Recombinant expression of Asparaginyl Endopeptidase from Rice in *Pichia pastoris* – a putative candidate for production of cyclic antimicrobial peptides

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The classes of AMPs we are interested in are rich in cysteines such as defensins, thionins, and also cyclic peptides called cyclotides. The cyclotides are families of backbone-cyclized cystine-knot-containing peptides from plants that possess the ability to kill vide range of microorganisms. Cyclic AMPs in general show higher stability and kill more efficiently. Some of them display even activity towards viruses and cancer cells.

Due to an increasing number of multi-resistant pathogens AMPs became highly interesting during the last decade. Some big pharmaceutical industries have started to investigate and produce AMPs. However, production can be costly and time consuming if done by conventional sold phase peptide synthesis. A cheaper alternative solution would be expression of the gene encoded AMP recombinant in microorganisms. However, recombinant production of backbone-cyclized AMPs is still a problem.

It is known that asparaginyl endopeptidases (AEP) are involved in the biosynthesis of cyclotides in plants. However, the precise mechanism is still unknown. In this project we want to study a asparaginyl endopeptidase from rice since rice is known to make cyclotides. In order to investigate the enzyme we need to express the cDNA genes (which we have already received) of the AEP from rice recombinant in *Pichia pastoris*.

If successful we will have produced the endopeptidase in a non-active form until pH is shifted to acidic. Since the AMP is secreted into the fermentation medium in its mostly inactive form it will not have a toxic effect on the expression host which should be beneficial for reaching a high production level.

If times allow it would be very interesting to test if the processed peptidase is not only cleaving peptide bond but also catalyses bond formation.