VKM Vitenskapskomiteen for mat og miljø
Norwegian Scientific Committee for Food and Environment

VKM Bulletin 2024: 10

Risk assessment of *Psychrobacter* sp. as plant protection product - Nemaslug®

Åsa Frostegård, Nana Y. Boahene, Christer Magnusson, Kjetil K. Melby, Siamak Yazdankhah, Beatrix Alsanius, Jorunn Børve, Paal Krokene, Mogens Nicolaisen, Line Nybakken, Johan A. Stenberg, Iben M. Thomsen, Sandra A.I. Wright, May-Guri Sæthre

Scientific Opinion of the Panel on Plant Health of the Norwegian Scientific Committee for Food and Environment

VKM Bulletin 2024: 10
Risk assessment of *Psychrobacter* sp. as plant protection product - Nemaslug®

Scientific Opinion of the Panel on Plant Health of the Norwegian Scientific Committee for Food and Environment
20.08.2024

ISSN: 2704-1689
Norwegian Scientific Committee for Food and Environment (VKM)
Postboks 222 Skøyen
0213 Oslo
Norway

Phone: +47 21 62 28 00
Email: vkm@vkm.no

vkm.no

Cover photo: Colourbox

Suggested citation: VKM, Åsa Frostegård, Nana Y. Boahene, Christer Magnusson, Kjetil K. Melby, Siamak Yazdankhah, Beatrix Alsanius, Jorunn Børve, Paal Krokene, Mogens Nicolaisen, Line Nybakken, Johan A. Stenberg, Iben M. Thomsen, Sandra A.I. Wright, May-Guri Sæthre (2024). Risk assessment of *Psychrobacter* sp. as plant protection product - Nemaslug®. Scientific Opinion of the Panel on Plant Health of the Norwegian Scientific Committee for Food and Environment. VKM Bulletin 2024:10, ISSN: 2704-1689. Norwegian Scientific Committee for Food and Environment (VKM), Oslo, Norway.

Risk assessment of *Psychrobacter* sp. as plant protection product - Nemaslug®

Preparation of the opinion

The Norwegian Scientific Committee for Food and Environment (Vitenskapskomiteen for mat og miljø, VKM) appointed a project group to draft the opinion. The project group consisted of three VKM members and two VKM members of staff. The Committee, by the Panel on Plant Health appointed specifically for the assignment, assessed and approved the final opinion.

Authors of the opinion

The authors have contributed to the opinion in a way that fulfils the authorship principles of VKM (VKM, 2019). The principles reflect the collaborative nature of the work, and the authors have contributed as members of the project group and the Panel on Plant Health, appointed specifically for the assignment.

Members of the project group (in alphabetical order after chair of the project group):

Åsa Frostegård – Chair of the project group. Member of the Panel on Microbial Ecology in VKM. Affiliation: 1) VKM; 2) Norwegian University of Life Sciences (NMBU)

Nana Y. Boahene – Project leader, VKM staff. Affiliation: VKM.

Christer Magnusson – Member of the Panel on Plant Health in VKM. Affiliation: 1) VKM; 2) NIBIO.

Kjetil K. Melby – Member of the Panel on Genetically Modified Organisms - Medicinal Products in VKM. Affiliation: 1) VKM; 2) University of Oslo (UiO) 3) Oslo University Hospital.

Siamak Yazdankhah – VKM staff. Affiliation: VKM

Members of the Panel on Plant Health (in alphabetical order before chair of the Panel):

Beatrix Alsanius – Affiliation: 1) VKM; 2) SLU.

Jorunn Børve – Affiliation: 1) VKM; 2) NIBIO.

Paal Krokene – Affiliation: 1) VKM; 2) NIBIO.

Christer Magnusson – Affiliation: 1) VKM; 2) NIBIO.

Mogens Nicolaisen – Affiliation: 1) VKM; 2) Aarhus University.

Line Nybakken – Affiliation: 1) VKM; 2) NMBU.

Johan A. Stenberg – Affiliation: 1) VKM; 2) SLU.

Iben M. Thomsen – Affiliation: 1) VKM; 2) University of Copenhagen.

Sandra A.I. Wright – Affiliation: 1) VKM; 2) University of Gävle.

May-Guri Sæthre – Chair of the Panel on Plant Health. Affiliation: 1) VKM; 2) Norges forskningsråd.

Competence of VKM experts

Persons working for VKM, either as appointed members of the Committee or as external experts, do this by virtue of their scientific expertise, not as representatives for their employers or third-party interests. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

Table of Contents

Summary

Background: The Norwegian Food Safety Authority (NFSA) requested the Norwegian Scientific Committee for Food and Environment (VKM) to conduct a risk assessment of the bacterium *Psychrobacter* sp. used in Nemaslug®. The plant protection product Nemaslug®, approved for use in Norway since 2005, contains the nematode *Phasmarhabditis hermaphrodita*. This nematode carries a symbiotic bacterium that acts as the active biocontrol organism by producing toxins that kill snails upon infection. The background for this assignment is that it was revealed that the bacterium in Nemaslug® is not *Moraxella osloensis*, upon which the previous approval was based. Instead, it has been identified as a species in the bacterial genus *Psychrobacter*, most likely *Psychrobacter faecalis*, alternatively *Psychrobacter pulmonis*. The present assessment will form the basis for the Norwegian Food Safety Authority's decision on whether Nemaslug® should retain its approval for use in Norway or not.

Methods: The draft assessment was conducted by a project group appointed by VKM consisting of three VKM members and two VKM members of staff. Data and information were gathered from the application provided by the NFSA and the previous report by VKM. A thorough literature search was conducted to gather additional relevant information.

Results: Species of *Psychrobacter* have a global distribution but are predominantly found in cold environments. However, strains of *P. faecalis* and *P. pulmonis* have been isolated from pigeon and lamb and can grow at 36-37 °C. Reports on the occurrence of *Psychrobacter* sp. in Norway are scarce, with the majority of the isolates originating from arctic and marine environments. The potential for spread and establishment of *Psychrobacter* sp. in the environment under Norwegian conditions is considered low. There is limited information on the health hazards of *Psychrobacter* sp. in humans, but it is generally regarded as a low-risk pathogen. The risk to non-target organisms, particularly molluscs, from Nemaslug® is uncertain and requires further research.

Uncertainty: There is uncertainty regarding the species affiliation of the bacterium used in Nemaslug®, although it is most likely *P. faecalis*. The taxonomic relationship between *P. faecalis* and *P. pulmonis* makes differentiation between the species based on 16S rRNA gene sequencing difficult. Further taxonomic analyses using complete genome sequencing or multilocus sequence analysis (MLSA) is recommended. There is also uncertainty regarding the effects of Nemaslug® on non-target molluscs.

Conclusion: The human health risk from using Nemaslug® is considered low, although a risk for highly infection-prone patient groups cannot be ruled out. There is no evidence of human health issues arising from the consumption of food products treated with Nemaslug®. The occurrence of *Psychrobacter* sp. in Norway is limited, and the potential for its spread and establishment in the environment is considered to be low. The risk to non-target organisms, particularly to molluscs, posed by Nemaslug® is uncertain and requires further research.

Data gaps: Further taxonomic analysis using MLSA, or complete genome sequencing should be required to confirm the species affiliation of the bacterial component of Nemaslug®. Knowledge on antibiotic resistance, as well as pathogenicity, in the genus *Psychrobacter* is largely lacking. More research is also needed to assess the effects of Nemaslug® on non-target organisms, especially on molluscs.

Key words: VKM, risk assessment, Norwegian Scientific Committee for Food and Environment, Norwegian Food Safety Authority, *Psychrobacter* sp*., Phasmarhabditis hermaphrodita,* Nemaslug®

Sammendrag på norsk

Bakgrunn: Vitenskapskomiteen for mat og miljø (VKM) har på oppdrag fra Mattilsynet gjort en risikovurdering av bakterien *Psychrobacter* sp. brukt i Nemaslug®. Plantevernmiddelet Nemaslug®, som er godkjent for bruk i Norge siden 2005, inneholder nematoden *Phasmarhabditis hermaphrodita*. Nematoden bærer en symbiotisk bakterie som fungerer som den aktive biokontrollorganismen ved å produsere giftstoffer som dreper snegler ved infeksjon. Bakgrunnen for oppdraget er at det ble avdekket at bakterien i Nemaslug® ikke er *Moraxella osloensis*, som den tidligere godkjenningen er basert på. Den er i stedet blitt identifisert som en art i bakterieslekten *Psychrobacter*, mest sannsynlig *Psychrobacter faecalis*, alternativt *Psychrobacter pulmonis*. Denne vurderingen skal danne grunnlag for Mattilsynets beslutning om hvorvidt Nemaslug® skal beholde sin godkjenning for bruk i Norge eller ei.

Metode: VKM satte ned en prosjektgruppe til å utføre risikovurderingen. Data og informasjon er hentet fra søkeren via Mattilsynet og VKMs forrige rapport om Nemaslug®. Deretter er det gjennomført et grundig litteratursøk for å innhente ytterligere relevant informasjon.

Resultater: Artene i slekten *Psychrobacter* har en global utbredelse og finnes hovedsakelig i kalde omgivelser. Stammer av *P. faecalis* og *P. pulmonis* har blitt isolert fra due og lam, og kan vokse ved 36-37 grader Celsius. Det er få rapporterte funn av *Psychrobacter* sp. i Norge og disse isolatene kommer hovedsakelig fra arktiske og marine miljøer. Potensialet for spredning og etablering av *Psychrobacter* sp*.* i miljøet under norske forhold vurderes som lavt. Det er begrenset informasjon om helsefarene ved *Psychrobacter* sp*.* for mennesker, men det regnes generelt som et lavrisiko-patogen. Risikoen som Nemaslug® utgjør for ikke-målorganismer, spesielt bløtdyr, er usikker og krever videre forskning.

Usikkerhet: Det er usikkerhet knyttet til hvilken bakterieart som er brukt i Nemaslug®, selv om det mest sannsynlig er *P. faecalis*. Det taksonomiske forholdet mellom *P. faecalis* og *P. pulmonis* gjør at det er vanskelig å skille mellom artene basert på 16S rRNA-gensekvensering. Videre taksonomisk analyse ved bruk av multilocus sekvensanalyse (MLSA) eller fullstendig genomsekvensering anbefales. Det er også usikkerhet rundt hvilken effekt Nemaslug® har på ikke-målorganismer blant mollusker.

Konklusjon: Selv om informasjonen om helsefarene knyttet til *Psychrobacter* sp. hos mennesker er begrenset, betraktes den generelt som et lavrisikopatogen. Helserisikoen for mennesker ved bruk av Nemaslug® anses derfor som lav, men risiko for pasientgrupper som er svært mottakelige for infeksjoner kan ikke utelukkes. Det er ingen bevis for helseproblemer som oppstår ved inntak av matvarer behandlet med Nemaslug®. Forekomsten av *Psychrobacter* sp. i Norge er begrenset, og potensialet for spredning og etablering i miljøet betraktes som lavt. Risikoen som Nemaslug® utgjør for ikke-målorganismer, spesielt mollusker, er usikker og krever videre forskning.

Manglende data: Videre taksonomisk analyse ved hjelp av MLSA og fullstendig genomsekvensering er nødvendig for å bekrefte artstilhørigheten til bakterien som inngår i Nemaslug®. Kunnskap om antibiotikaresistens, samt patogenisitet, i slekten

Psychrobacter er i stor grad mangelfull. Mer forskning er også nødvendig for å vurdere effekten av Nemaslug® på ikke-målorganismer blant mollusker.

Nøkkelord: VKM, risikovurdering, Vitenskapskomiteen for mat og miljø, Mattilsynet, *Psychrobacter* sp*., Phasmarhabditis hermaphrodita,* Nemaslug®

Abbreviations and glossary

Abbreviations

MaldiToF MS	Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry
MLSA	Multilocus Sequence Analysis
NFSA	Norwegian Food Safety Authority
VKM	Norwegian Scientific Committee for Food and Environment

Glossary

Gastropods: A large taxonomic class (latin Gastropoda) of invertebrate animals within the phylum of molluscs (Mollusca). Gastropods are commonly known as slugs and snails.

Molluscs: A large taxonomic phylum (latin Mollusca) of invertebrate animals comprising five taxonomic classes, including the class Gastropoda (slugs and snails).

Psychrotrophs: Cold tolerant bacteria and archaea that can grow at low temperatures (0-7 °C) but have a maximum growth temperature above 20 °C.

Psychrophiles: ”Cold loving” bacteria and archaea adapted to grow at 0 °C or even below and with a maximum temperature for growth at 20 °C or below.

Background as provided by the Norwegian Food Safety Authority

Psychrobacter sp., most likely *P. faecalis*, is the symbiotic bacterium of the beneficial nematode *Phasmarhabditis californica* and the effective organism in the product **Nemaslug® 2.0.** Approval of this product was applied for and rejected 08.04.2022.

The evaluation process of Nemaslug® 2.0 uncovered that the earlier approved product **Nemaslug®,** like Nemaslug® 2.0**,** contains the symbiotic bacterium *Psychrobacter* sp., and not *Moraxella osloensis* as the applicant stated earlier. *Moraxella osloensis* was evaluated in a report by VKM in 2008. In order to keep the approval for Nemaslug® to the Norwegian market, *Psychrobacter* sp. has to be risk assessed. The outcome of the assessment will influence whether the product Nemaslug® will keep its approval or not.

Terms of reference as provided by the Norwegian Food Safety Authority

With focus on the bacteria *Psychrobacter* sp., The Norwegian Food Safety Authority would like an assessment of the following:

- The human health risk by using the plant protection product Nemaslug®.
- The human health risk by consumption of food products treated with Nemaslug®.
- The occurrence of the bacteria in Norway, and whether it is naturally occurring.
- The organisms' potential to establish and spread in the environment under Norwegian climatic conditions, specified by the use of Nemaslug® in greenhouses and outdoors. The risk of effects on indigenous species under Norwegian climatic conditions should also be assessed.
- Uncertainty about taxonomy which can make the risk assessment difficult.

1 Methodology and Data

The following sections describe the strategy used for retrieving and compiling the information used in this assessment.

1.1 Data and information gathering

Specific information regarding the biocontrol product Nemaslug® was retrieved and reviewed from attachments to the application and through correspondence with the applicant as provided by the Norwegian Food Safety Authority (NFSA). Additional data and information were retrieved from VKM's report from 2021 (VKM et al., 2021).

1.2 Literature search and selection

A specific search strategy was drafted in cooperation with an experienced librarian, who performed an initial literature search on bacteria belonging to the genus *Psychrobacter*, see Appendix I for details. The following databases were searched: Ovid Medline, Embase, Web of Science, the Cochrane Database of systematic reviews, CAB Abstracts, Cinahl, and Epistemonikos. The search resulted in a total of 240 references after duplicates were discarded.

Screening of titles and abstracts was undertaken pairwise and blinded by members of the project group, employing the Rayyan tool for systematic literature screening. The screening was based on the set of inclusion and exclusion criteria agreed on by the project group beforehand, with reference to the terms of reference provided by the Norwegian Food Safety Authority for this assessment (see Tables 1 and 2). Eligible publications, a total of 11, were exported to an Endnote library and made available to the members of the project group.

Additional manual searches for relevant articles and grey literature that were not picked up by the main search were also performed in PubMed and Google Scholar to strengthen the knowledge base of the assessment.

Table 1: Overview of eligibility criteria

Context	Research articles on *Psychrobacter* sp*.* and /or Nemaslug®.
Language	Publications in English, Norwegian or other Scandinavian language.
Date	No limits
Type of publications	Primary studies, review articles and reports

Table 2: Overview of exclusion criteria

Context	Research articles that do not include *Psychrobacter* sp. and /or Nemaslug®.
Language	Publications not in English, Norwegian or other Scandinavian language.
Date	Not relevant
Type of publications	Letter to editor, comments, conference abstract, posters

1.3 Product and trade name

The product Nemaslug® (BASF Agricultural Specialities) is intended for biocontrol of slugs. It contains nematodes carrying symbiotic bacteria that are the active biocontrol organisms (Stenberg et al., 2021; Tan et al., 2001).

1.3.1 Associated organisms

The nematode in Nemaslug® is *Phasmarhabditis hermaphrodita.* The nematode is distributed as a third stage infective dauers/juveniles. The bacterium used when growing *P. hermaphrodita* for production of Nemaslug® was earlier described as *Moxarella osloensis,* based on phenotyping methods, but more recent sequencing of the 16S rRNA gene reclassified it as *Psychrobacter* sp., most likely *P. faecalis* or possibly *P. pulmonis*. These two species are phylogenetically very close and cannot be separated by 16S rRNA gene sequencing (Deschaght et al., 2012).

The nematode acts as a vector, while the current understanding is that the bacteria produce toxins upon infestation of the target organisms. The bacterium should thus be viewed as the active biocontrol organism (Stenberg et al., 2021; Tan et al., 2001), even though the mechanism of biocontrol is completely dependent on the vector.

1.3.2 Natural distribution

The nematode *P. hermaphrodita* is globally widespread and has been reported from several European countries as well as from Egypt, Chile, Iran, New Zealand, USA and Canada (Rae et al., 2023). In a study from Norway, the nematode was present in 14 out of 32 locations (44 %) (Ross et al. 2015). One reason for the wide distribution of this species is most likely that the nematodes have established in new areas following use of Nemaslug® (Howe et al., 2020).

The bacterial genus *Psychrobacter* is considered to have a global distribution but has predominantly been reported from low-temperature environments, including marine waters, sediments and soils in polar areas. Most *Psychrobacter* species have an optimal growth temperature of 20 to 30 °C, but many grow at temperatures down to 4 °C. The type of strain of the species *P. faecalis* was isolated from pigeon feces in Germany and has been reported to grow at temperatures up to 36 °C (Kämpfer et al., 2002). *P.*

pulmonis was first isolated in Spain from lungs of lambs and was reported to grow at 37 °C (Vela et al., 2003).

Assessment

2 Introduction

Nematodes of the species *Phasmarhabditis hermaphrodita* are bacterial feeders and detritivores living in leaf litter, compost and organic soils where they consume dead invertebrates, slug faeces and associated bacteria. They are also facultative parasites of slugs and snails, including those that are pests of agricultural and horticultural plants. During periods of food shortage, the nematodes can form developmentally arrested nonfeeding juveniles (dauer-juveniles) that move locally in the soil in search for new food sources. When encountering a slug, the dauer-juvenile infects the slug by entering through natural openings at the rear of the slug's mantle. Once inside the body, the dauer-juvenile may stay inactive until the slug dies and then develop to regular feeding juvenile stages, which commence their development while feeding on bacteria flourishing on the slug cadaver. Still, however, it is unclear as to what extent the nematodes are responsible of killing the slug by their own activity. The juveniles develop into self-fertilizing female hermaphrodites (males are uncommon or absent) that lay eggs and allow the population to increase. When the slug cadaver is depleted, new dauer-juveniles are formed that leave to search for new food sources (Rae et al., 2023; Wilson et al., 2005).

2.1 Purpose and scope

This assessment is intended to update the risk assessment of the bacteria in the biocontrol product Nemaslug®, considering new information regarding the taxonomy of the bacteria that has been obtained from the applicant. Risk assessment of the nematode *Phasmarhabditis hermaphrodita* is not part of the terms of reference in this report.

2.2 Relevance for use as plant protection product

Chemical bait pellets containing metaldehyde are commonly used for controlling slugs, but there are concerns regarding leakage of this noxious compound into watersheds as well as toxic effects on diverse non-target organisms, including vertebrates. A biological control alternative is to apply the parasitic nematode *P. hermaphrodita*, which has been used as a biological control agent in several countries since its introduction on the market in 1994 (Pieterse et al., 2017; Rae et al., 2023).

2.3 Hazard identification

Hazard identification is implicit in the terms of reference.

2.4 Hazard characterization

2.4.1 The bacterial component

The bacterium associated with the nematode *P. hermaphrodita,* used in the version of Nemaslug® evaluated here*,* was earlier reported to be the species *Moraxella osloensis* (Tan et al., 2001). The process resulting in slug death was initially described for the grey garden slug, *Deroceras reticulatum* (Tan et al., 2002). Infective juveniles of *P. hermaphrodita* invade the slug's shell cavity and release bacteria. These bacteria likely act as the primary lethal agents to the slugs, possibly through the release of a lipopolysaccharide-based endotoxin. As the slug weakens and dies, the juvenile nematodes feed on it and mature into self-fertilizing hermaphrodites. They then produce the next generation of infective juveniles, which vacate the corpse to seek new hosts. While the details of the infection mechanisms remain unclear, a recent compilation of results from various studies involving diverse molluscs from around the world revealed susceptibility to *P. hermaphrodita* infection in twelve out of 22 slug species and eight out of 21 snail species (Rae et al., 2023).

The development of the Nemaslug® as a product for biological control of slugs included the testing of a range of bacterial species isolated from the nematode *P. hermaphrodita* (Rae et al., 2023 and references therein). The bacterium *Moraxella osloensis* was chosen due to its consistent production of high yields of pathogenic nematodes. Recently, genome sequencing revealed that the bacterium associated with the commercially available biocontrol nematode *P. hermaphrodita* in Nemaslug® is not *M. osloensis,* as has been claimed during 25 years of commercial use, but instead is a species in the genus *Psychrobacter* (Sheehy et al., 2022). Supporting this, sequencing results (16S rRNA gene sequencing) provided by BASF Agricultural Specialities identified the bacterium in the Nemaslug® product as *Psychrobacter* sp., most likely *P. faecalis* or possibly *P. pulmonis*.

The genus *Psychrobacter* encompasses cold-tolerant, Gram-negative, non-spore-forming gamma-proteobacteria of the family *Moraxellaceae* (Bowman, 2006). Currently, the List of Prokaryotic names with Standing Nomenclature (LPSN) includes more than 40 *Psychrobacter* species (Parte et al., 2020) isolated from permafrost (Bakermans et al., 2006), seawater, and marine sediments (Matsuyama et al., 2015; Yoon et al., 2005), marine animals such as crustaceans and fish (Kristiansen et al., 2013; Romanenko et al., 2009), marine mammals (Apprill et al., 2014), and food products (Bjerke et al., 2019; Bjørkevoll et al., 2003). Some *Psychrobacter* species have been isolated from warm-blooded animals, including pigeon (Kämpfer et al., 2002), pig digestive tract (Koh et al., 2015), human gut (Lagier et al., 2016), and lamb lungs (Vela et al., 2003). The genus *Psychrobacter* is closely related to the genus *Moraxella*, although there are some differences in genetic properties. Bacteria belonging to *Psychrobacter* have, for example, larger genomes (Welter et al., 2021).

The species *P. faecalis* was described as a novel species by Kämpfer et al. (2002). It has been reported to grow well at temperatures between 4 and 36 °C. So far, no studies have demonstrated any cases of infections to any species (Sheehy et al., 2022). *P. pulmonis* was isolated from lungs of lambs and described as a novel species by Vela et al (2003), able to grow at 5 to 37 °C. Both *P. faecalis* and *P. pulmonis*, as well as a

couple of other *Psychrobacter* species, may cause opportunistic infections in mammals (Deschaght et al., 2012).

An important observation made by Welter et al. (2021) is that organisms within the *Psychrobacter* genus fall into two ecotypes. One is the flexible ecotype (FE) capable of growth at 37 °C, and the other is the restricted ecotype (RE), consisting of psychrotrophs or psychrophiles that can't grow at temperatures above 20 °C. This distinction could account for the variations in temperature ranges reported in the literature for this genus. The FE group tends to include more strains from mammalian sources, while the RE group generally consists of strains derived from other hosts, food products, and marine and terrestrial sources. Both ecotypes have been identified in mammals. The study by Welter et al. (2021) suggests that the genus *Psychrobacter* evolved from a mammalian-associated ancestor, potentially a pathobiont or a pathogen. Metagenome analyses of bacteria present in the nematode species *P. californica*, *P. neopapillosa*, and two strains of *P. hermaphrodita* revealed that both wild type and commercial strains of *Phasmarhabditis* harbored a diverse range of bacterial phyla. The most abundant belonged to Pseudomonadota, Bacillota, Actinobacteriota, and Bacteroidota (Sheehy et al., 2022). This suggests that multiple bacterial species could be causing mortality in various infected slugs/snails (a recent list of snails and slugs shown to be susceptible to infection by *Phasmarabditis* species is found in Rae et al. (2023).

2.4.2 Virulence factors

The bacterium used in Nemaslug®, earlier described as *Moraxella osloensis*, has been re-identified as being *Psychrobacter faecalis* or *P. pulmonis.* The producer of the biocontrol product has, however, not provided a definite identification. There are, to our knowledge, no reports on virulence factors in *P. faecalis* or *P. pulmonis.* Studies of *M. osloensis* have suggested that lipopolysaccharides (LPS) released from the bacterium play a role in the killing of snails. Other possible virulence factors have also been suggested (Sheehy et al., 2022; Tan et al., 2001).

2.4.3 Pathogenicity in humans

The pathogenic potential of *Psychrobacter* species in humans has not been studied systematically, but clinical cases have been reported (Bonwitt et al., 2018; Caspar et al., 2013; Deschaght et al., 2012; Kumaria et al., 2022; Wirth et al., 2012). We reviewed data from blood samples obtained from patients in the Oslo area suspected to have invasive bacterial infections. Data from 2012, 2013, and 2014 and from parts of 2022 and the entirety of 2023 were reviewed. In 2022, 696 bacterial isolates from blood (mostly) and some spinal fluids were obtained, and one isolate of *Moraxella osloensis* was detected. In 2023, 1740 isolates were analyzed, and one isolate of *M. osloensis* was detected. Specific isolates, such as *Moraxella* species, were mostly identified using Matrix Assisted Laser Desorption Ionization-Time of Flight mass spectrometry (MaldiToF MS). So far, no *Psychrobacter* species have been detected in blood cultures by a routine diagnostic laboratory providing services for hospitals in the Oslo area (K.K. Melby, personal communication, 06.06.2024). These results suggest that *P. faecalis/pulmonis* have a very low pathogenic potential in humans, but it should not be completely ruled

out that they may be pathogenic to the increasing number of highly infection-prone patients. This microbe may pose a challenge for these patients as well as newborns (Caspar et al., 2013; Kumaria et al., 2022; Ortiz-Alcantara et al., 2016). In addition, infection in an immunocompetent individual has been described (Caspar et al., 2013). Finally, it should be kept in mind that *Psychrobacter* species may be difficult to detect using routine methods, including MaldiToF MS (Bonwitt et al., 2018; Brauge et al., 2021). The scarcity of known infections with *Psychrobacter* species is reflected in the 2020 issue of Mandell Infectious Diseases, which does not comment on this microbe among the various microbes discussed (Bennett et al., 2020).

2.4.4 Antimicrobial resistance

Data provided by the producer show that the microbe included in Nemaslug® is generally sensitive to most antibiotics. Knowledge on antibiotic resistance in the genus *Psychrobacter* is largely lacking. Studies are limited to presentations of *in vitro* data from conventional antibiotic sensitivity tests performed in connection to clinical case investigations. A beta-lactamase test (beta-lactam inactivation) in a single case report was negative (Bonwitt et al., 2018). Data published on *Psychrobacter* sp. patient isolates which had been further examined (Bonwitt et al., 2018; Caspar et al., 2013; Le Guern et al., 2014; Leung et al., 2006) showed strains mostly being susceptible to both beta-lactam antibiotics (cell-wall construction inhibitors) and protein synthesis inhibitors (macrolides, aminoglycosides and tetracyclines) and DNA coiling (quinolones).

At present, studies have focus on detecting environmental resistance genes (ERG) and/or antibiotic resistance genes (ARG) in various microbiomes. Possible resistance in *Psychrobacter* sp. to antibiotics, including beta-lactams, has been reported (Kim et al., 2013; Santos et al., 2022) including suggestions as to mechanisms of action. They suggest that ARG is mainly based on antibiotic efflux (50%), antibiotic inactivation (33%) and antibiotic target alteration (8%) (Cuadrat et al., 2020). Reports on ARG have pointed on mobile gene elements in strains recovered from non-clinical environments (Aslam et al., 2018; Cuadrat et al., 2020; Kim et al., 2013). *Psychrobacter* may harbour various resistance gene markers (Cuadrat et al., 2020; Kim et al., 2013), and thus potentially act as a gene element reservoir given the microbe's adaptability to varying growth conditions like temperature.

Regular human exposure to the bacterial strain, or species, used in Nemaslug®, is likely. This includes humans and animals exposed to antibiotics. Regular antibiotic sensitivity testing should ensure that the strain remains sensitive to antibacterial agents we have at our disposal.

2.5 Regulatory status in Norway

The use and handling of biological agents in general, and in laboratories in particular, is regulated according to the pathogenicity of the microorganism in question. A list of microorganisms and their pathogenicity is given in the Norwegian Labour Inspection Authority Regulations on Biosafety (Lovdata - Forskrift om tiltaksverdier og grenseverdier for fysiske og kjemiske faktorer i arbeidsmiljøet samt smitterisikogrupper for biologiske faktorer (Lovdata, 2024)). Neither *P. faecalis* nor *P. pulmonis* are listed in the Norwegian

Labour Inspection Authority Regulations on Biosafety, implying that these bacteria are not perceived to be infectious. The nematode *Phasmarhabditis hermaphrodita* used in Nemaslug® is approved for use in Norway (Godkjente produkter og virksomheter for godkjenning av plantevernmidler, Mattilsynet).

3 Risk characterization

3.1 Occurrence and distribution in Norway

While *Psychrobacter* species are distributed worldwide, reports from Norway are scarce. Bacterial isolates identified as *Psychrobacter* have been reported from the Arctic, for example from guano on Svalbard, and from lobster caught in the North Sea, but the strains were not identified as *P. faecalis* and were generally only described as *Psychrobacter* spp. belonging to other groups within *Psychrobacter* (Bekaert et al., 2015; Dziewit et al., 2013). Bacteria in the genus *Psychrobacter* have also been identified in other commercial seafood products in Norway, such as in salt-cured cod (Bjørkevoll et al., 2003) and in brine from six Norwegian producers of "rakfisk", a fermented fish product (Bjerke et al., 2019).

3.2 Potential for spread, establishment and dispersal

The ability of a bacterial population to invade an environment is strongly dependent on the diversity of the resident microbial community (Mawarda et al., 2022). The microbial diversity of soils is very high compared to most other environments, and invading bacteria face several challenges, including competition for resources, predation, and infection by bacteriophages. In addition, different abiotic factors may affect the survival of new bacterial species. It is therefore considered unlikely that *Psychrobacter faecalis* will establish and spread in the environment under Norwegian conditions.

3.2.1 Climatic limitations

Bacteria belonging to the genus *Psychrobacter* are predominantly found in cold, non-polar environments, including Antarctic glacier mud and sediment, as well as in soils, sea ice, alpine soil, Siberian permafrost, and Arctic seawater (Lasa et al., 2017). A recent investigation by Welter et al. (2021) of 15 *Psychrobacter* species showed that they had a growth temperature range of 4 to 38 °C. Most species have a growth optimum around 20 °C, but *P. faecalis* and *P. pulmonis* can grow at 36-37 °C. There are thus no apparent climatic limitations for these bacteria in most Norwegian environments.

3.2.2 Dispersal ability

The bacterium *Psychrobacter faecalis* and/or *P. pulmonis* present in Nemaslug® will spread primarily together with its vector, the nematode *P. hermaphrodita*.

3.2.3 Taxonomic challenges

Based on 16S rRNA gene sequencing, the producer of the biocontrol product identifies the bacterium in Nemaslug® as *Psychrobacter faecalis* or, possibly, the closely related *Psychrobacter pulmonis*. The differences between the two species appear to be marginal (Deschaght et al., 2012). A more detailed taxonomic analysis is desirable.

3.3 Health hazards

3.3.1 Human health

A limited number of published case reports suggest that only certain strains of *Psychrobacter* are regarded as medically significant, opportunistic pathogens in humans, mammals and fish. The precise mechanisms of pathogenesis and the epidemiology of pathogenic strains remain largely unknown. Transmission typically occurs through environmental and nosocomial (hospital-acquired) contact, such as breathing aerosols and wound infections. Notable infections in humans include rare cases of chronic peritonitis, infant meningitis, bacteremia, ocular infections like keratoconjunctivitis, endocarditis, and infective arthritis (Bonwitt et al., 2018). There have also been instances of *Psychrobacter* strains causing infections in AIDS patients and lung infections in sheep. None of the *Psychrobacter* strains that cause disease in humans have so far been identified as *P. phaecalis* or *P. pulmonis*. However, it should be noted that both these bacterial species can be present in the human microbiome (Deschaght et al., 2012) and have also been isolated from warm-blooded animals such as pigeon and lamb (Welter et al., 2021).

Psychrobacter strains are generally sensitive to antimicrobial drugs and reports on antibiotic resistance in this genus are scarce. Resistance against β-lactams (penicillin) have been detected in strains of *P. phenylpyruvicus* and *P. immobilis* (Caspar et al., 2013), and resistance has also been reported against other antibiotics, for example against tetracycline and streptomycin in a *P. psychrophilus* strain (Petrova et al., 2009), and against gentamicin, tobramycin, ampicillin and lincomycin in a strain of *P. immobilis* (Lozano et al., 1994).

3.3.2 Animal health and biodiversity

There is limited understanding of the potential impacts of the nematode *Phasmarhabdites hermaphrodita* and its associated bacterial strains on non-target molluscs. However, a recent study reviewing results from investigations of the susceptibility of several slug and snail species to *P. hermaphrodita*, showed that 12 of 22 investigated slug species and 8 of 21 snail species were killed by the nematode (Rae et al., 2023). Increased mortality of non-target terrestrial gastropods was also shown in another recent study (Denver et al., 2024). Although the referred studies were performed in different parts of the world, it seems likely that Nemaslug® will kill some non-target molluscs also in Norway.

The type strains of the bacteria *Psychrobacter faecalis* and *P. pulmonis* were isolated from pigeon faeces and lamb lungs, respectively (Kämpfer et al., 2002; Vela et al., 2003). None of these animals did, however, show any sign of disease.

3.3.3 Potential for damage to plants

Psychrobacter faecalis and *P. pulmonis* are not known as plant pathogens and are not listed in the global database of the European Plant Protection Organization (EPPO Global Database and GD Desktop/2021-03-23).

4 Uncertainties

There is uncertainty concerning:

- the species affiliation of the bacterium. According to a 16S rRNA gene sequence analysis provided by the producer/applicant it likely belongs to *Psychrobacter faecalis,* but this method is unable to differentiate this species from closely related bacteria such as *Psychrobacter pulmonis*

- the degree of pathogenicity of *Phasmarhabditis hermafrodita* and its associated bacterium *Psychrobacter faecalis,* or *P. pulmonis*, to non-target molluscs.

5 Conclusions (with answers to the terms of reference)

5.1 Human health risk by using the plant protection product Nemaslug®

The 2020 issue of the reference textbook for infectious diseases, Mandell Infectious Diseases (Bennett et al., 2020), does not comment on *Psychrobacter* species. Also, no *Psychrobacter* species have so far been detected by the routine diagnostic laboratory that provides services for hospitals in the Oslo area. However, infection in an immunocompromised individual has been described. Based on these findings, the pathogenic potential of *Psychrobacter* species in humans appears to be low but should not be completely ruled out, considering the increasing number of highly infection-prone patient groups. There is thus a small, but not negligible, risk that the bacterium present in Nemaslug® may pose a challenge for such patients as well as for newborns and the elderly. Moreover, the difficulty in detecting *Psychrobacter* species using routine methods should be kept in mind.

There is currently no evidence suggesting that the nematode *Phasmarhabditis hermaphrodita* can infect or harm humans.

5.2 Human health risk by consumption of food products treated with Nemaslug®

If Nemaslug® is used in food production for human consumption, the pathogenic potential of the bacterial strain used is important. We are not aware of any reported human health issues arising from the use of Nemaslug® in food production.

5.3 The occurrence of the bacteria in Norway

While *Psychrobacter* species are distributed worldwide, reports from Norway are scarce. Bacterial isolates identified as *Psychrobacter* have been reported from the Arctic, for example from guano on Svalbard, and from lobster caught in the North Sea, but the strains were not identified as *P. faecalis* and were generally only described as *Psychrobacter* species belonging to other groups within *Psychrobacter* (Bekaert et al., 2015; Dziewit et al., 2013). Bacteria of the genus *Psychrobacter* have also been identified in other commercial seafood products in Norway such as salt-cured cod (Bjørkevoll et al., 2003) and brine from six Norwegian producers of "rakfisk", a fermented fish product (Bjerke et al., 2019).

According to the Department of Microbiology, Institute of Clinical Medicine, University of Oslo/Oslo University Hospital, no case of *Psychrobacter* sp has ever been detected in the routine diagnostic laboratory providing services for hospitals in the Oslo area, suggesting that the occurrence of *Psychrobacter* species in clinical samples in Norway is low or negligible (see Chapter 2.4.3 for more details).

5.4 The potential to survive and spread in the environment under Norwegian conditions if Nemaslug® were to be used in greenhouses and outdoors. Risk to non-target organisms under Norwegian conditions

The wide distribution of the nematode *Phasmarhabditis hermaphrodita* in large parts of the world is partly attributed to its use as biocontrol agent. It is likely that the use of Nemaslug® will expand the distribution of this nematode also in Norway, particularly when used outdoors in fields or gardens. Knowledge about effects on non-target organisms has hitherto been limited. However, a recent study (Rae et al., 2023) reviewing results from investigations of the susceptibility of several slug and snail species to *P. hermaphrodita* and their bacteria, showed that 12 of 22 slug species and 8 of 21 snail species were killed. Increased mortality of non-target terrestrial gastropods was also shown in another recent study (Denver et al., 2024). Although the referred studies were performed in different parts of the world, it seems likely that Nemaslug® will kill non-target molluscs also in Norway.

Psychrobacter phaecalis and *P. pulmonis* were isolated from healthy, warm-blooded animals (pigeon and lamb, respectively), and can also be parts of the human microbiome (Deschaght et al., 2012) but are not known to cause disease (Welter et al., 2021). The pathogenic potential of *Psychrobacter* species in humans appears to be low but a risk to highly infection-prone patient groups cannot be ruled out.

5.5 Uncertainty about taxonomy which can influence the risk assessment

There is uncertainty concerning the species affiliation of the bacterium that is the active component of Nemaslug®, although it likely belongs to the species *Psychrobacter faecalis* or *Psychrobacter pulmonis*. The close relationship between these and related bacteria makes it impossible or difficult to differentiate these organisms based on only 16S rRNA gene sequencing. For this to be done satisfactorily, a more thorough taxonomic analysis based on complete genome sequencing or multilocus sequence analysis (MLSA) is needed.

6 Data gaps

The close taxonomic relationship between *Psychrobacter faecalis* and *Psychrobacter pulmonis* makes it difficult or impossible to differentiate between these organisms based only on 16S rRNA gene sequencing. A more thorough taxonomic analysis would require complete genome sequencing or multilocus sequence analysis (MLSA).

Knowledge on antibiotic resistance, as well as pathogenicity, in the genus *Psychrobacter* is largely lacking.

There are ecological concerns among malacologists (mollusc researchers) about the use of nematodes for biocontrol purposes and more research on effects of nematodes on native (non-target) molluscs is recommended (Christensen et al., 2021; Mc Donnell et al., 2023; Rae et al., 2023).

7 References

Apprill, A., Robbins, J., Eren, A. M., Pack, A. A., Reveillaud, J., Mattila, D., Moore, M., Niemeyer, M., Moore, K. M., & Mincer, T. J. (2014). Humpback whale populations share a core skin bacterial community: towards a health index for marine mammals? *PLoS One*, *9*(3), e90785. https://doi.org/10.1371/journal.pone.0090785

Aslam, B., Wang, W., Arshad, M. I., Khurshid, M., Muzammil, S., Rasool, M. H., Nisar, M. A., Alvi, R. F., Aslam, M. A., & Qamar, M. U. (2018). Antibiotic resistance: a rundown of a global crisis. *Infection and drug resistance*, 1645-1658.

Bakermans, C., Ayala-Del-Río, H. L., Ponder, M. A., Vishnivetskaya, T., Gilichinsky, D., Thomashow, M. F., & Tiedje, J. M. (2006). *Psychrobacter cryohalolentis* sp. nov. and Psychrobacter arcticus sp. nov., isolated from Siberian permafrost. *Int J Syst Evol Microbiol*, *56*(Pt 6), 1285-1291. https://doi.org/10.1099/ijs.0.64043-0

Bekaert, K., Devriese, L., Maes, S., & Robbens, J. (2015). Characterization of the dominant bacterial communities during storage of Norway lobster and Norway lobster tails (*Nephrops norvegicus*) based on 16S rDNA analysis by PCR-DGGE. *Food Microbiol*, *46*, 132-138. https://doi.org/10.1016/j.fm.2014.06.022

Bennett, J. E., Dolin, R., & Blaser, M. J. (2020). *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*. Elsevier Health Sciences.

Bjerke, G. A., Rudi, K., Avershina, E., Moen, B., Blom, H., & Axelsson, L. (2019). Exploring the Brine Microbiota of a Traditional Norwegian Fermented Fish Product (Rakfisk) from Six Different Producers during Two Consecutive Seasonal Productions. *Foods*, *8*(2). https://doi.org/10.3390/foods8020072

Bjørkevoll, I., Olsen, R. L., & Skjerdal, O. T. (2003). Origin and spoilage potential of the microbiota dominating genus *Psychrobacter* in sterile rehydrated salt-cured and dried salt-cured cod (Gadus morhua). *Int J Food Microbiol*, *84*(2), 175-187. https://doi.org/10.1016/s0168-1605(02)00418-x

Bonwitt, J., Tran, M., Droz, A., Gonzalez, A., & Glover, W. A. (2018). *Psychrobacter sanguinis* Wound Infection Associated with Marine Environment Exposure, Washington, USA. *Emerg Infect Dis*, *24*(10), 1942-1944. https://doi.org/10.3201/eid2410.171821

Bowman, J. P. (2006). The Genus *Psychrobacter*. In M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer, & E. Stackebrandt (Eds.), *The Prokaryotes: A Handbook on the Biology of Bacteria Volume 6: Proteobacteria: Gamma Subclass* (pp. 920-930). Springer New York. https://doi.org/10.1007/0-387-30746-X_35

Brauge, T., Trigueros, S., Briet, A., Debuiche, S., Leleu, G., Gassilloud, B., Wilhelm, A., Py, J., & Midelet, G. (2021). MALDI-TOF Mass Spectrometry Fingerprinting Performance Versus 16S rDNA Sequencing to Identify Bacterial Microflora From Seafood Products and Sea Water Samples [Brief Research Report]. *Frontiers in Marine Science*, *8*. https://doi.org/10.3389/fmars.2021.650116

Caspar, Y., Recule, C., Pouzol, P., Lafeuillade, B., Mallaret, M. R., Maurin, M., & Croize, J. (2013). *Psychrobacter arenosus* bacteremia after blood transfusion, France. *Emerg Infect Dis*, *19*(7), 1118-1120. https://doi.org/10.3201/eid1907.121599

Christensen, C. C., Cowie, R. H., Yeung, N. W., & Hayes, K. A. (2021). Biological control of pest non-marine molluscs: A pacific perspective on risks to non-target organisms. *Insects*, *12*(7), 583.

Cuadrat, R. R. C., Sorokina, M., Andrade, B. G., Goris, T., & Dávila, A. M. R. (2020). Global ocean resistome revealed: Exploring antibiotic resistance gene abundance and distribution in TARA Oceans samples. *Gigascience*, *9*(5). https://doi.org/10.1093/gigascience/giaa046

Denver, D., Howe, D. K., Colton, A. J., Richart, C. H., & Mc Donnell, R. J. (2024). The biocontrol nematode *Phasmarhabditis hermaphrodita* infects and increases mortality of Monadenia fidelis, a non-target terrestrial gastropod species endemic to the Pacific Northwest of North America, in laboratory conditions. *PLoS One*, *19*(3), e0298165. https://doi.org/10.1371/journal.pone.0298165

Deschaght, P., Janssens, M., Vaneechoutte, M., & Wauters, G. (2012). *Psychrobacter* isolates of human origin, other than *Psychrobacter phenylpyruvicus*, are predominantly *Psychrobacter faecalis* and *Psychrobacter pulmonis*, with emended description of *P. faecalis*. *Int J Syst Evol Microbiol*, *62*(Pt 3), 671-674. https://doi.org/10.1099/ijs.0.032631-0

Dziewit, L., Cegielski, A., Romaniuk, K., Uhrynowski, W., Szych, A., Niesiobedzki, P., Zmuda-Baranowska, M. J., Zdanowski, M. K., & Bartosik, D. (2013). Plasmid diversity in arctic strains of *Psychrobacter* spp. *Extremophiles*, *17*(3), 433-444. https://doi.org/10.1007/s00792-013-0521-0

EPPO Global Database and GD Desktop/2021-03-23. https://www.eppo.int/RESOURCES/eppo_databases/global_database

Howe, D. K., Ha, A. D., Colton, A., De Ley, I. T., Rae, R. G., Ross, J., Wilson, M., Nermut, J., Zhao, Z., Mc Donnell, R. J., & Denver, D. R. (2020). Phylogenetic evidence for the invasion of a commercialized European *Phasmarhabditis hermaphrodita* lineage into North America and New Zealand. *PLoS One*, *15*(8), e0237249. https://doi.org/10.1371/journal.pone.0237249

Kim, M., Kwon, T. H., Jung, S. M., Cho, S. H., Jin, S. Y., Park, N. H., Kim, C. G., & Kim, J. S. (2013). Antibiotic resistance of bacteria isolated from the internal organs of edible snow crabs. *PLoS One*, *8*(8), e70887. https://doi.org/10.1371/journal.pone.0070887

Koh, H. W., Kim, M. S., Lee, J. S., Kim, H., & Park, S. J. (2015). Changes in the Swine Gut Microbiota in Response to Porcine Epidemic Diarrhea Infection. *Microbes Environ*, *30*(3), 284-287. https://doi.org/10.1264/jsme2.ME15046

Kristiansen, M., & Ringø, E. (2013). Evaluation of prebiotic and probiotic effects on the intestinal gut microbiota and histology of atlantic salmon.

Kumaria, A., Crusz, S. A., Lister, M., Kirkman, M. A., & Macarthur, D. C. (2022). *Psychrobacter piechaudii* shunt infection: first report of human infection. *Child's Nervous System*, *38*(7), 1385-1388. https://doi.org/10.1007/s00381-021-05401-7

Kämpfer, P., Albrecht, A., Buczolits, S., & Busse, H. J. (2002). *Psychrobacter faecalis* sp. nov., a new species from a bioaerosol originating from pigeon faeces. *Syst Appl Microbiol*, *25*(1), 31-36. https://doi.org/10.1078/0723-2020-00099

Lagier, J. C., Khelaifia, S., Alou, M. T., Ndongo, S., Dione, N., Hugon, P., Caputo, A., Cadoret, F., Traore, S. I., Seck, E. H., Dubourg, G., Durand, G., Mourembou,

G., Guilhot, E., Togo, A., Bellali, S., Bachar, D., Cassir, N., Bittar, F., Delerce, J., Mailhe, M., Ricaboni, D., Bilen, M., Dangui Nieko, N. P., Dia Badiane, N. M., Valles, C., Mouelhi, D., Diop, K., Million, M., Musso, D., Abrahão, J., Azhar, E. I., Bibi, F., Yasir, M., Diallo, A., Sokhna, C., Djossou, F., Vitton, V., Robert, C., Rolain, J. M., La Scola, B., Fournier, P. E., Levasseur, A., & Raoult, D. (2016). Culture of previously uncultured members of the human gut microbiota by culturomics. *Nat Microbiol*, *1*, 16203. https://doi.org/10.1038/nmicrobiol.2016.203

Lasa, A., & Romalde, J. L. (2017). Genome sequence of three *Psychrobacter* sp. strains with potential applications in bioremediation. *Genom Data*, *12*, 7-10. https://doi.org/10.1016/j.gdata.2017.01.005

Le Guern, R., Wallet, F., Vega, E., Courcol, R. J., & Loïez, C. (2014). *Psychrobacter sanguinis*: an unusual bacterium for nosocomial meningitis. *Journal of Clinical Microbiology*, *52*(9), 3475-3477.

Leung, W. K., Chow, V. C. Y., Chan, M. C. W., Ling, J. M. L., & Sung, J. J. Y. (2006). *Psychrobacter bacteraemia* in a cirrhotic patient after the consumption of raw geoduck clam. *Journal of Infection*, *52*(6), e169-e171.

Lovdata. (2024). *Forskrift om tiltaksverdier og grenseverdier for fysiske og kjemiske faktorer i arbeidsmiljøet samt smitterisikogrupper for biologiske faktorer.* https://lovdata.no/dokument/SF/forskrift/2011-12-06-1358/KAPITTEL_9#KAPITTEL_9

Lozano, F., Florez, C., Fecio, F., Gamboa, F., Gómez-Mates, J., & Martín, E. (1994). Fatal *Psychrobacter immobilis* infection in a patient with AIDS. *Aids*, *8*(8), 1189.

Matsuyama, H., Minami, H., Sakaki, T., Kasahara, H., Watanabe, A., Onoda, T., Hirota, K., & Yumoto, I. (2015). *Psychrobacter oceani* sp. nov., isolated from marine sediment. *Int J Syst Evol Microbiol*, *65*(Pt 5), 1450-1455. https://doi.org/10.1099/ijs.0.000118

Mawarda, P. C., Lakke, S. L., van Elsas, J. D., & Salles, J. F. (2022). Temporal dynamics of the soil bacterial community following *Bacillus* invasion. *Iscience*, *25*(5).

Mc Donnell, R. J., D, K. H., & D, R. D. (2023). First Report of the Gastropod-Killing Nematode, *Phasmarhabditis californica*, in Washington State, U.S.A. *J Nematol*, *55*(1), 20230013. https://doi.org/10.2478/jofnem-2023-0013

Ortiz-Alcantara, J. M., Segura-Candelas, J. M., Garces-Ayala, F., Gonzalez-Duran, E., Rodriguez-Castillo, A., Alcantara-Perez, P., Wong-Arambula, C., Gonzalez-Villa, M., Leon-Avila, G., Garcia-Chequer, A. J., Diaz-Quinonez, J. A., Mendez-Tenorio, A., & Ramirez-Gonzalez, J. E. (2016). Fatal *Psychrobacter* sp. infection in a pediatric patient with meningitis identified by metagenomic next-generation sequencing in cerebrospinal fluid. *Arch Microbiol*, *198*(2), 129-135. https://doi.org/10.1007/s00203-015-1168-2

Parte, A. C., Sardà Carbasse, J., Meier-Kolthoff, J. P., Reimer, L. C., & Göker, M. (2020). List of Prokaryotic names with Standing in Nomenclature (LPSN) moves to the DSMZ. *Int J Syst Evol Microbiol*, *70*(11), 5607-5612. https://doi.org/10.1099/ijsem.0.004332

Petrova, M., Gorlenko, Z., & Mindlin, S. (2009). Molecular structure and translocation of a multiple antibiotic resistance region of a *Psychrobacter psychrophilus* permafrost strain. *FEMS microbiology letters*, *296*(2), 190-197.

Pieterse, A., Malan, A. P., & Ross, J. L. (2017). Nematodes that associate with terrestrial molluscs as definitive hosts, including *Phasmarhabditis hermaphrodita* (Rhabditida: Rhabditidae) and its development as a biological molluscicide. *J Helminthol*, *91*(5), 517-527. https://doi.org/10.1017/S0022149X16000572

Rae, R., Sheehy, L., & McDonald-Howard, K. (2023). Thirty years of slug control using the parasitic nematode *Phasmarhabditis hermaphrodita* and beyond. *Pest Manag Sci*, *79*(10), 3408-3424. https://doi.org/10.1002/ps.7636

Romanenko, L. A., Tanaka, N., Frolova, G. M., & Mikhailov, V. V. (2009). *Psychrobacter fulvigenes* sp. nov., isolated from a marine crustacean from the Sea of Japan. *International Journal of Systematic and Evolutionary Microbiology*, *59*(6), 1480-1486.

Santos, A., Burgos, F., Martinez-Urtaza, J., & Barrientos, L. (2022). Metagenomic characterization of resistance genes in Deception Island and their association with mobile genetic elements. *Microorganisms*, *10*(7), 1432.

Sheehy, L., Cutler, J., Weedall, G. D., & Rae, R. (2022). Microbiome Analysis of Malacopathogenic Nematodes Suggests No Evidence of a Single Bacterial Symbiont Responsible for Gastropod Mortality. *Front Immunol*, *13*, 878783. https://doi.org/10.3389/fimmu.2022.878783

Stenberg, J. A., Sundh, I., Becher, P. G., Björkman, C., Dubey, M., Egan, P. A., Friberg, H., Gil, J. F., Jensen, D. F., & Jonsson, M. (2021). When is it biological control? A framework of definitions, mechanisms, and classifications. *Journal of Pest Science*, *94*(3), 665-676.

Tan, L., & Grewal, P. S. (2001). Pathogenicity of *Moraxella osloensis*, a bacterium associated with the nematode *Phasmarhabditis hermaphrodita*, to the slug *Deroceras reticulatum*. *Appl Environ Microbiol*, *67*(11), 5010-5016. https://doi.org/10.1128/aem.67.11.5010-5016.2001

Tan, L., & Grewal, P. S. (2002). Endotoxin Activity of *Moraxella osloensis* against the Grey Garden Slug, *Deroceras reticulatum*. *Applied and Environmental Microbiology*, *68*(8), 3943-3947. https://doi.org/doi:10.1128/AEM.68.8.3943-3947.2002

Vela, A. I., Collins, M. D., Latre, M. V., Mateos, A., Moreno, M. A., Hutson, R., Dominguez, L., & Fernandez-Garayzabal, J. F. (2003). *Psychrobacter pulmonis* sp. nov., isolated from the lungs of lambs. *Int J Syst Evol Microbiol*, *53*(Pt 2), 415-419. https://doi.org/10.1099/ijs.0.02413-0

VKM. (2019). *Kriterier for forfatterskap og faglig ansvar i VKMs uttalelser* https://vkm.no/download/18.48566e5316b6a4910fc2dbd6/1561035075341/VKMs%20forfatterskapskriterier_revidert%20versjon%2020.06.2019.pdf

VKM, Stenberg, J. A., Melby, K. K., Magnusson, C., Nielsen, A., Rydning, J., Wendell, M., Alsanius, B., Krokene, P., Nicolaisen, M., Thomsen, I. M., Wright, S. A. I., & Rafoss, T. (2021). *Risk assessment of the biocontrol product Nemaslug 2.0 with the active organisms Phasmarhabditis californica (strain P19D) and Moraxella osloensis. Scientific Opinion of the Panel on Plant Health. VKM Report 2021:16, ISBN: 978-82-8259-370-0, ISSN: 2535-4019. Norwegian Scientific Committee for Food and Environment (VKM), Oslo, Norway*. https://vkm.no/english/riskassessments/allpublications/healthandenvironmentalrisksofusingnemaslug20.4.54e8ddba17a4da12c4d492c8.html

Welter, D. K., Ruaud, A., Henseler, Z. M., De Jong, H. N., van Coeverden de Groot, P., Michaux, J., Gormezano, L., Waters, J. L., Youngblut, N. D., & Ley, R. E. (2021). Free-Living, Psychrotrophic Bacteria of the Genus *Psychrobacter* Are Descendants of Pathobionts. *mSystems*, *6*(2). https://doi.org/10.1128/mSystems.00258-21

Wilson, M. J., & Grewal, P. S. (2005). Biology, production and formulation of slug-parasitic nematodes. In *Nematodes as biocontrol agents* (pp. 421-429). CABI Publishing Wallingford UK.

Wirth, S. E., Ayala-Del-Río, H. L., Cole, J. A., Kohlerschmidt, D. J., Musser, K. A., Sepúlveda-Torres, L. D. C., Thompson, L. M., & Wolfgang, W. J. (2012). *Psychrobacter sanguinis* sp. nov., recovered from four clinical specimens over a 4-year period. *Int J Syst Evol Microbiol*, *62*(Pt 1), 49-54. https://doi.org/10.1099/ijs.0.029058-0

Yoon, J. H., Lee, C. H., Kang, S. J., & Oh, T. K. (2005). *Psychrobacter celer* sp. nov., isolated from sea water of the South Sea in Korea. *Int J Syst Evol Microbiol*, *55*(Pt 5), 1885-1890. https://doi.org/10.1099/ijs.0.63682-0

8 Appendix I

8.1 Documentation of Search Strategy

Document