RESEARCH PROGRAMME

BELGIAN SCIENCE POLICY

Bilateral Cooperation

Bilateral Agreement with China

FINAL REPORT

Proposal's title: ***DETECTION OF INFECTIOUS NOROVIRUSES IN SHELLFISH AND ANTI-VIRAL POTENTIAL STUDY OF SEAFOOD***
Proposal's acronym: NoroShell
Contract no: **BL/02/C57**

For the PARTNERSHIP: the coordinator

Prof. dr. Mieke Uyttendaele

DATE: 10/01/2014

ADMINISTRATIVE REPORT

For partner 1, Gent University:

Modifications to the contract: Annex 6

List of personnel working on the project (in charge of the project or not):

Personnel working on the project, funded by the budget:

Dan Li, Ph.D Student

Dept. Food safety & food quality, Faculty of Bio-Science Engineering, UGent

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Overview of performed tasks regarding each work package:

WP1: Monthly sample collection and screening.

1.1 Protocol establishment and training.
1.2 Sampling planning and contact with shellfish farms.
1.3 Monthly sample collection and detection.of NoV.
1.4 Further confirmation of positive samples.
1.5 Data analysis and publication.

WP2: Anti-NoVs study of compounds from sea food.

2.1 Literature study and preliminary screening of anti-viral components using MNV-1 as a surrogate.
2.2 Further study of anti-viral mechanisms of selected components using human NoVs.
2.3 Anti-viral effect with the presence of food matrix.
2.4 Data analysis and publication.

List of seminars/symposia:

Informal meeting (with international partners): August 2012, meeting with Chinese researcher Dr. Qing-Juan Tang in Gent for the training and determination of viral detection protocols.

Participation of Coordinator (Mieke Uyttendaele) to FSA-UK Norovirus in food discussion meeting in London, January 13-14th 2013, UK.

(http://www.food.gov.uk/news-updates/news/2012/nov/noro_jan

Extended Guidance committee (on invitation only) on virus work at UGent, Belgium (including work to be presented on BELSPO_China collaboration, EU Veg-i-Trade & SPF Public Health TRAVIFOOD project): June 13th 2013.

Fifth International Conference on Caliciviruses. Dan Li, Ambroos Stals, Mieke Uyttendaele. Noroviruses in shellfish products of Belgian food markets: detection, interpretation, and confirmation. October 12-15, 2013, Beijing, China.

Extended Guidance committee (on invitation only) on virus work at Brussels, Belgium (including work to be presented on BELSPO_China collaboration, EU Veg-i-Trade & SPF Public Health TRAVIFOOD project): December 10th 2013.

For partner 2, Ocean University of China :

Modifications to the contract: NOT APPLICABLE

List of personnel working on the project (in charge of the project or not):

Personnel working on the project, not funded by the budget:

Qing-Juan Tang, MD, associate professor

College of Food Science and Engineering,

Ocean University of China

Juan Wu, master student

College of Food Science and Engineering,

Ocean University of China

Overview of performed tasks regarding each workpackage:

WP1: Monthly sample collection and screening.

1.1 Protocol establishment and training.
1.2 Sampling planning and contact with shellfish farms.
1.3 Monthly sample collection and detection.of NoV.
1.4 Further confirmation of positive samples.
1.5 Data analysis and publication.

List of seminars/symposia:

Workshop in Qingdao, China, May 26th 2013, for dissemination of knowledge and results in China.

SCIENTIFIC REPORT

Short description of the project:

In recent years, noroviruses (NoVs), which are the major agent of acute gastroenteritis, are more and more identified as a great threat for human health worldwide (Noda et al., 2008). Being transmitted via the faecal-oral route, NoVs outbreaks are frequently associated with bivalve shellfish, which can bioaccumulate NoVs at high levels from surrounding marine and estuarine waters, and tend to be eaten raw or lightly cooked, (Le Guyader et al., 2006).

Study of NoVs has been hampered by the lack of suitable animal models and the inability to propagate in cell cultures (Duizer et al., 2004). Reverse transcription polymerase chain reaction (RT-PCR), which cannot discriminate between inactivated and infectious viral particles, is still the most commonly used detection method (Richards, 1999). This may provide potentially false-positive results and thus the contamination condition of infectious NoVs in foods is actually unknown.

Currently, anti-viral activities of extracts and compounds isolated from natural products have already been reported towards several severe pathogenic viruses such as human immunodeficiency virus (HIV), herpes simplex virus (HSV), hepatitis B virus (HBV), etc. (Mukhtar et al., 2008). However, anti-NoVs effects of natural specific seafood products are hardly investigated.

In this project, **firstly, NoVs will be screened from popular shellfish species in Belgium and China.** Shellfish samples belonging to different categories (oysters and mussels included) will be collected from Belgium and China. NoVs will be extracted from dissected digestive tissues of shellfish using the standard method of Europe (Lees et al., 2010). As a validation of this new binding RT-PCR technique to specifically distinguish presence of infectious NoV, the detection result of binding based RT-PCR will be compared with traditional RT-PCR. If positive results are obtained and contamination with viral strains with a high contamination level are identified it will be attempted to genotype these viral strains by DNA sequencing. Also during analysis, in parallel to infectious NoV detection, hygiene indicators *E.coli* will be analysed in order to investigate their potential to be used as NoVs contamination indicators. **Secondly, a few compounds mainly isolated from seafood (e.g. fucoidan, chondroitinsulfate and gangliosides from sea cucumber, polysaccharides from squid ink) will be investigated for potential anti-NoV activity**. In the first stage, the preliminary screening of anti-viral products will be performed by using murine norovirus (MNV-1). MNV-1 is the most related NoV strain to the human NoVs that can be cultivated in the laboratory (Wobus et al., 2004). In the second stage, the key results obtained using MNV-1 will be confirmed by using human NoVs isolated from faecal samples of NoVs infected patients. Finally, to judge the potential practical application as a preservative or additional hurdle factor to contribute to inactivate infectious NoV in foods, the anti-viral effect of some selected promising seafood extracts will be further investigated for their antiviral activity to NoV in the presence of a food matrix.

Results obtained:

- A protocol for NoVs detection in shellfish has been established based on the standard method of Europe (Annex 1) and the Chinese partner was trained with the same techniques by August 2012. The NoV screening results of Belgium fishery products have been summarized in Annex 1, presented in Fifth International Conference on Caliciviruses (October 12-15, 2013, Beijing, China) and submitted to **International Journal of Food Microbiology** to be published. The screening results generated from the Chinese partner is summarized in Annex 2.

- A literature review (**Inactivation of food-borne viruses using natural biochemical substances)** was composed based on the study of numerous previous work on Web of Science. The manuscript has been published on **Food Microbiology** (Annex 3).

- The anti-NoV effect of several substances were investigated by plaque assay using MNV-1 as a surrogate and by binding-based RT-qPCR using authentic human NoV. After the screening of the potential anti-NoV substances, grape seed extract (OligoProanthocyanidinas as the main functioning component) is the only one within the tested substances that showed promising anti-NoV properties (Annex 4). The mechanism of the anti-viral effect was studied by testing the specific binding properties and the morphology of human NoV capsid proteins. In order to evaluate the application potentials of grape seed extract, the anti-viral effect with the presence of food matrix was explored in practical scenarios stimulating the working environment in food industry. The results have been published in **Applied and Environmental Microbiology** (Annex 5).

ANNEXES (planning tables, mission reports, data structure systems, articles ...)

Annex 1 : Article 1, Noroviruses in shellfish and fishery products offered at Belgian food market: detection, interpretation and confirmation.

Annex 2 : Screening results of Noroviruses from Chinese partner.

Annex 3 : Article 2, Inactivation of Food-Borne Viruses using Natural Biochemical Substances.

Annex 4 : Screening results for the anti-NoV substances.

Annex 5 : Article 3, Effect of Grape Seed Extract on Human Norovirus GII.4 and Murine Norovirus 1 in Viral Suspensions, on Stainless Steel Discs, and in Lettuce Wash Water.

Annex 6 : Modification of contract.
