1	Production of chitooligosaccharides by polyhydroxyalkanoates-
2	immobilized recombinant chitosanase
3	陳楷雯 (5109)
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5	Outline
6	1. Introduction
7	2. The preparation of PHA-CSN
8	3. The characterization of PHA-CSN
9	4. Hydrolysis products of chitosan
10	5. Conclusion
11	Abstract
12	Immobilization of enzymes to solid supports or their self-assembly into insoluble
13	particles enhances their applicability by strongly improving properties such as stability
14	in changing environments, re-usability and applicability in continuous biocatalytic
15	processes.
16	In this study, the csn gene encoded chitosanase CSN was fused to the phaC gene
17	from Ralstonia eutropha H16 by pipe cloning method. Then, the fused gene was co-
18	expressed with the phaAB gene to produce polyhydroxyalkanoate (PHA) nanoparticles
19	for the immobilization of CSN. The enzyme was immobilized on the surface of PHA
20	nanoparticles to form PHA-CSN. These nanoparticles possess enzymatic properties
21	similar to those of free CSN. PHA-CSN has the highest activity at 45°C and pH 5.5 in
22	the presence of 4 mM Mn ²⁺ . The main hydrolysis products of chitosan catalyzed by PHA-
23	CSN are chitobiose and chitotroise.

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