Production of chitin and chitosan oligomers and their use as bioprotective agents in vegetable cultivation BL/12/V23

(Geographic) study area : Vietnam

Context and objectives

Agriculture is an important part in Vietnam development and economy. Around 60% of the Vietnamese population is engaged in agricultural activities. Meanwhile, in rural zones, there are more and more problems of poverty. Agriculture is still mostly a family business and therefore farming units are rather small entities. Each farmer produces in general a stable crop (mostly rice) and vegetables, the latter having a higher added value. However the quality of these vegetables is questionable, in particular because of the inappropriate use of chemical pesticides exposing farmers to chronic poisoning. The vegetables on the markets are often contaminated with unacceptable levels of pesticide residues causing every year poisoning of thousands Vietnamese consumers. Moreover intense use of pesticides is threatening exportation opportunities for vegetables from Vietnam. Therefore if the farmers would improve the quality of the produced vegetables, this will improve their socio-economical situation in a durable way. The objective of this project is to develop an alternative range of natural low cost biopesticides based on mixtures of chitin/chitosan oligomers produced from shrimp shells.

Methodology

- Screening of the Vietnam Type Culture Collection (VTCC) for microorganisms secreting high chitinase activity and/or high chitin deacetylase activity
- Preparation of chitin and chitosan from shrimp shell waste. Use of these substrates for the production of oligomers with enzymatic preparations from VTCC selected microorganisms
- Characterization of oligomers produced by enzymatic way. Development and adaptation of methods for measuring the degree of polymerization (DP) and the degree of acetylation (DA)
- Antifungal activity and biological activity in planta
- Validation of the technology and scaling-up of the production
- Scientific exchanges and technology transfer

Scientific Results

- **Screening of VTCC**

On 2350 strains of Actinomycetes and Fungi from VTCC screened, 266 strains showed chitinase activity and 5 strains chitin deacetylase activity (CDA). After several successive screenings, 6 strains (4 Streptomyces and 2 Fungi) were selected for their high chitinase activity and 4 strains (3 Streptomyces and 1 Fungus) for their high CDA activity. Medium composition and culture conditions in reactor were optimized.

- **Preparation of chitin, chitosan and oligomers**

Chitin and chitosan were prepared from shell waste from shrimp farming in Vietnam. These substrates were used for oligomer preparation using chitinase and chitin deacetylase from selected VTCC microorganisms.

- **Characterization of chitin/chitosan oligomers**

As plant protective activity is mainly influenced by oligomer length (DP) and acetylation degree (DA), analytical methods were developed and/or adapted to measure these characteristics : separation and analysis by TLC (Thin Layer Chromatography) and HPLC (High Performance liquid Chromatography) for measuring DP and quantifying oligomers; UV spectrometry method and microassay of free amines for measuring DA. The techniques have been applied to the oligosaccharides produced by enzymatic way.

- **Antifungal and biological activity of chitin/chitosan oligomers**

Bioassays were performed against the pathogenic fungus *Botrytis cinerea* to quantify the fungicide effect of oligomers. The technique is based on the quantification of inhibition of spore germination and growth of the pathogen on microplate. The bioprotective effect was assessed on tomato plants treated with chitin/chitosan oligomers and challenged by the pathogen. Preliminary tests provided interesting and encouraging results : up to 60% protection and up to 70% spore germination inhibition. The experiments must be repeated to confirm the results.

- **Validation and scaling-up**

Experiments must be carried out to validate the technology. Next step will be the process scaling-up and field trials on tomato and other vegetables.

- **Scientific exchanges and technology transfer**

Three Vietnamese scientists have been trained to technology and analytical methods through extended stages in Belgium (ULG). A master thesis was carried out jointly with the Vietnam National University of Hanoi and the University of Liege.

Products and services

Publications

Dao Thi Luong1, Nguyen Anh Tuan, Nguyen Thi Van, Le Thi Hoang Yen, Marie-France Jaspar-Versali, Jacques Dommes and Duong Van Hop. Study in an Actinomycetes producing chitinase and chitin deacetylase. ICBiotech Annual report Journal Osaka University, Japan (submitted).

Le Thi Hoang Yen, Nguyen Anh Tuan, Le Viet Hung, Nguyen Thi Hong Nhung and Duong Van Hop. Screening for chitinolytic fungi isolated in Vietnam and optimizing cultural conditions for the production of chitinase by Trichoderma reesei VN09-F0060. ICBiotech Annual report Journal Osaka University, Japan (submitted).

Jaspar-Versali Marie-France, Bouazza Naïm, Mariutto Martin, Duong Van Hop and Jacques Dommes. Preparation of chitosan oligomers by non-specific enzymes (in preparation).

International conference

Luong Thi Dao. Screening and 16S rRNA analysis of the Actinomycetes producing chitin deacetylase and chitinase. 9th Asia-pacific Chitin and Chitosan Symposium, Nha Trang, Vietnam, August 3-6, 2011.

Thi Hoa,g Yen Le. Screening and optimising of cultural conditions for production of chitinase by fungi isolated in Vietnam and recognition of this chitinase by zymogram. 9th Asia-pacific Chitin and Chitosan Symposium, Nha Trang, Vietnam, August 3-6, 2011.

Master Thesis

Study on characterization of chitinase from Streptomyces. Master Thesis Major Biotechnology presented by Nguyen Thanh Huong, Vietnam national University of Hanoi and Liege University, 2011.

Ideas for future research

The method of chitin/chitosan oligomer production by enzymatic way is promising. The oligomers show antifungal and bioprotective activities on infected tomato plants. However research should be pursued to confirm the bioprotection activity on other vegetables, to carry out field trials and to develop a formulation suitable for application by Vietnamese farmers.

Execution

Period: 01/12/2009 – 31/05/2012

Laboratory/network:

Belgian Coordinator:
Prof. Jacques Dommes
Plant Molecular Biology and Biotechnology Unit
Department of Life Science (B22)
University of Liege (ULG)
Email : j.dommes@ulg.ac.be

Vietnames partner:
Dr. Duong Van Hop
Institute of Microbiology and Biotechnology
Vietnam National University
Hanoi, Vietnam
Email: hopdv@vnu.edu.vn

Discipline

Biotechnology
Agriculture
Environment
Materials (bio- etc.)
Integrated Plant Protection