V Simpósio Ibero-americano de Quitina
Sociedade Ibero-americana de Quitina
Marzo, 2010, Santiago, Chile

HYDROLYSIS OF CHITOSAN USING CRUDE ENZYMES PRODUCED BY *Paenibacillus ehimensis*

M.G.B. PAGNONCELLI[1], N.K. ARAÚJO[2], C.F. ASSIS[3], S. RODRIGUES[4], G.R. MACEDO[5]

[1] Departamento de Farmácia, Universidade Federal do Rio Grande do Norte, Natal, Brasil. e-mail: giobinder@ufrnet.br
[2] Departamento de Bioquímica, Universidade Federal do Rio Grande do Norte, Natal, Brasil. e-mail: nakar_rn@hotmail.com
[3,5] Núcleo Tecnológico, Universidade Federal do Rio Grande do Norte, Natal, Brasil
[4] Departamento de Farmácia, Universidade Federal do Ceará, Fortaleza, Brasil.

Chitosan is a deacetylated derivative of chitin**.** Chitosan and its derivatives showed functional properties making them useful in many fields including, food, cosmetics, medicine and pharmaceuticals**.** However, its poor solubility makes the chitosan difficult to be used in food and biomedical applications. On the other hand, chitosan oligosaccharides (COS) are readily soluble in water due to their shorter chain lengths and free amino groups in the D-glucosamine units [1].

There are two methods for hydrolysis of chitosan: chemical and enzymatic. Chemical hydrolysis is carried out at high temperatures under highly acidic conditions, resulting in a large amount of glucosamine (chitosan monomer), due to the difficulties in the process control. Therefore, this method produces low yields of pentamers and hexamers. Enzymatic hydrolysis has some advantages in producing COS. Chitosanases can catalyze the hydrolysis under mild conditions [2].

Chitosanses are expensive to be utilized in large-scale industrial applications, because enzyme hydrolysis requires multi-steps, particularly, enzyme preparation and purification. The aim of this work was to obtain COS using a crude enzyme obtained by *Paenibacillus ehimensis*.

For chitosanase production culture media consisting peptone 6.0 $g.L^{-1}$, yeast extract 6.0 $g.L^{-1}$, glucose 1.0 $g.L^{-1}$, K_2HPO_4 1.0 $g.L^{-1}$, $MgSO_4.7H_2O$ 0.5 $g.L^{-1}$ and colloidal chitosan 2.0 $g.L^{-1}$ (pH 7.0). The medium was inoculated with 10 % of a pre-culture in exponential growing phase and incubated on an orbital shaker at 32 ℃ and 120 rpm for 24 h.

To obtain the hydrolytic product, 2 mL of 1% soluble chitosan and 2 mL of supernatant (crude enzymes) was incubated at 55 ℃ during 2 h. The reducing sugars was measuring by DNS method and the COS mixture was subjected to TLC, using silica gel plate F-250 (Merck 60).

After incubation for 10 minutes, the reducing sugars in the reaction medium become to rise and around 35 g glucosamine per 100 g chitosan was generated after 3 hours.

The products obtained by enzymatic hydrolysis of soluble chitosan using the crude chitosanases from *Paenibacillus ehimensis* (supernatant) were analyzed by TLC. Chitosan was hydrolyzed to $(GlcN)_2$ and $(GlcN)_6$ at the initial stage of the reaction. After incubation for 2 hours, amounts of $(GlcN)_2$ and $(GlcN)_3$ in the hydrolysates were increased , as shown in Figure 1.

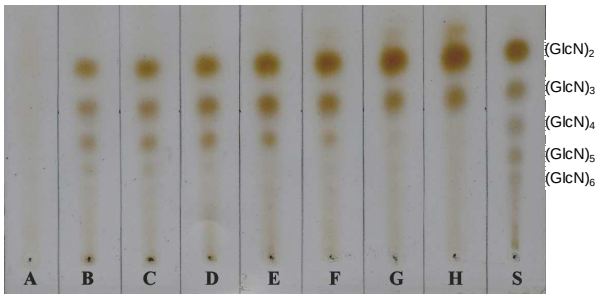

Fig 1. TLC profiles of chitooligosaccharides produced in the hydrolysis of soluble chitosan by crude chitosanase from *Paenibacillus ehimensis*. Lane A-H, present hydrolysates obtained after enzyme reaction for 10, 20, 30 min, 1, 2, 3, 6 and 9 h; Lane S, COS standard.

This study demonstrated that COS could be produced by crude enzymes from the *Paenibacillus ehimensis*. The use of crude enzymes instead purified ones, are of industrial interest because enzyme purification steps are expensive steps in industrial enzyme production. This crude chitosanase showed good industrial potential.

ACKNOWLEDGEMENTS

Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq– Brasil)

REFERENCES

1. Li, J.; Du, Y.; Yang, J.; Feng, T.; Li, A. and Chen, P. Preparation and characterization of low molecular weight chitosan and chito-oligomers by a commercial enzyme. *Polym. Degrad. Stab.*, 87, 441-448, 2005.
2. Ming, M.; Kuroiwa, T.; Ichikawa, S.; Sato, S. and Mukataka, S. Production of chitosan oligosaccharides by chitosanase directly immobilized on an agar gel-coated multidisk impeller. *Biochem. Eng. J.,* 28, 289–294, 2006.