**BABEȘ-BOLYAI UNIVERSITY**
**FACULTY OF BIOLOGY AND GEOLOGY**
**DOCTORAL SCHOOL OF INTEGRATIVE BIOLOGY**

# DOCTORAL THESIS

## Synthesis of polyhydroxyalkanoates in the extremely halotolerant bacterium *Halomonas elongata*

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**Keywords**

Polyesters, polyhydroxyalkanoate, halotolerant, Halomonas, biodegradable, thermostable, renewable sources, unsterile.

**Summary**

Polyhydroxyalkanoates (PHAs) synthesis in prokaryotes is a trait generally associated with stressors especially nutrient limitation. Once synthesized these polyesters are stored in the cells and later used as carbon and energy source. Members of the *Halomonas* genus have been systematically studied for their application potential including PHAs synthesis. Ectoine production in *H. elongata* is a feature already exploited in contrast to the PHA synthesis that is less studied at this bacterium.

This doctoral thesis aimed:

i) to investigate the PHAs synthesis in the environmental *H. elongata* 2FF strain and to assess the physio-chemical characteristics of the produced polyester.

ii) to explore the ability of the type strain *H. elongata* to convert industrial by-products (as carbon sources) into polyhydroxybutyrate (PHB).

iii) to evaluate the mechanical properties of the PHB films manufactured from the storage polyester synthesized by *H. elongata* type strain.

In **Chapter I** we review the general knowledge on PHA structures, synthesis, and perspectives in applicative fields with a special focus on the PHAs production in *Halomonas* spp.

In **Chapter II** we describe the isolation of an extremely halotolerant *H. elongata* from Fără Fund hypersaline lake (Transylvanian Basin, Romania) that can produce up to 0.95 g/L PHB when cultured in a double nutrient-limited medium. The polyester produced by the environmental *Halomonas* sp. strain was extracted and purified to 96%, had a crystallinity degree of 39%, and was identified as PHB. Furthermore, the polyester was shown to have industrially relevant thermal properties (melting temperature ($T_m$) = 168°C).

Since glucose is an expensive carbon source, the following step was to evaluate the ability of *H. elongata* type strain to produce PHAs using low-value feedstocks (**Chapter III**). The selected low-value feedstocks were sugar cane molasses (SCM), sugar beet molasses (SBM), glycerol, lactose, and corn steep liquor. The preferred substrate for PHB production in the type strain *H. elongata* grown at 8% w/v salinity was SCM, and the maximum production reached 2.36 g/L PHB in 96 h with a productivity rate of 0.021 g/L/h.

In **Chapter IV** we explore the ability of *H. elongata* type strain to produce PHB under high salinity (8%) and nonsterile conditions as a prerequisite for a likely cost-effective process. The extracted PHB was used to manufacture PHB films. Based on the solution intercalation methods three PHA membranes were produced: one made out of PHB extracted from *H. elongata* (PHBh), another membrane fabricated from commercial PHB and the third membrane from commercial polyhydroxybutyrate-*co*-hydroxyvalerate (PHBV). The PHBh film presented a higher hardness (43.78 MPa), Young`s Modulus (1.28 GPa), tensile stress (9.99 MPa) and tensile strain 1.39 % than those measured for the films fabricated from commercial PHB and PHBV.

**Chapter V** outlines the main achievements of the present thesis and includes general concluding remarks and perspectives of our experimental findings. We demonstrated unequivocally that *H. elongata* 2FF can produce polyhydroxybutyrate PHB when cultured in nutrient limiting media. This was the first report which answered the question "*which type of PHA is produced by Halomonas elongata?*". Moreover, a comprehensive chemical and physical evaluation of PHB synthesized in *Halomonas* spp. was reported by us.

We performed growth kinetics analyses on this environmental strain and suggested that has been capable to produce 40% PHB from the cell dry mass when cultured on a double nutrient-limited medium. We demonstrated for the first time that *Halomonas elongata* is capable to convert carbon substrates like molasses (both sugar cane and sugar beet molasses), lactose, or glycerol into PHB. Our findings demonstrated that this strain is a feasible candidate for the production of PHB from low-value feedstocks. Finally, we tested for the first time the ability of this strain to produce the PHB using glucose as a carbon source under nonsterile cultivation conditions.

As perspectives, scaling up the production of PHB in a bioreactor where the cultivation conditions can be controlled and further optimized seems the way to go. The evaluation of additional carbon sources (cheese whey, starch, coffee spent ground, etc.) for PHB production using *Halomonas* spp. is needed to broaden the range of potential economically cheap substrates that might be used for industrial PHA production at high salinity conditions. Furthermore, insightful knowledge on the genetic and biochemical mechanisms of PHB synthesis in connection with cell metabolism in *H. elongata* and closely related species could be gained by applying state-of-the-art transcriptomics and metabolomics approaches. Genetic engineering of *Halomonas* spp. could be employed for overproduction of PHB or production of alternative polyesters as precursors for bioplastics.

Overall, we depicted the ability of an environmental *H. elongata* strain to produce PHB on a double nutrient-limiting medium, showing the importance of saline lakes as natural sources for microorganisms with applicative potential. Then we demonstrated the PHB synthesis in *H. elongata* type strain grown on alternative carbon sources thus suggesting its potential to be used in the next generation of industrial biotechnology. Finally, we demonstrated that when cultured in nonsterile conditions, *H. elongata* can produce PHB from glucose and no contamination was noted upon a reasonably prolonged time. The extracted PHB could then be used for film manufacturing with promising mechanical features. Based on these findings we can state that *H. elongata* is an attractive candidate for PHA production at an industrial scale and therefore could be used to develop sustainable technologies that follow the principles of a sustainable circular economy.