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DISSERTATION SUMMARY

Characterization of a putative respiratory complex in the hyperthermophilic archaeon, *Thermococcus litoralis*

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Thermococcus litoralis, isolated from a shallow submarine thermal spring in Italy, is a hyperthermophilic archaeon. It is an obligate heterotroph, growing at temperatures between 55 ºC and 98 ºC, with an optimum around 85 ºC, and it is able to reduce sulfur.

While isolating the genes of the soluble hydrogenase I of *T. litoralis,* we found open reading frames in the upstream region, in the opposite transcriptional direction to the hydrogenase genes. So far, a region of approximately 10 kb was isolated from a partial genomic library and sequenced. In this region, 8 orfs, namely *leg1-8* were found. Their genomic organization – overlap of the start and stop codons with the subsequent orfs, or the existence of just 2-3 bp long gaps – suggests that they form an operon.

Upstream from the first orf, a typical archaeal promoter can be assigned, containing BoxA and BoxB conserved regions. Typical ribosomal binding sites precede all of the open reading frames. Downstream from the *leg8* orf, there is a possible hairpin structure forming sequence motif, which can be a transcriptional termination signal. Attempts are being made to determine the length of the transcript(s) by Northern analysis.

Based on the deduced amino acid sequence, prediction programs suggest that proteins coded by *leg 1,2,6,7,8* are soluble, while *leg 3,4,5* code for transmembrane proteins.

Comparing these sequences to a protein sequence database, we cannot precisely determine the function of the proteins coded by these putative genes. All proteins, found to be similar in the database comparisons, are the members of different energy conserving respiratory systems, such as the NADH:ubiquinone oxidoreductase complex or the formate hydrogen lyase system of *E. coli.*

The aim of the project is to determine the physiological role of this putative enzyme complex. The lack of a recombinant genetic system for these organisms makes impossible to produce and analyze mutants. Without knowing the function of the proteins, mutagenesis with chemical agents is also impossible, because we are unable to screen the mutant library. A possible solution is the complete or partial isolation of the putative protein complex to determine its activity.

In order to follow the isolation of the complex, we decided to raise antibodies against the LEG2, LEG3 and LEG8 proteins. LEG2 and LEG8 were overexpressed in *E. coli* using the pET protein expression system. The His-tag containing recombinant proteins were purified by metal affinity chromatography.

All of our attempts to overexpress LEG3 failed. LEG3 appears to be an integral membrane protein, thus its hydrophobicity likely caused the difficulties in overexpression. To overcome this problem, a 10 amino acid stretch of the C-terminal end of LEG3 was chosen with the help of antigenic region determining programs. This 10mer peptide was custom-synthesized and conjugated to KLH.

With these antibodies, we will be able to follow the purification steps and, with Western hybridization experiments, we can determine the cellular location of these proteins and probable expression regulation can be studied under different growth conditions. The antibodies can also be used in immunoelectrophoresis, which makes possible to link the activity (stained in native gel) to the product of the leg orfs with the use of antibodies.

In parallel with the antibody production, we are making efforts to detect enzyme activities in the membrane fraction of *T. litoralis*, which can be inhibited with well known respiratory chain inhibitors, such as piericidin A.

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