PE 22 - Secretion of Chitosanases

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All known chitosanases are extracellular enzymes. Their amino acid sequences are tagged by a signal peptide that directs the nascent protein to the secretion apparatus. Most chitosanases are secreted via the SEC pathway. Their signal peptides consist of three domains: a positively charged N-terminal domain, a hydrophobic core region and a C-terminal end including a recognition site for cleavage by a signal peptidase.
Examination of putative chitosanase sequences found in fully sequenced genomes revealed that two sequences from streptomycetes, SCO0677 from *S. coelicolor* A3(2) and SAV2015 from S. avermitilis have a twin-arginine motif in their signal sequences. These motifs direct the proteins to the recently discovered TAT pathway of secretion, thought to be dedicated to the translocation of fully folded proteins.
We compared the secretory properties of SCO0677 chitosanase with the enzyme from *Streptomyces sp.* N174, one of the best known chitosanases. Chimaeric genes were constructed linking the segments encoding the mature parts of both proteins to SEC-type or TAT-type signal peptides and expressed in Streptomyces lividans. The production level was monitored and the proteins have been purified.
Both proteins were secreted more efficiently when directed by their original-type signal peptide. However, once secreted by the "right" pathway or the "wrong" pathway and purified, the enzymes had the same specific activity. The type of signal peptide influenced the production level but not the activity. A whole series of TAT signal peptides (first extracted in silico from the *S. coelicolor* genome) has been tested with SCO0677 chitosanase. A signal peptide from the SCO0624 protein of unknown function directed the most efficient secretion of SCO0677 chitosanase. Implications of these results for biotechnology will be discussed.