Novel L-asparaginases as potential therapeutic agents and antimicrobial targets: structural and functional studies of enzymes with dual implications for drug design

L-Asparaginases are enzymes (i.e. biocatalysts) that catalyze a very simple reaction: splitting of the amino acid L-asparagine to L-aspartate and ammonia. Through a series of serendipitous experiments, it was established that when L-asparaginase is injected to the blood of a **leukemia patient**, certain types of leukemia, most notably **acute childhood leukemia, can be cured with nearly 100% efficiency**. This effect was explained by noting that some cancer cells are unable to synthesize the non-essential nutrient, L-asparagine. Thus, when the injected L-asparaginase clears the circulating pools of this nutrient, healthy cells will survive, while cancer cells, at prolonged treatment, will be starved to death. However, the currently administrated bacterial asparaginases cause serious side effects, thus new therapeutic asparaginases are urgently needed.

Recently, we have characterized a novel and unique L-asparaginase from the soil bacterium *Rhizobium etli*, which fixes atmospheric nitrogen in symbiosis with legume plants. Our preliminary results suggest a completely new catalytic mechanism and open up exciting avenues for further research, including not only search for enzymes of **medicinal importance** (**antileukemic agents**) but also studies of macromolecular targets of **economic interests** (**potential antifungal targets in agriculture**) (Fig. 1). The latter aspect is connected with our observation that asparaginases similar to *Rhizobium etli* enzymes are selectively present in some pathogenic fungi that cause both serious **human infections** as well as catastrophic plant diseases **devastating plantations worldwide**. Identification of potential inhibitors of such enzymes will foster the development of **new classes of fungicides** that can help fighting fungal pathogens and save many crops that are **essential for human diet** (potato, bananas, tomatoes, apples, etc.). This is an urgent issue because **climate changes** often lead to emergence of serious natural disasters destroying large-scale plantations. Moreover, the increase of global temperature often results in the migration of pathogenic species from their local habitats to other parts of the world.

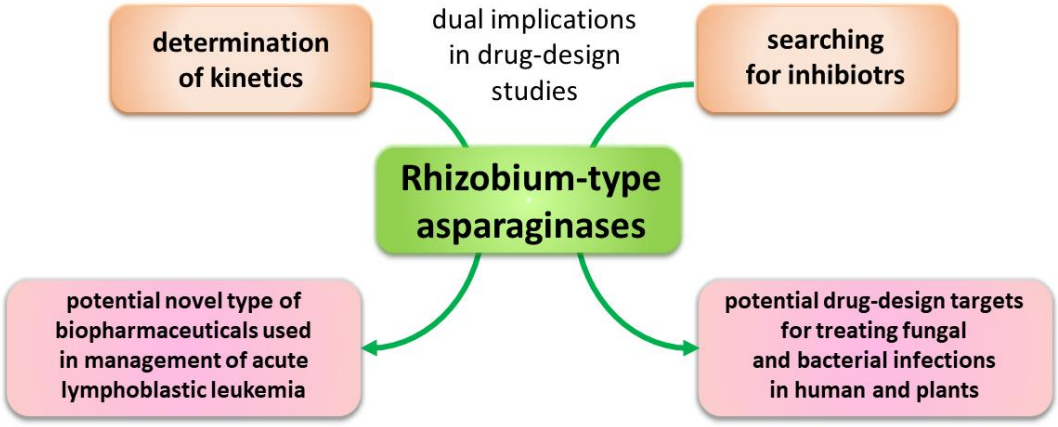

Fig. 1. Flowchart of the project goals and explanation of the dual role of asparaginases in drug design studies: as enzymes used as bio-drugs for the treatment of leukemia, and as targets for antifungal compounds (inhibitors) for combating diseases caused by pathogenic fungi.

The **aims of this project comprise full structural and functional characterization** of selected novel Rhizobium-type asparaginases: the **model enzymes** from *Rhizobium etli* (ReAI and ReAII) and homologs of ReAII present in some **pathogenic fungi**. The results will elucidate the details of the catalytic mechanism probing the new enzymes as **new antileukemic agents**, with improved efficiency and **minimized adverse side effects**. This is, however, not the only potential of Rhizobium-type asparaginases. In the second lane of our research, we will be searching for their inhibitors, hoping to find promising compounds that could be developed into **fungicides for combating pathogenic fungi of people and agricultural crops**. Our research will significantly advance structural biology, biochemistry, enzymology, and pharmacology.