Chapter 2
Objective

Due to the silencing of many natural toxins under common growth conditions, a robust and unambiguous technique is required to screen different fermentation approaches and identify the defined molecular trigger for the secretion of metabolites that affect biological maintenance. Once a suitable condition is established, the downstream purification and characterization of the respective secondary metabolite is a straightforward process. With regard to the plentitude of identified natural proteasome inhibitor classes and the conserved character of the UPS in various species, it is likely that the application of such a technique will yield many novel and auspicious lead structures.

Therefore, the aim of this thesis was the development of a methodology to unambiguously detect proteasome-inhibiting agents in crude culture broths. In order to avoid all biochemical artifacts, the assay was intended to be based on a natural and subunit-specific proteasome substrate. With regard to the unreliability of common proteasome assays in heterogeneous mixtures, the substrate was devised to enable NMR readout by regiospecific atom labeling.

After the establishment of a suitable peptide sequence, the assay was to be verified by analyzing media of *P. syringae* containing defined quantities of the irreversible syrbactin inhibitor SylA in order to probe the detection limits and analyze the signal-to-noise ratio of the technique. In turn, the developed tool was envisioned to be applied for the analyses of cultures derived from other organisms suggested to produce syrbactins due to genetic similarities with *P. syringae*. As the group of syrbactins comprises the extremely potent GlbA, it was likely that the isolation of the respective molecules would identify other at least equally potent compounds and grant deeper insight into their binding mechanisms. From the list of potential organisms, it was intended to analyze cultures of *P. luminescens* because the S1 insect pathogen can be handled easily especially compared to the equally promising but highly virulent *Burkholderia* species. Moreover, it was described that *P. luminescens* produces intensely colorful substances and would therefore represent a case of hardship for the approval of the developed technique.

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Due to the silencing of the respective gene cluster in common cultures of the bacterium, the assay was to be utilized for the identification of a suitable growth condition to trigger the biosynthesis of the toxin. The major purpose of the research project was to pave the way for the isolation and structural characterization of the proteasome inhibiting compound from natural source. Subsequently, it was aspired to investigate the molecular binding mechanism and in vivo potency of the natural compound by the means of crystallographic complex structure analysis with the 20S proteasome as well as cell culture experiments for the evaluation of cytotoxicity and intracellular pathway affection.

In summary, the introduction of an orthogonal methodology into the field of proteasome assays aimed to disclose the broad range of still undiscovered inhibitors by offering a methodology for their detection already during the fermentation procedure of the organism. Exemplifying the approach on a real-case scenario, it was envisioned to pursue the whole process from the detection to the isolation of the inhibitor and eventually characterize its in vitro and in vivo properties by performing multidisciplinary experiments.

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