Development of a real-time PCR method coupled with a selective pre-enrichment step for quantification of Morganella morganii and Morganella psychrotolerans in fish products

Histamine fish poisoning is common and due to toxic concentrations of histamine often produced by Gram-negative bacteria in fin-fish products with a high content of the free amino acid histidine. The genus Morganella includes two species previously reported to cause incidents of histamine fish poisoning. Morganella morganii and Morganella psychrotolerans are both strong producer of histamine. However, little is known about the occurrence and critical stages for fish contamination with these bacteria. To elucidate contamination routes of Morganella, specific real-time quantitative PCR (RTi qPCR) methods for quantification of M. morganii and M. psychrotolerans have been developed. Selective primers amplified a 110 bp region of the vasD gene for M. psychrotolerans and a 171 bp region of the galactokinase gene for M. morganii. These primer-sets showed high specificity as demonstrated by using purified DNA from 23 other histamine producing bacteria and 26 isolates with no or limited histamine production. The efficiency of the qPCR reactions on artificially contaminated fish samples were 100.8% and 96.3% respectively. The limit of quantification (LOQ) without enrichment was 4 log CFU/g. A quantitative enrichment step with a selective Medium was included and improved the sensitivity of the methods to a LOQ of below 50 CFU/g in seafood. RTi qPCR methods with or without enrichment were evaluated for enumeration of Morganella species in naturally contaminated fresh fish and lightly preserved seafood from Denmark. These new methods will contribute to a better understanding of the occurrence and histamine production by Morganella species in fish products, information that is essential to reduce the unacceptably high frequency of histamine fish poisoning. (C) 2015 Elsevier B.V. All rights reserved.
Histamine and other biogenic amines

General information
State: Published
Organisations: National Food Institute, Division of Industrial Food Research
Authors: Ababouch, L. (Ekstern), Emborg, J. (Intern), Dalgaard, P. (Intern)
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Publisher: Food and Agriculture Organization of the United Nations, FAO
Editors: Ryder, J., Iddya, K., Ababouch, L.
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Number: 574
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A new predictive tool for the global seafood sector – SSSP v. 3.1 from 2009

General information
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Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources, Section for Software and GIS development
Authors: Dalgaard, P. (Intern), Mejldholm, O. (Intern), Emborg, J. (Intern), Cowan, B. J. (Intern)
Publication date: 2009
Event: Abstract from 3rd Joint Trans-Atlantic Fisheries Technology Conference, Copenhagen, Denmark.
Main Research Area: Technical/natural sciences
Source: orbit
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Publication: Research › Conference abstract for conference – Annual report year: 2009

Histamine fish poisoning – new information to control a common seafood safety issue

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Dalgaard, P. (Intern), Emborg, J. (Intern)
Number of pages: 789
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Title of host publication: Foodborne Pathogens: Hazards, risk analysis and control
Interaction between histamine producing bacteria and prediction of biogenic amine formation in seafood

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Dalgaard, P. (Intern), Jakobsen, L. C. (Intern), Emborg, J. (Intern)
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Event: Abstract from 7th Symposium of Food Microbiology, Helsingør, Denmark.
Main Research Area: Technical/natural sciences
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Source-ID: 253810
Publication: Research › Book chapter – Annual report year: 2009

Interaction between histamine producing bacteria and prediction of biogenic amine formation in seafood

General information
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Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Dalgaard, P. (Intern), Jakobsen, L. C. (Intern), Emborg, J. (Intern)
Publication date: 2009
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Bibliographical note
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Interaction between histamine producing bacteria and prediction of biogenic amine formation in seafood

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Dalgaard, P. (Intern), Jakobsen, L. C. (Intern), Emborg, J. (Intern)
Publication date: 2009
Event: Abstract from 96th Annual Meeting of International Association for Food Protection (IAFP), 12-15 July, Grapevine, Texas, USA, .
Main Research Area: Technical/natural sciences

Mathematical models for growth, heat inactivation and histamine formation by Morganella psychrotolerans and Morganella morganii were studied to evaluate the importance of these bacteria in seafood. Curves for growth and histamine formation by M. psychrotolerans in broth and seafood were generated at constant and changing storage temperatures (n=12). Observed and predicted times to formation of 100, 500 and 2000 ppm histamine were used for evaluation of an existing M. psychrotolerans histamine formation model [Emborg, J., Dalgaard, P., 2008-this issue-this issue. Modelling and predicting the growth and histamine formation by Morganella psychrotolerans. International Journal of Food Microbiology. doi:10.1016/j.jfoodmicro.2008.08.016] Growth rates for M. psychrotolerans and M. morganii were determined at different constant temperatures from 0 degrees C to 42.5 degrees C whereas heat inactivation was studied between 37.5 degrees C and 60 degrees C. A M. morganii growth and histamine formation model was developed by combining these new data (growth rate model) and data from the existing literature (maximum population density and yield factor for histamine formation). The developed M. morganii model was evaluated by comparison of predicted growth and histamine formation with data from the existing literature. Observed and predicted growth rates for M. psychrotolerans, at constant temperatures, were similar with bias and accuracy factor values of 1.15 and 1.45, respectively (n = 11). On average times to formation of critical concentrations of histamine by M. psychrotolerans were acceptably predicted but the model was not highly accurate. Nevertheless, predictions seemed useful to support decisions concerning safe shelf-life in relation to formulation, storage and distribution of chilled seafood. Parameters for the effect of temperature on growth and inactivation of M. psychrotolerans and M. morganii differed markedly with T-min of -8.3 to -5.9 degrees C vs. 0.3 to 2.8 degrees C, T-opt of 26.0 to 27.0 degrees C vs. 35.9 to 37.2 degrees C and T-max 32.0 to 33.3 degrees C vs. 44.0 to 47.4 degrees C. D-50 degrees C of 5.3 min vs. 13.1 min and z-values of 6.8 degrees C and 7.2 degrees C. At temperatures above similar to 15 degrees C M. morganii grew faster than M. psychrotolerans. Bias- and accuracy factor-values of 1.41 and 2.44 (n = 93) showed the predicted growth of M. morganii to be faster than previously observed in fresh fish and broth. In agreement with this, predicted times to formation of critical histamine concentrations by M. morganii were on
average shorter than observed in fresh fish. A combined model was suggested to predict histamine formation by both psychrotolerant and mesophilic Morganella during storage of fresh fish between 0 degrees C and 37 degrees C.
Histamine and biogenic amines: formation and importance in seafood

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Dalgaard, P. (Intern), Emborg, J. (Intern), Kjølby, A. (Ekstern), Sørensen, N. (Ekstern), Ballin, N. (Ekstern)
Pages: 292-324
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Place of publication: Cambridge
Publisher: British Welding Research Association
Editor: Børresen, T.
Main Research Area: Technical/natural sciences
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Source-ID: 225181
Publication: Research - peer-review › Book chapter – Annual report year: 2008

Histamine fish poisoning – new controls for a common seafood safety issue

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Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Dalgaard, P. (Intern), Emborg, J. (Intern)
Publication date: 2008
Event: Abstract from 5th Open SEAFOODplus conference, Copenhagen 8-10 June 2008, Denmark
Main Research Area: Technical/natural sciences

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Modelling of growth and inactivation between 0 °C and 55 °C for the important psychrotolerant and histamine producing bacterium Morganella psychrotolerans

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Modelling the effect of temperature, carbon dioxide, water activity and pH on growth and histamine formation by *Morganella psychrotolerans*

A mathematical model was developed to predict growth and histamine formation by *Morganella psychrotolerans* depending on temperature (0-20 degrees C), atmosphere (0-100% CO2), NaCl (0.0-6.0%) and pH (5.4-6.5). Data from experiments with both sterile tuna meat and Luria Bertani broth was used to develop the mathematical growth and histamine formation model. The expanded Logistic model with a growth dampening parameter (m) of 0.7 was used as primary growth model. A primary model for histamine formation during storage was obtained by combining the expanded Logistic growth model with a yield factor (Y-His/CFU). 120 maximum specific growth rate (mu(max))-values were generated for M. psychrotolerans and used to model the combined effect of the studied environmental parameters. A simple cardinal parameter type secondary model was used to model the effect of the four parameters on mu(max). The maximum population density (log N-max) was correlated with log (Y-His/CFU) and a simple constrained polynomial (quadratic) secondary model was developed for the effect of the environmental conditions on these model parameters. The developed model describes the effect of initial cell concentrations, storage conditions and product characteristics on histamine formation. This is a significant progress compared to previously available models for the effect of storage temperature only.
Biogenic amine formation and microbial spoilage in chilled garfish (Belone belone belone) - effect of modified atmosphere packaging and previous frozen storage

Abstract
Aims: To evaluate biogenic amine formation and microbial spoilage in fresh and thawed chilled garfish. Methods and Results: Storage trials were carried out with fresh and thawed garfish fillets at 0 or 5°C in air or in modified atmosphere packaging (MAP: 40% CO2 and 60% N2). During storage, sensory, chemical and microbial changes were recorded and histamine formation by isolates from the spoilage microflora was evaluated at 5°C. Photobacterium phosphoreum was responsible for histamine formation (>1000 ppm) in chilled fresh garfish. The use of MAP did not reduce the histamine formation. Strongly histamine-producing P. phosphoreum isolates formed 2080-4490 ppm at 5°C, whereas below 60 ppm was formed by other P. phosphoreum isolates. Frozen storage inactivated P. phosphoreum and consequently reduced histamine formation in thawed garfish at 5°C markedly. Conclusions: Photobacterium phosphoreum can produce above 1000 ppm of histamine in chilled fresh garfish stored both in air and in MAP. Freezing inactivates P. phosphoreum, extends shelf life and markedly reduces histamine formation in thawed MAP garfish during chilled storage.

Significance and Impact of the Study: At 5°C, more than 1000 ppm of histamine was formed in garfish; thus even when it is chilled this product represents a histamine fish-poisoning risk.
Formation of histamine and biogenic amines in cold-smoked tuna: An investigation of psychrotolerant bacteria from samples implicated in cases of histamine fish poisoning

Two outbreaks and a single case of histamine fish poisoning associated with cold-smoked tuna (CST) were reported in Denmark during 2004. The bacteria most likely responsible for histamine formation in CST implicated in histamine fish poisoning was identified for the first time in this study. Product characteristics and profiles of biogenic amines in the implicated products were also recorded. In the single poisoning case, psychrotolerant Morganella morganii -like bacteria most likely was responsible for the histamine production in CST with 2.2% ñ 0.6% NaCl in the water phase (WPS). In outbreak 1, Photobacterium phosphoreum most likely formed the histamine in CST with 1.3% ñ 0.1% WPS. In outbreak 2, which involved 10 persons, the bacteria responsible for histamine formation could not be determined. The measured concentrations of WPS were very low compared with those of randomly collected commercial samples of CST and cold-smoked blue marlin (4.1 to 12.7% WPS). Challenge tests at 5øC with psychrotolerant M. morganii and P. phosphoreum in CST with 4.4% WPS revealed growth and toxic histamine formation by the psychrotolerant M. morganii -like bacteria but not by P. phosphoreum. In a storage trial with naturally contaminated CST containing 6.9% WPS, lactic acid bacteria dominated the microbiota, and no significant histamine formation was observed during the shelf life of about 40 days at 5øC and of about 16 days at 10øC. To prevent toxic histamine formation, CST should be produced with >5% WPS and distributed with a declared 5øC shelf life of 3 to 4 weeks or less.

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State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Emborg, J. (Intern), Dalgaard, P. (Intern)
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Main Research Area: Technical/natural sciences

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Scopus rating (2016): CiteScore 1.68 SJR 0.759 SNIP 0.82
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Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 0.91 SNIP 0.957 CiteScore 1.94
Web of Science (2014): Indexed yes
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Scopus rating (2013): SJR 1.083 SNIP 1.087 CiteScore 2.11
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Scopus rating (2012): SJR 1.09 SNIP 0.981 CiteScore 2.03
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Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 0.972 SNIP 0.963 CiteScore 1.96
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
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Scopus rating (2010): SJR 1.006 SNIP 0.946
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 1.104 SNIP 1.118
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 1.123 SNIP 1.026
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 1.038 SNIP 1.122
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 1.136 SNIP 1.212
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 1.089 SNIP 1.131
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 1.266 SNIP 1.388
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 1.324 SNIP 1.63
Web of Science (2003): Indexed yes
Scopus rating (2002): SJR 1.158 SNIP 1.392
Web of Science (2002): Indexed yes
Scopus rating (2001): SJR 1.097 SNIP 1.504
Scopus rating (2000): SJR 1.119 SNIP 1.389
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http://www.ingentaconnect.com.globalproxy.cvt.dk/content/iafp/jfp/2006/00000069/00000004/art00027;jsessionid=464rino
dk8tg4.alice
Hornfisk og histaminforgiftning - årsag og forebyggelse

General information
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Authors: Dalgaard, P. (Intern), Emborg, J. (Intern)
Pages: 4-12
Publication date: 2006
Main Research Area: Technical/natural sciences

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Original language: Danish
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http://www.aqua.dtu.dk/Publikationer/Fisk-og-hav.aspx

Morganella psychrotolerans sp. nov., a histamine-producing bacterium isolated from various seafoods

Mesophilic Morganella morganii (n=6) and psychrotolerant M. morganii-like isolates from various seafoods (n=13), as well as clinical M. morganii isolates (n=3), were characterized by using a polyphasic approach including multi-locus sequencing. Based on the phylogenetic analysis, the 22 strains were divided into two distinct groups comprising mesophilic and psychrotolerant isolates, respectively. This classification was supported by DNA-DNA hybridization studies, whereby a psychrotolerant isolate (strain U2/3T) showed 41.0 and 17.8 % relatedness to the type strains of the mesophilic species Morganella morganii subsp. morganii (strain LMG 7874T) and Morganella morganii subsp. sibonii (strain DSM 14850T), respectively. Analysis of the 16S rRNA gene sequences showed a similarity of 98.6 % between mesophilic and psychrotolerant isolates. However, fragments of seven protein-encoding housekeeping genes (atpD, dnaN, gyrB, hdc, infB, rpoB and tuf) all showed less than 90.9 % sequence similarity between the two groups. The psychrotolerant isolates grew at 0-2 °C and also differed from the mesophilic M. morganii isolates with respect to growth at 37 °C and in 8.5 % (w/v) NaCl and fermentation of D-galactose. The psychrotolerant strains appear to represent a novel species, for which the name Morganella psychrotolerans sp. nov. is proposed. The type strain is U2/3T (=LMG 23374T=DSM 17886T).

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Authors: Emborg, J. (Intern), Dalgaard, P. (Intern), Ahrens, P. (Intern)
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Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Multi locus sequencing used for identification of a new species of Morganella associated with outbreaks of histamine poisoning
**Significant histamine formation in tuna (Thunnus albacares) at 2 degrees C - effect of vacuum- and modified atmosphere-packaging on psychrotolerant bacteria**

Occurrence and importance of psychrotolerant histamine producing bacteria in chilled fresh tuna were demonstrated in the present study. The objective was to evaluate microbial formation of histamine and biogenic amines in chilled fresh tuna from the Indian Ocean and stored either vacuum-packed (VP) or modified atmosphere-packed (MAP). Firstly, biogenic amines and the dominating microbiota were determined in VP tuna involved in an outbreak of histamine fish poisoning in Denmark. Secondly, the microbiota of fresh MAP tuna was evaluated at the time of processing in Sri Lanka and chemical, microbial and sensory changes were evaluated during storage at 1-3 degrees C. To explain the results obtained with naturally contaminated tuna the effect of VP and MAP on biogenic amine formation by psychrotolerant bacteria was evaluated in challenge tests at 2 degrees C and 10 degrees C. The VP tuna that caused histamine fish poisoning had a histamine concentration of > 7000 mg/kg and this high concentration was most likely produced by psychrotolerant Morganella morganii-like bacteria or by Photobacterium phosphoreum. Similar psychrotolerant M. morganii-like bacteria dominated the spoilage microbiota of fresh MAP tuna with 60% CO2/40% N2 and formed > 5000 mg/kg of histamine after 24 days at 1.7 degrees C. These psychrotolerant bacteria were biochemically similar to M. morganii subsp. morganii and
their 16S rDNA (1495 bp) showed > 98% sequence similarity to the type strain of this species. Toxic concentrations of histamine were produced at 2.1 degrees C in inoculated VP tuna by both the psychrotolerant M. morganii-like bacteria (7400 +/- 1050 mg/kg) and R. phosphoreum (4250 +/- 2050 mg/kg). Interestingly, MAP with 40% CO2/60% O2, in challenge tests, had a strong inhibitory effect on growth and histamine formation by both the psychrotolerant M. morganii-like bacteria and R. phosphoreum. In agreement with this, no formation of histamine was found in naturally contaminated fresh MAP tuna with 40% CO2/60% O2 during 28 days of storage at 1.0 degrees C. To reduce current problems with histamine fish poisoning due to VP tuna it is suggested, for lean tuna loins, to replace vacuum packaging with MAP containing similar to 40% CO2 and similar to 60% O2. (c) 2005 Elsevier B.V. All rights reserved.
Microbial spoilage and formation of biogenic amines in fresh and thawed modified atmosphere-packed salmon (Salmo salar) at 2 degrees C

Aims: To evaluate the microbial spoilage, formation of biogenic amines and shelf life of chilled fresh and frozen/thawed salmon packed in a modified atmosphere and stored at 2 degrees C.

Methods and Results: The dominating microflora, formation of biogenic amines and shelf life were studied in two series of storage trials with naturally contaminated fresh and thawed modified atmosphere-packed (MAP) salmon at 2 degrees C. Photobacterium phosphoreum dominated the spoilage microflora of fresh MAP salmon at more than 106 cfu g-1 and the activity of this specific spoilage organism (SSO) limited the shelf life of the product to ca 14 and 21 d in the two experiments. Despite the high levels of P. phosphoreum, less than 20 mg kg-1 histamine was observed in fresh MAP salmon prior to sensory spoilage. Freezing eliminated P. phosphoreum and extended the shelf life of MAP salmon at 2 degrees C by 1-2 weeks. Carnobacterium piscicola dominated the spoilage microflora of thawed MAP salmon and probably produced the ca 40 mg kg-1 tyramine detected in this product at the end of its shelf life.

Conclusions: Photobacterium phosphoreum dominated the spoilage microflora of fresh MAP salmon but produced only small amounts of biogenic amines in this product. The elimination of P. phosphoreum by freezing allowed this bacteria to be identified as the SSO in fresh MAP salmon. Significance and Impact of the Study: The identification of P. phosphoreum as the SSO in fresh MAP salmon facilitates the development of methods to determine and predict the shelf life of this product, as previously shown with fresh MAP cod. [Journal Article; In English; England].

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Emborg, J. (Intern), Laursen, B. (Ekstern), Rathjen, T. (Ekstern), Dalgaard, P. (Intern)
Pages: 790-799
Publication date: 2002
Main Research Area: Technical/natural sciences

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Projects:

**Detection of strongly histamine-producing and psychrotolerant bacteria in seafood**
Postdoc project in collaboration between the Predictive Microbiology group at DTU Aqua and DTU Systems Biology.
Funded by the Danish Research Council for Technology and Production Sciences

National Food Institute
Division of Industrial Food Research
Section for Aquatic Microbiology and Seafood Hygiene
Period: 01/01/2007 → 31/03/2010
Number of participants: 2
Acronym: Hiproba
Project participant:
**Emborg, Jette (Intern)**
Project Manager, academic:
**Dalgaard, Paw (Intern)**
**Financing sources**  
Source: Public research council  
Name of research programme: Danish Research Council for Technology and Production Sciences  
Project

**Biogene Aminer i fiskeprodukter - betydning af kuldetolerante bakterier**

Department of Systems Biology  
Period: 01/05/2004 → 26/10/2007  
Number of participants: 6  
PhD Student:  
Emborg, Jette (Intern)  
Supervisor:  
Ahrens, Peter (Intern)  
Main Supervisor:  
Dalgaard, Paw (Intern)  
Examiner:  
Martinussen, Jan (Intern)  
Bover I Cid, Sara (Ekstern)  
Knøchel, Susanne (Intern)

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Source: Internal funding (public)  
Name of research programme: Eksternt EU-finansieret  
Project: PhD