A novel and improved microbial-chemical process to obtain chitin, chitosan and chito-oligosaccharides form shrimp waste

P.E Regalado-Infante, N.G. Rojas-Avelizapa**, R. Núñez-Pastrana*, R.C. Llarena-Hernandez*, A.M. Rivas-Castillo*** and L.I. Rojas-Avelizapa**

- **Facultad de Ciencias Biológicas y Agropecuarias, Universidad Veracruzana. Peñuela, Amatlán de los Reyes, Veracruz. MÉX.*
- *(E-mail: luzrojas@uv.mx; pregalado@uv.mx)*

*** Centro de Investigación en Ciencia Aplicada y Tecnología Avanzada del Instituto Politécnico Nacional, Unidad Querétaro, Qro., MÉX.*
****Universidad Tecnológica de La Zona Metropolitana del Valle de México, Miguel Hidalgo y Costilla 5, Fracc. Los Héroes Tizayuca, 43816, Hidalgo, MÉX.*

Introduction

Figure 1. Shrimp waste in Alvarado, Ver., Méx.

The biodegradation of crustacean shells is very slow. There is an urgent need to process and use shrimp waste, which contains several bioactive compounds such as chitin, pigments, amino acids and fatty acids. Since 1998 to 2021, the volume of shrimp captured in México has varied within 80 to 220 K tons where 40-50 % of such volume represents crustacean wastes. In Mexico, a minimal proportion of such material is used to manufacture chicken feed and food flavors, while in other countries the main use is for the obtention of chitin and chitosan. Both polymers are widely used in industries such as medical, pharmaceutical, food and effluent treatments.

The obtention of chitin-chitosan and derivatives, require that the crustacean wastes being subjected to aggressive chemical processes with acids and alkali, for removing both minerals and proteins and deacetylation.
For this reason, other alternatives have been proposed, such as the use of microorganisms to obtain chitin and chitosan.
Based on previous information, the aim of the present study was to produce chitin, chitosan, and chito-oligosaccharides from chitosan, through microbial processes using both *B. thuringiensis* strains LBM1 and LBM2 reducing the use of chemical treatments.

Results & Discussion

The fermentation of shrimp wastes by *Bacillus thuringiensis* LBM1 showed the production of high levels of proteolytic activity (350 U/ml), and very low levels of chitinolytic activity (0.12 U/ml) (Fig. 2).

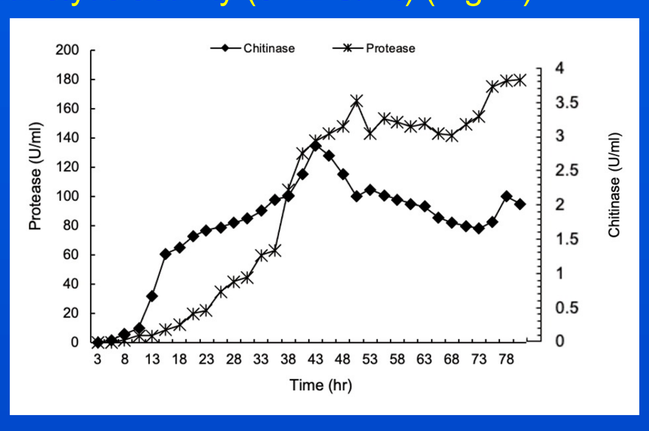

Figure 2. Kinetics of protease and chitinase production by *Bacillus thuringiensis* strain BLM1, cultured in media with 2% ground shrimp waste in tap water at 28 °C and 180 rev/min.

It should be pointed out that when shrimp waste fermentation by LBM1 was finished, a solid remanent consisting of 35.78 % of the solid substrate initially added. In fact, it was possible to obtain good quality chitin from de remnant substrate due to proteolytic enzyme action (Fig. 3).

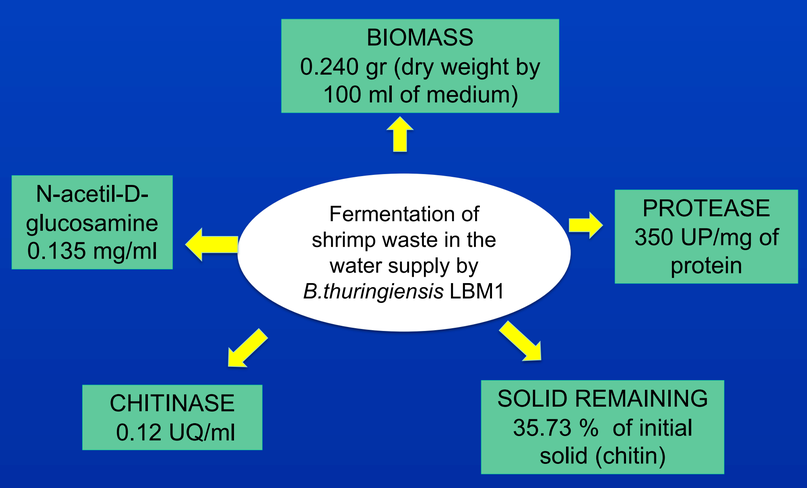

Figure 3. Material balance after fermentation of shrimp waste by *Bacillus thuringiensis* LBM1*

Table 2 shows the results of the bromatological analysis of raw material at initial time of fermentation and up to 96 h. Likewise, a decrease in the percentage of protein is observed at the end of the fermentation time, due to the fact that LBM1 strain is highly proteolytic (350 UP/mg protein), while the crude fiber (chitin) remains constant throughout the process, this is due to the fact that this microorganism produces chitinases in a very low proportion (0.2 UQ/mg chitin)

Time (hour)	Humidity (%)	Ashes (%)	Protein (%)	Ethereal extract (%)	Raw fiber (%)
0	4.35	32.88	26.31	1.74	34.72
24	1.97	36.88	22.87	4.14	34.13
48	1.85	38.08	20.25	4.63	35.20
72	1.80	39.09	17.98	5.12	34.29
96	1.77	50.10	7.29	5.11	35.73

Once the fermentation was finished, the resulting material (Chitin) underwent a single chemical treatment with 50% NaOH for 4 h at 60 °C under agitation conditions to deacetylate chitin and obtain chitosan. Finally said chitosan was characterized by means of a potentiometric titration, finding a degree of deacetylation of 78.26%.

Figure 4. shrimp waste transformation process

Finally, the chito-oligosaccharides were produced by enzymatic saccharification of colloidal chitosan, with the crude chitosanase produced by the Bt LBM2 strain grown in a medium with 2% chitosan suspended in a synthetic medium for 90 h at 50 °C, and analysed by Chromatographic separation in Sephadex G-15 and Thin layer chromatography

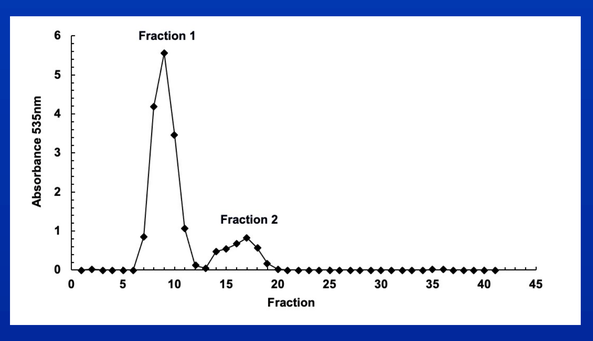

Figure 5. Chromatographic analysis of the chito-oligosaccharides contained in a mixture produced by enzymatic hydrolysis of colloidal chitosan by the extracellular chitosanase of Bt-LBM2.

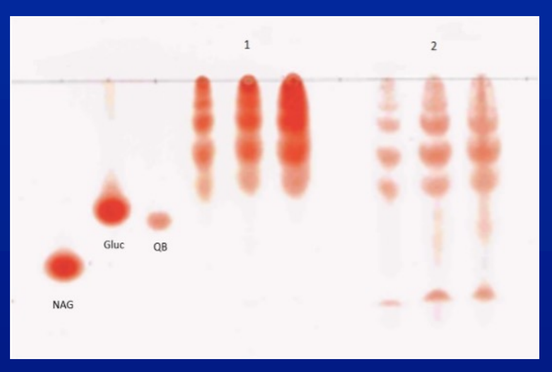

Figure 6. Chito-oligosaccharides present in the two fractions obtained by exclusion chromatography in Sephadex G-15 of the enzymatic hydrolysate of colloidal chitosan by the extracellular chitosanase of Bt-LBM2.

Conclusions

The results showed that, *B. thuringiensis* LBM1 was able to deproteinize shrimp shell. The resulting chitin, as the desired product was easily converted to chitosan by deacetylation in a 50 % NaOH solution at 60 °C during 4 h. We got a chitosan with a good degree of deacetylation about 78.26 %. Finally, the saccharification the colloidal chitosan with the chitosanase from *B. thuringiensis* LBM2 produced about 6 chito-oligosaccharides, which included mono, di, tri and tetrasaccharides.

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