SUMMARY

During this M.D. thesis experimental evidence was provided on the involvement of polyamines on the positive regulation that AtoS-AtoC/Az two-component signal transduction system exerts on poly-(R)-3-ydroxybutyrate (cPHB) biosynthesis in *E. coli.*

The AtoS-AtoC/Az system is induced by acetoacetate and regulates positively the transcription of the *atoDAEB* operon, encoding the enzymes that control the short-chain fatty acid catabolism in *E. coli*. The AtoC/Az response regulator is the polyamine-inducible, non-competitive inhibitor of Ornithine decarboxylase, the key enzyme of polyamine biosynthesis. Polyamines are indispensable cellular components implicated in many physiological functions. Recently, new roles have been attributed to AtoS-AtoC/Az in *E. coli* K-12. AtoS-AtoC/Az is, also, involved in the regulation of cPHB biosynthesis, the only member of the polyhydroxyalkanoate family, which is synthesized in *E. coli*. Spermidine enhances the cPHB amounts only in cells expressing the AtoS-AtoC/Az system, as well. N^8-acetylspermidine enhances the cPHB biosynthesis upon AtoS-AtoC/Az overproduction in a lower extent than spermidine. cPHB is up to attribution of many physiological roles in *E. coli* cells, such as calcium homeostasis by forming calcium channels on cytoplasmic membranes, competence for genetic transformation, protection of the complexed proteins from proteolysis and participation in DNA organization.

The requirement of the expression of both the components of AtoS-AtoC/Az system in the presence of spermidine, for the enhanced cPHB biosynthesis in *E. coli*, was initially elucidated. The HPLC-analysed cPHB amounts, proved that the extrachromosomal overproduction of the AtoS sensor kinase in Δ*atoSC* cells, was unable to complement the Δ*atoSC* phenotype, concerning the cPHB biosynthesis, even upon the presence of spermidine, resulting in lower cPHB levels compared to the *atoSC*$^+$ isogenic strain counterpart. The overproduction of the intracellular levels of AtoS kinase retained the basal levels of cPHB accumulation in *atoSC*$^+$ cells as well, in the

presence of spermidine. The *in trans* introduction of AtoC/Az in Δ*atoSC* cells, caused a limited increasement of cPHB in polyamine presence, without the complementation of the Δ*atoSC* phenotype. A similar enhancement, at lower levels however, than the respective amounts accumulated under the overproduction of the whole AtoS-AtoC/Az system, was obtained in AtoC/Az-overproducing *atoSC*$^{+}$ cells, as well.

The AtoS-AtoC/Az system regulates positively the cPHB biosynthesis upon spermidine induction through its direct effects on the *atoDAEB* operon transcription in *E. coli*, since the extrachromosomal introduction of AtoS-AtoC/Az system in Δ*atoSC* cells without the presence of the functional *atoDAEB* operon, could not restore the diminished capacity of the Δ*atoSC* strain for cPHB production.

Mutations altering the putative phoshorylation sites of AtoC/Az, diminished or abrogated the cPHB induction. The requirement of the response regulator phoshorylation was studied in the presence of spermidine as well as upon acetoacetate induction. The relative effects of D55G and H73L mutations on cPHB accumulation upon acetoacetate induction were inversed as to their effects on *atoDAEB* operon expression, as well as to their relative effects on cPHB in spermidine presence, i.e. histidine substitution had a more pronounced effect on the reduction of the enhanced cPHB that is achieved upon the overproduction of the wild-type protein, compared to the aspartate residue mutation. The introduction of both the AtoC/Az mutations, resulted in total abrogation of the AtoS-AtoC/Az overexpression effect phenotype.

The manipulation of the intracellular amounts of AtoS-AtoC/Az in *E. coli* cells, altered the intracellular localization of cPHB, between the membranes and the cytoplasm, compared to the basal levels biosynthesis and localization. This alteration depends on the signal (acetoacetate or spermidine) that is recognized and induces the signal transduction. Acetoacetate caused the accumulation of cPHB initially in the membranes and following the growth phases, in the cytoplasm. Spermidine, however, caused the accumulation of cPHB in the membranes, where it was further localized with an exception of a short interval of increased cytoplasmic amounts of the biopolymer.