# ABSTRACT

RTX (Repeat in ToXin) superfamily consists of many proteins divided into several groups according to their different functions and characteristics: toxins, metalloproteases, lipases, proteins of the S-layer, bacteriocins and proteins with unknown function. However, all of them can be characterized by the following features: i) they contain tandemly repeated (6-50) nonapeptide glycine-rich calcium-binding consensus sequences GGXGXDX[L/I/V/W/Y/F]X (where X is any amino acid residue) in the C-terminal part of the protein. The presence of these repeats is a *sine qua non* condition for RTX protein family membership; ii) secretion from the cell occurs without a periplasmic intermediate by a mechanism which involves recognition of a signal sequence at the C-terminus of the protein by membrane-associated proteins that export the toxin across a channel spanning the entire bacterial envelope directly to the outside of the cell (Type I Secretion System); iii) the genes for protein synthesis, activation and secretion are mostly grouped together on the chromosome and form *rtx* operons. RTX toxins are the largest protein group of the RTX family. To this group belong mostly the proteins with molecular weight ranging from 100 to 200 kDa, with posttranslational fatty acid acylation mediated by a specific activating protein. Posttranslational modification is crucial for the activation of the toxin and its function, in that it allows close contact of the toxin with the cytoplasmic membrane of host cells. The contact can be realized in two different, but not necessarily independent manners: i) reversible passive adhesion, followed by the exposure of the hydrophobic core of the toxin to the lipidic membrane and formation of a transmembrane pore, accompanied with irreversible conformational change in the toxin structure; ii) binding to the specific receptor and subsequent insertion into cell membrane and eventual internalisation of the toxin by receptor-mediated endocytosis.

Most RTX toxins create pores in the cell membrane of target cells, provoking eventual cell death (apoptosis or necrosis) and they appear to require the presence of calcium ions for cytotoxic activity. The toxins exhibit various modes of action and vary in target cell specificity.

RTX toxins can be further divided into two categories based on their target cell specificity (cytotoxins and leukotoxins). It is also known that they play an important role in specificity of individual pathogens to the various host organisms. RTX toxins are produced by a broad range of Gram-negative bacteria, including the genera of *Neisseria, Actinobacillus, Bordetella, Escherichia, Klebsiella, Moraxella, Vibrio*, etc. *In vitro*, RTX toxins display mainly cytotoxic and often also hemolytic activity. From the immunological point of view, RTX toxins are a valuable immunoprophylactic tool, while being mostly one of the main virulence factors of given pathogenic bacteria and having the ability to induce complex immune responses of the host that also lead to antibody production.

This doctoral thesis is devoted to studies on selected RTX proteins, analyzing their biochemistry of action and use as antigens for immunoprophylactic vaccination.



**Keywords:** *Actinobacillus pleuropneumoniae*, ApfA, Apx toxins, FrpC, immunity, cloning, meningococcal infection, mutagenesis, *Neisseria meningitidis*, pleuropneumonia, antibodies, purification, recombinant proteins, SPM, protein cleavage, TbpB, vaccine, calcium.