionts were analysed by means of HPLC and TLC to detect the secondary chemistry profile. DNA-analyses of the ITS region were performed to assure mycobiont authenticity. Exposing mycobiont cultures to the stress parameters induced the production of secondary compounds in higher quantities but in some cases different ratios. A further series of experiments focused on the effect of diffractaic and barbatic acids on the growth of the symbiotic photobiont *Trebouxia jamesii*; usnic acid was similarly tested. Pure substances were obtained from mycobiont cultures by performing a "preparative TLC" A determined quantity of algae was incubated on BBM plates containing three different concentrations of the pure lichen metabolites. The growth of the photobionts was monitored over a period of one month to evaluate the impact of each substance on the cultured algae. While diffractaic and usnic acid

had no noticeable effect, barbatic acid strongly inhibited algal growth and resulted in cell death.

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