

SEARCH FOR BIOACTIVE MOLECULES IN AZORES

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Abstract

CIRN is involved in a project for search and valorisation of bioactive molecules produced by different organisms in Azores and also in a project for the identification of pharmacogenetically relevant polymorphisms in the azorean population.

1) Search for natural products

This search was initially carried out in terrestrial plants, and more recently has also began to include marine organisms, such as macroalgae and sponges. We are particularly interested in compounds with antitumour activity, although other bioactivities, such as antimicrobial, antioxidant and insecticidal have also been studied.

Among the most relevant results are the *in vitro* antitumour activity of compounds isolated from endemic plants *Euphorbia stygiana* [1] and *Juniperus brevifolia* [2]. We are currently studying the mechanisms of action of these compounds. Concerning the studies with macroalgae, extracts with a pronounced cytotoxicity towards tumour cell lines have also been found. Sponge extracts displaying cytotoxicity have also been found, and are also currently being studied.

2) Search for proteases and encoding genes

Currently we have a large collection of microorganisms isolated mostly from soil, but also from volcanic events. Our research had focused essentially an insect parasitic nematode, which parasitic phase releases a large set of proteins that are active in the host. We are using genomic and proteomic approaches to analyse the released proteins. An express sequence tags database of the parasitic phase was performed with about 10 000 clones. 2 500 were sequenced and 86% identified [3,4]. On the other hand, secreted / excreted proteins (SP) were separated by 2DE and 84 spots were identified. Proteases represent about 90% of the identified proteins but there are others such as one protease inhibitor. Assays *in situ* hybridization showed that these proteases were expressed *de novo* by the nematode parasitic stage, thus suggesting they were triggered by the host presence. Moreover the expression of all of them was life cycle dependent as shown by qRT-PCR. These finds suggested that these proteins have a specific function in the parasitic process. Four proteases belonging to serine proteases have been purified and full cDNAs sequenced. Studies in the biological activities of these proteases proved they had a role in the pathogenic process. Sc-Chym caused an important reduction of pro-phenoloxidase activity thus blocking the recognition of foreign bodies by

the host [5]; Sc-Tryp was reducing the ability of haemocytes to recognize invaders by modifying the cytoskeleton [6]; Sc-SP-1 caused destruction of the basal membrane of mid-gut epithelial tissue; and Sc-SP-3 was shown to be toxic against *Sf9* insect cells by inducing cell apoptosis [7]. Bioinformatic analysis of Sc-SP-3 showed that, beside the catalytic domain, this protease had a CCP domain, which was predicted to interact with cell specific targets. Another full cDNA encoding a metalloprotease was sequenced. In this protease it was predicted a catalytic and a ShKT domain. The last is an ion channel toxin with high homology with anemones channel toxins.

In what concerns the identification of active peptides in *Bacillus thuringiensis* azorean isolates we identified 79 spherical, 190 bipyramidal and one rod shape isolates. These isolates have been checked for insecticidal activities and for the presence of the corresponding *cry* genes. So far five isolates were detected with insecticidal activity but without the corresponding *cry* genes, thus suggesting we are dealing with new *cry* genes. So far any isolate was shown to have *cyt* genes however preliminary assays showed two isolates have peptides inducing cytotoxicity. Again this find suggests we are in presence of new peptides.

3) Search for pharmacogenetically relevant polymorphisms in the azorean population.

The efficacy of most drug treatments can be improved if taking into account genomic variation, both in the context of the pipeline of drug development as well as the clinical prescription of medications according to the patient's constitution. Many pharmacologically relevant DNA polymorphisms show variable allele frequencies among populations. The knowledge of their frequency distribution may lead to the identification of at-risk groups (individuals more prone, for example, to adverse effects). Information derived from this sort of study being potentially of use in laying foundations that can be applied to the improvement of the general healthcare quality.

We have initiated the study of several loci namely, TYMS (Thymidylate synthetase) – TSER, MTHFR - C677T, and MDR1 (=ABCB1)-C3435T, associated to colorectal, gastric and breast cancers therapy.

References

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