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Publications:

Modulation of the tissue regenerative process in fish by ß-glucans

Immune modulators are compounds capable of interacting with the immune system and thereby modifying the host response. This interaction enhances non-specific defense mechanisms, improving health and promoting survival. are glucose polysaccharides present in sea weed, bacteria, fungi and cereal but not in animals. are commonly used as immune modulators, but the mechanisms through which the modulation is achieved remains to be understood. Wound healing and tissue regeneration are essential mechanisms to ensure the survival and health of any organism. Studies from the mammalian systems have shown the importance of fibroblasts, macrophages, reactive oxygen species (especially hydrogen peroxide) and certain cytokines during wound healing processes. In fish however, only a few studies have been devoted tissue regeneration and modulation of cell proliferation during wound healing, even though mechanical injury as well as numerous diseases can severely damage fish tissues. The work presented examines for the first time the immunomodulatory effects of during wound healing processes in fish. Experiments have been conducted both in vivo and in vitro and results clearly show the immunomodulatory effects of during the wound healing process. The wound healing process was monitored using image analysis, real time PCR and proteomics.
The study showed that treatment enhanced wound closure in fish, probably due to the enhancement of a localized inflammatory response. The modulatory effect of on wound healing seems to be orchestrated by the immune system, since as direct effect on fibroblast proliferation were observed. Furthermore, production of ROS may influence the fate of tissue regeneration, and differences in ROS patterns could be one of the possible ways in which fish alert the immune system to drive the immune response towards pathogen eradication or tissue repair.

General information

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Organisations: National Food Institute, Division of Industrial Food Research, Technical University of Denmark
Authors: Nielsen, M. E. (Intern), Jiménez, N. I. V. (Intern), Przybylska, D. A. (Intern), Schmidt, J. (Intern), Hasselbalch Volke Hougaard, C. (Ekstern), Wulff, T. (Intern)
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β-glucan enriched bath directly stimulates the wound healing process in common carp (Cyprinus carpio L.)

Wound healing is a complex and well-organized process in which physiological factors and immune mechanisms are involved. A number of different immune modulators have been found to enhance the non-specific defence system in

vertebrates, among which β-glucans are the most powerful and extensively investigated.
The aim of the present study was to investigate the biological impact of two different commercially available β glucan containing products on the wound healing process in carp. Throughout a two week experiment fish were kept either untreated (control), or in water supplemented with the two different types of β-glucans. The wound healing process was monitored using a multispectral visualisation system. The correlation between wound closure and immune response was investigated by measuring the gene expression patterns of IL-1b, IL-6 family member M17, IL-8 and Muc5b, and measurement of production of radical oxygen species. PAMPs/DAMPs stimulation caused by the wounding and or β-glucans resulted in an inflammatory response by activating IL-1b, IL-6 family member M17 and IL-8 and differences in the expression pattern were seen depending on stimuli. IL-1b, IL-6 family member M17 and IL-8 were activated in all wounds regardless of treatment. Expression of all three interleukins was highly up regulated in control wounded muscle already at day 1 post-wounding and decreased at subsequent time-points. The reverse was the case with control wounded skin, where expression increased from day 1 through day 14. The results for the β-glucan treated wounds were more complex. The images showed significantly faster wound contraction in both treated groups compared to the control. The obtained results clearly demonstrated that a β glucan enriched bath promotes the closure of wounds in common carp and induce a local change in cytokine expression.

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Mucosal immune response in common carp (Cyprinus carpio L.): Host pathogen interactions in relation to beta-glucan stimulation

Control of fish diseases is a great concern in aquaculture because of losses in the production. Drug choices for the treatment of common infectious diseases are becoming increasingly limited and expensive and, in some cases, unavailable due to the emergence of drug resistance in bacteria and fungi. This is why number of biological compounds, as an alternative to the drugs, has been used to reduce the risk of diseases and improve fish welfare by enhancement of non-specific defence system. Among them, ß-glucans, naturally occurring polysaccharides found in the cell wall of plants, bacteria and fungi, are some of the most powerful and extensively investigated immune modulants. ß-glucans have been proven to enhance the immune system and pharmacologically they are classified as biological response modifiers (BRM). The focus of the present thesis was on: 1. creation of a model for the examination of the biological impact of two commercially available ß-glucan enriched products on the wound healing process in common carp (Cyprinus carpio L.) in sterile, controlled conditions; 2. investigation of potential impact of intravenously injected ß-glucan on mucosal immune response and immunoglobulin switch-like process in common carp. In order to reach these objectives, different methods were used such as real-time quantitative PCR (RT-PCR) in order to measure the expression of immunerelated genes involved in wound healing process, ELISA for specific antibody detection, cortisol assay for measurement of stress level, respiratory burst assay for radical production measurement and image analysis.
The results of this study showed that previous infections gave rise to changes regarding texture quality parameters in fresh fish meat, and were a starting point for use of immune modulators such as ß-glucans. Further work showed that bath in two commercially available ß-glucan enriched products, specifically MacroGard and 6.3 kDa oat fiber, had a direct positive effects on the wound closure in common carp and promoted faster wound healing compared to non-treated fish. We showed the immunological and regenerative response following stimulation with PAMPs and DAMPs in controlled conditions, without the exposure to pathogens, which resulted in an inflammatory response by activating IL-1b, IL-6 and IL-8. Local differences in expression pattern dependent on stimulation by DAMPs alone or DAMPs/PAMPs combination. In addition, the absence of marked differences on the respiratory burst activity in head-kidney cells supports the idea of a localized immune response to the site of injury. Due to direct and constant contact between skin and ß-glucan, bath treatment was an obvious choice to investigate. However, intravenous injection of ß-glucan showed it has a biological effect on skin as well. Once again we observed immunological changes taking place in skin of common carp, with IgM/IgZ immunoglobulin switch-like process. Finally, ß-glucan seems to work in dose-dependent manner, with elevating cortisol level when injected at high doses.

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Ontogeny of the carp (Cyprinus carpio L.) innate immune system: Gene expression and experimental limitations

The objective of this study was to investigate the ontogeny of the immune system in common carp (Cyprinus carpio, L.). The work has been focused on innate immune responses during the wound healing processes and how the innate immune response develops with age and size of the fish.
Newly hatched carp were brought to the facilities at DTU and kept in aquaria at 25°C. They were initially fed Artemia nauplii, and later switched to commercial dry granulate feed. Carp were anaesthetised and then experimentally wounded

at days 10, 16, 24, 47 and 94 days post-hatch. Sampling was carried out at day 1, 3 and 7 post-wounding and samples were stored in RNA later for isolation of RNA. The physical tissue damage was performed using a sterile needle, which penetrated the skin and the underlying musculature in an area above the lateral line of the left side of fish. Carps at the age of 10, 16 and 24 days post-hatch were stored and processed whole, whereas just the muscle (the left (wound area) and right filet (internal control)) was sampled for the two latter time-points. mRNA was extracted from the samples, cDNA was synthesised and gene expression was quantified using real-time RT-PCR. The investigated genes were IL-1β, IL-6, TNF-α, SAA, Hsp70, TGF-β and the mucins Muc2c and Muc5bc.
It can be generally concluded that the response of the investigated genes appeared to be faster and more pronounced at earlier life stages. However, care should be taken when comparing the results for the fish wounded on days 10, 16 and 24 post-hatching with the fish wounded on days 47 and 94 post-hatching since the way of sampling differed. However, it is inherently difficult to standardise experimental procedures for fish that increase 1000-fold during the course of the experiment. The results and experimental pitfalls will be further discussed.

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Previous bacterial infection affects textural quality parameters of heat-treated fillets from rainbow trout (Oncorhynchus mykiss)
Sensory quality of fish meat is influenced by many parameters prior to slaughter. In the present study, it was examined if previous infections or damages in the muscle tissue influence product quality parameters in fish. Fillets from rainbow trout (Oncorhynchus mykiss) reared in seawater at a commercial fish farm were sensory evaluated for more than a year after recovery following physical tissue damage or infection by the bacterial pathogens Yersinia ruckeri and Vibrio anguillarum. The effect of vaccination was also included as some fish were vaccinated before bacterial challenge. The fish fillets were sensory examined as heat-treated and cold-smoked. Heat-treated fillets from nonvaccinated fish previously infected by V. anguillarum had changed textural characteristics and were less flaky, had a lower oiliness and a higher toughness and fibrousness in comparison with control fish. This article was the first to describe a correlation between previous infections in fish and changes in sensory-quality parameters. PRACTICAL APPLICATIONS. This work contributes with knowledge about sensory-quality parameters of fish meat after recovery from infections and physical-tissue damage. Because the results demonstrate an influence on the texture from previous disease, the practical potentials of the results are valuable for the aquaculture industry. In order to minimize the effects of previous diseases on the sensory quality regarding the texture, these fishes should be processed as cold-smoked instead of being sold as raw meat. The established correlation between disease history stresses the importance of disease prevention in aquaculture production, e.g., vaccination of the fish.

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Scopus rating (2010): 0.419 0.594
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Beta-glucan bath promote wound healing in common carp (Cyprinus carpio L.)
β-glucans are well known for their ability to modulate the immune system. These polysaccharides, derived from fungi, plants and bacteria cell wall [1] potently trigger inflammatory response in infected host [2]. The effects of β-glucans depend on the origins, route of administration, molecular weight, water solubility, degree of branching and polymer length [3]. In vitro studies in mammals have shown that β-glucans directly activate leukocytes by increasing phagocytosis, cytotoxicity, antimicrobial and antiviral activity and reactive oxygen production. In addition, β-glucans affect the wound healing process [1, 4]. Previous studies have shown that β-glucans stimulate production of pro-inflammatory mediators, cytokines and chemokines like e.g. IL-8, IL-1b, or IL-6 [5]. Studies in higher vertebrates clearly show that both PAMPs (pathogen associated molecular pattern) and DAMPs (danger-associated molecular pattern) cause inflammation. The aim of this study was to investigate capability to modulate immune parameters during the wound healing processes of two commercially available β–glucans. In in vivo study, carps of ~50g were anaesthetised and wounded with 5mm biopsy punches. During the extent of the experiment the fish were kept in ordinary tap water or in tap water supplemented with two different β–glucans to a final concentration of 0.1µg/ml. Skin and muscle tissue from wounded and unwounded areas were collected at 24 hours, 3 days and 2 weeks post injury and subjected to real-time RT-PCR for measuring the expression of immune and wound healing related genes (e.g. IL-1β, IL-6, IL-8). The visual healing of the wounds was registered using a multispectral imaging device called a VideometerLab (Hørsholm, Denmark). Our results clearly show that both types of β–glucans promoted faster wound healing.

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Authors: Przybylska, D. A. (Intern), Schmidt, J. (Intern), Nielsen, M. E. (Intern)

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Only small fractions of soluble ß-glucan modulate the mucosal immune system in carp (Cyprinus carpio L.)

For decades the ability of β-glucans to modulate immunity through activation of innate cellular components has been observed. However, toxicological effects associated with the systemic administration and dose-related immune-suppression has also been described. The superior aim of this study is to understand the effect of β-glucan induced modulation in carp in relation to tissue regeneration, mucosal immunity and host-pathogen interactions. Expression profiles of immune related genes will be measured in fresh water specie – common carp (Cyprinus carpio L.). The methodology of the project involves the usage of real-time quantitative PCR to quantify expression of genes of interest (IL-1β, IL-10, TNF-α, IL-6). This study will provide further understanding on the effect of β-glucan as a modulator which could improve fish welfare and health as well as having an economic potential production for the aquaculture industry.

General information

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Authors: Przybylska, D. A. (Intern), Nielsen, M. E. (Intern)
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ß-glucans accelerate the closing of open wounds in carp, Cyprinus carpio

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Organisations: Division of Industrial Food Research, National Food Institute, DTU Data Analysis, Department of Informatics and Mathematical Modeling
Authors: Schmidt, J. (Intern), Przybylska, D. A. (Intern), Ljungqvist, M. G. (Intern), Dissing, B. S. (Intern), Ersbøll, B. K. (Intern), Nielsen, M. E. (Intern)
Publication date: 2011
Event: Poster session presented at Tissue Repair & Regeneration, New London, NH, .
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Beta-glucans promote wound healing in common carp (Cyprinus carpio L.)

β-glucans are well known for their ability to modulate the immune system. These polysaccharides, derived from fungi, plants and bacteria cell wall [1] potently trigger inflammatory response in infected host [2]. The effects of β-glucans depend on the origins, route of administration, molecular weight, water solubility, degree of branching and polymer length [3]. In vitro studies in mammals have shown that β-glucans directly activate leukocytes by increasing phagocytosis, cytotoxicity, antimicrobial and antiviral activity and reactive oxygen production. In addition, β-glucans affect the wound healing process [1, 4]. Previous studies have shown that β-glucans stimulate production of pro-inflammatory mediators, cytokines and chemokines like e.g. IL-8, IL-1b, or IL-6 [5]. Studies in higher vertebrates clearly show that both PAMPs (pathogen associated molecular pattern) and DAMPs (danger-associated molecular pattern) cause inflammation. The aim of this study was to investigate capability to modulate immune parameters during the wound healing processes of two commercially available β–glucans. In in vivo study, carps of ~50g were anaesthetised and wounded with 5mm biopsy punches. During the extent of the experiment the fish were kept in ordinary tap water or in tap water supplemented with two different β–glucans to a final concentration of 0.1µg/ml. Skin and muscle tissue from wounded and unwounded areas were collected at 24 hours, 3 days and 2 weeks post injury and subjected to real-time RT-PCR for measuring the expression of immune and wound healing related genes (e.g. IL-1β, IL-6, IL-8). The visual healing of the wounds was registered using a multispectral imaging device called a VideometerLab (Hørsholm, Denmark). Our results clearly show that both types of β–glucans promoted faster wound healing.

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Effects of beta-glucans on wound healing in carp, Cyprinus carpio

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Mucosal immune response in common carp: host pathogen interactions in relation to β-glucan stimulation

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PAMPs and DAMPs stimulate the expression of pro-inflammatory cytokines in vitro in fibroblasts from fish.
The recognition of PAMPs by immune cells relies on conserved PRRs such as TLRs, NLRs and RLRs leading to activation of NFB signaling pathways. These receptors are activated upon stimulation by different ligands such as bacterial or viral components. The binding of ligands to the receptors activates downstream signalling pathways, which subsequently leads to expression of pro-inflammatory cytokines and chemokines. DAMPs released from necrotic cells may also bind to and activate similar downstream signalling events. In teleosts it was found that mechanical damage of the muscle tissue using sterile needles induced a very rapid expression of the pro-inflammatory cytokines IL-1β, IL-8 and IL-10 as measured by real-time PCR. The results imply that cells located in the muscular tissue in addition to recruited cells are involved in the observed increased cytokine / chemokine expression. It is believed that this expression to a large extend is mediated by fibroblasts in the musculature. To investigate this, a fibroblast cell-line (RTHDF1) from the rainbow trout was stimulated with either LPS from E. coli, cell debris or supernatant from sonicated fibroblasts. Whereas LPS stimulation resulted in a significant up-regulation of the expression of IL-1β, IL-8 and IL-10 and stimulation with supernatant from sonicated cells led to a significant up-regulation of IL-1β and IL-10, while debris only stimulated the expression of IL-1β. TLR-2 and -4 are not described from salmonid fishes; however TLR-3, -5 and -9 are described in this evolutionary lineage of the bony fishes. The expression of TLR-3 and -9 receptors were significantly up-regulated following physical damage of muscle tissue as well as in stimulated fibroblasts, where LPS induced both TLR-3 and -9, supernatant

from sonicated cells only TLR-9 while debris caused no induction. The present study reinforce the idea that fibroblasts are able to react to PAMPs and DAMPs and that non-immune cell-types play an important role in the inflammatory reaction per se. From an evolutionary perspective the facilitation of an inflammatory response through recognition of PAMPs and DAMPs by non immune cells seems plausible. 1Ossum, C.G., et al. (2004) Characterization of a novel fibroblast-like cell line from rainbow trout and responses to sublethal anoxia. Journal of Fish Biology, 64, 1103-1116. This work was supported by The Directorate for Food, Fisheries and Agri Business grant nr. 3310-06-00116 and by the EU Training network on protective immune modulation in warm water fish by feeding glucans (NEMO), grant nr. 214505.

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The immune response differentially regulates Hsp70 and glucocorticoid receptor expression in vitro and in vivo in common carp (Cyprinus carpio L.)

Heat shock or stress proteins and glucocorticoids (cortisol) regulate a sequential pro-inflammatory and anti-inflammatory cytokine expression profile to effectively kill pathogens, whilst minimizing damage to the host. Cortisol elicits its effects through the glucocorticoid receptor (GR) for which Hsp70 and Hsp90 are required as chaperones. In common carp, (Cyprinus carpio) duplicated glucocorticoid receptor genes and splice variants with different cortisol sensitivities exist. We investigated the expression profiles of heat shock proteins Hsp70, Hsc70, Hsp90α and Hsp90β and the three different variants of GR in vitro in and in vivo to define their role in immune modulation. A rapid transient induction of GR1 (a and b) and Hsp70 was seen after LPS treatment in vitro in head kidney phagocytes, whereas cortisol treatment did not affect constitutive or LPS-induced expression of Hsp70 or GR1 expression. In vivo zymosan-induced peritonitis upregulated GR and Hsp70 expression which appears to increase sensitivity for cortisol-induced immune modulation. Indeed, the increased GR and Hsp70 expression correlates with inhibition of both LPS- and zymosan-induced expression of pro-inflammatory cytokines. Infection with the blood parasite T. borreli decreases GR1a expression in thymus, but increases GR2 expression in spleen. Differentially regulated expression of Hsp70 and of glucocorticoid receptor variants with different cortisol sensitivities, underlines their physiological importance in a balanced immune response.
Keyword: Heat shock protein,Immune modulation,Common carp,Glucocorticoid receptor,Teleost

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Authors: Stolte, E. H. (Ekstern), Chadzinska, M. (Ekstern), Przybylska, D. A. (Intern), Flik, G. (Ekstern), Savelkoul, H. F. (Ekstern), Verburg-van Kemenade, B. L. (Ekstern)
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Scopus rating (2006): 0.95 1.3
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Projects:

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National Food Institute
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Number of participants: 5
Phd Student:
Przybylska, Dominika Alicja (Intern)
Main Supervisor:
Nielsen, Michael Engelbrecht (Intern)
Examiner:
Nielsen, Henrik Hauch (Intern)
Hoole, David (Ekstern)
Raida, Martin (Intern)

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Source: Internal funding (public)
Name of research programme: Marie Curie (EU-stipendium)
Project: PhD

Activities:

NEMO-partners meeting
Dominika Alicja Przybylska (PhD. Student)
National Food Institute, Division of Industrial Food Research

Details
Date: 4 May 2011 → 9 May 2011
Activity: Other research and communication activities › Other

15th EAFP Conference on Diseases of Dish and Shellfish
Dominika Alicja Przybylska (PhD. Student)
National Food Institute, Division of Industrial Food Research

Details
Date: 10 Sep 2011 → 16 Sep 2011

Description
β-glucans are well known for their ability to modulate the immune system. These polysaccharides, derived from fungi, plants and bacteria cell wall [1] potently trigger inflammatory response in infected host [2]. The effects of β-glucans depend on the origins, route of administration, molecular weight, water solubility, degree of branching and polymer length [3]. In vitro studies in mammals have shown that β-glucans directly activate leukocytes by increasing phagocytosis, cytotoxicity, antimicrobial and antiviral activity and reactive oxygen production. In addition, β-glucans affect the wound healing process [1, 4]. Previous studies have shown that β-glucans stimulate production of pro-inflammatory mediators, cytokines and chemokines like e.g. IL-8, IL-1b, or IL-6 [5]. Studies in higher vertebrates clearly show that both PAMPs (pathogen associated molecular pattern) and DAMPs (danger-associated molecular pattern) cause inflammation. The aim of this study was to investigate capability to modulate immune parameters during the wound healing processes of two commercially available β–glucans. In in vivo study, carps of ~50g were anaesthetised and wounded with 5mm biopsy punches. During the extent of the experiment the fish were kept in ordinary tap water or in tap water supplemented with two different β–glucans to a final concentration of 0.1µg/ml. Skin and muscle tissue from wounded and unwounded areas were collected at 24 hours, 3 days and 2 weeks post injury and subjected to real-time RT-PCR for measuring the expression of immune and wound healing related genes (e.g. IL-1β, IL-6, IL-8). The visual healing of the wounds was registered using a multispectral imaging device called a VideometerLab (Hørsholm, Denmark). Our results clearly show that both types of β–glucans promoted faster wound healing.
Place: Split, Croatia
Activity: Lecture and oral contribution

7th Histopathology Workshop
Dominika Alicja Przybylska (PhD. Student)
National Food Institute, Division of Industrial Food Research

Details
Date: 17 Sep 2011

Description
Place: Split, Croatia
Activity: Lecture and oral contribution

Poster presentation
Dominika Alicja Przybylska (PhD. Student)
National Food Institute, Division of Industrial Food Research

Details
Date: 11 Apr 2010 → 15 Apr 2010
Event: **Poster presentation : Only small fractions of soluble ß-glucan modulate the mucosal immune system in carp (Cyprinus carpio L.)** *11/04/2010 - 15/04/2010*
Activity: Participation in conference/workshop/course/seminar › Participation in workshop, seminar, course

NEMO-partners meeting
Dominika Alicja Przybylska (PhD. Student)
National Food Institute, Division of Industrial Food Research

Details
Date: 15 Apr 2010 → 18 Apr 2010
Activity: Other research and communication activities › Other

NEMO-partners meeting
Dominika Alicja Przybylska (PhD. Student)
National Food Institute, Division of Industrial Food Research

Details
Date: 20 Sep 2010 → 24 Sep 2010
Activity: Other research and communication activities › Other

Poster presentation
Dominika Alicja Przybylska (PhD. Student)
National Food Institute, Division of Industrial Food Research

Details
Date: 3 May 2009 → 5 May 2009
Event: **Poster presentation : ß-glucan as immune modulator in Common Carp (Cyprinus carpio)** *03/05/2009 - 05/05/2009*
Activity: Participation in conference/workshop/course/seminar › Participation in workshop, seminar, course

NEMO-partners meeting
Dominika Alicja Przybylska (PhD. Student)
National Institute of Aquatic Resources

Details
Institution/organisation/company information: Lyngby/ Copenhagen
Date: 9 Sep 2009 → 11 Sep 2009
Activity: Other research and communication activities › Other

Oral presentation: Mucosal Immunology and Pathology in Carp– Glucan and Host Pathogen Interactions
Dominika Alicja Przybylska (Participant)
National Food Institute, Division of Industrial Food Research

Details
Date: 9 Sep 2009 → 11 Sep 2009
Activity: Other research and communication activities › Other