

Prolonged Synthesis of Chitin Polysaccharide from Chlorovirus System

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Abstract : Chlorella viruses or chloroviruses contain a gene that encodes a function for chitin synthesis, which is expressed early in viral infection to produce chitin polysaccharide, a polymer of β -1, 4-linked GlcNAc, on the outside of Chlorella cell wall. Interestingly, chlorovirus system is an eco-friendly system which converts CO₂ and solar energy from the environment into useful materials. However, infected Chlorella cells are lysed at the final stage of viral infection, and this phenomenon is caused by the breaking down of polysaccharide. To postpone the lysing period and prolong the synthesis of chitin polysaccharide on cells, the slow growing virus incorporated with aphidicolin treatment, an inhibitor of DNA synthesis, was investigated. In this study, a total of 25 virus isolates from water samples in Japan region were analyzed for CHS (the gene for CH synthase) gene by PCR (polymerase chain reaction). The accumulation and appearance of chitin polysaccharide on infected cells were detected by biotinylated chitin-binding proteins WGA (wheat germ agglutinin)-biotin for chitin in conjunction with avidin-Cy 2 or Cy 3 and investigated by fluorescence microscopy, observed as green or yellow fluorescence over the cell surface. Among all chlorovirus isolates, cells infected with CNF1 revealed the accumulation of chitin over the cell surface within 30 min p.i. and continued to accumulate on cells until 4 h p.i. before cell lyses which was 1.6 times longer accumulation period than cells infected with CVK2 (prototype virus). Furthermore, addition of aphidicolin could extend the chitin accumulation on cells infected with CNF1 until 8 h p.i. before cell lyses. Whereas, CVK2-infected cells treated with aphidicolin could prolong the chitin synthesis only for 6 h p.i. before cell lyses. Therefore, chitin synthesis by Chlorella-virus system could be prolonged by using slow-growing viral isolates and with aphidicolin.

Keywords : chitin, chlorovirus, Chlorella virus, aphidicolin

Conference Title : ICBBSE 2017 : International Conference on Biotechnology and Biological Systems Engineering

Conference Location : London, United Kingdom

Conference Dates : March 14-15, 2017