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Current knowledge of the health and environmental risks of microbial-based cleaning products

Scientific opinion of the Panel on Microbial Ecology of the Norwegian Scientific Committee for Food and Environment

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Current knowledge of the health and environmental risks of microbial-based cleaning products

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Current knowledge of the health and environmental risks of microbial-based cleaning products

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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.

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Summary

In 2019, The Norwegian Environment Agency (NEA) requested the Norwegian Scientific Committee for Food and Environment (VKM) to extend the VKM report from 2016 entitled "Health and environmental risk assessment of microbial cleaning products". VKM was asked to give an update on the microorganisms most commonly used in microbial cleaning products and the potential health and environmental effects of their release. VKM was also asked to suggest a documentation checklist covering information a declarant should specify in a declaration.

Methodology

VKM appointed a working group consisting of members of the Panel on Microbial Ecology. The Panel on Microbial Ecology has reviewed and revised the draft prepared by the working group. Two independent external experts with long-standing experience within the field have also reviewed the report.

The assessment is primarily based on available scientific literature (articles, reports) published within the last five years, and identified by systematic literature search employing specific terms as well as defined inclusion criteria in Pubmed, Web of Science, Scopus, Medline, and Google scholar.

Results

VKM considers, similar to the situation in 2016, that there seems to be insufficient information when it comes to specification of the microbial content of the product. Knowledge of the identity of the microorganisms at the species and preferable strain level is a prerequisite for conducting environmental and health risk assessment of microbial-based cleaning products (MBCPs). According to the examined literature, the most frequently used microorganisms belong to genus *Bacillus,* followed by members of the genera *Bifidobacterium, Lactobacillus*, *Rhodopseudomonas* and *Saccharomyces*. Some of these are represented by members which have long histories of safe use in the food industry and as supplements. However, due to the general lack of transparency and third-party assessment on the detailed chemical and microbial formulation of the products, the potential for misclassification (e.g. *Bacillus* spp.) and contamination with pathogens (human, animal, plant) needs to be taken into consideration.

Release scenarios include disposal into sewage, with major knowledge gaps on the possible short and long-term impact on local microbial ecosystems. Studies from hospitals indicate that certain MBCPs may have long-term effects on surfaces, preventing the recontamination, persistence and spread of pathogenic microorganisms and opportunists. Although the cleaning effects are considered to be due to the biochemical properties of the microorganisms, further research is needed on potential synergistic and antagonistic cleaning effects of mixtures of species/strains as well as the mechanism of potential inhibitory effects

on unwanted microorganisms, in particular to better understand the impact on animal and plant health (terrestrial, freshwater and marine).

Documentation checklist

VKM suggests a list of documentation that should be provided by the applicant upon declaration to enable environmental and health risk assessment. The list covers relevant information on the identification and characterization of microorganisms, process quality control and product labelling.

Conclusions

There are still considerable knowledge gaps, uncertainties regarding effect/safety, mechanisms of action, and insufficient transparency on the content of microbial-based cleaning products to the strain level which limit our ability to conduct data-driven environmental and health risk assessments.

Key words: VKM, risk assessment, Norwegian Scientific Committee for Food and Environment, Norwegian Environment Agency, microbial-based cleaning products

Sammendrag på norsk

I 2019 ba Miljødirektoratet VKM om å utvide følgende rapport fra 2016: "Health and environmental risk assessment of microbial cleaning products". VKM ble bedt om å finne oppdatert informasjon om hvilke mikrober som brukes i mikrobielle rengjøringsmidler, og potensielle konsekvenser disse mikrobene kan ha for helse og miljø. VKM ble også bedt om å foreslå en sjekkliste som bør vedlegges som dokumentasjon når mikrobielle rengjøringsmidler skal risikovurderes.

Metodikk

VKM utnevnte en arbeidsgruppe bestående av medlemmer av faggruppen for mikrobiell økologi. Faggruppen har gått gjennom og revidert utkastet fra arbeidsgruppen. To uavhengige eksterne eksperter med lang erfaring innenfor fagfeltet, har også gått gjennom rapporten.

Faggruppens bedømmelse bygger på tilgjengelig vitenskapelig litteratur (artikler og faglige rapporter) publisert i løpet av de siste 5 årene, funnet ved bruk av spesifikke søketermer og inklusjonskriterier i Pubmed, Web of Science, Scopus, Medline og Google scholar.

Resultat

Som i 2016 finner VKM at det generelt er utilstrekkelig informasjon om det mikrobielle innholdet i rengjøringsprodukter. Identifisering av mikrober til artsnivå, og fortrinnsvis til stammenivå, er en forutsetning for å kunne vurdere miljø– og helsemessige konsekvenser ved bruk av rengjøringsmidler basert på levende mikrober. Ifølge tilgjengelig litteratur, tilhører de mest brukte mikrobene slektene *Bacillus, Bifidobacterium, Lactobacillus*, *Rhodopseudomonas* and *Saccharomyces*, hvorav arter i slekten *Bacillus* ser ut til å være hyppigst benyttet. Flere av slektene har arter som har vært i bruk i matvareindustrien i lang tid. På grunn av manglende innsyn i produksjonsmetoder, og mangel på kvalitetskontroll fra uavhengige parter som kan fastslå detaljert kjemisk og mikrobiell sammensetning av produkter, må vi ta potensialet for feilklassifisering av organismer (som f.eks. arter av *Bacillus*), og kontaminering med patogener med i vurderingen.

Mikrobielle rengjøringsprodukter kan f.eks. slippes ut i miljøet gjennom kloakk. Det er stor mangel på kunnskap om hvordan dette vil påvirke mikrobielle økosystemer på lokalt nivå, både på kort og lang sikt. Studier fra sykehus tyder på at noen produkter er effektive over lang tid på overflater, ved at de blokkerer kolonisering med patogene mikroorganismer og opportunistiske patogener. Selv om rengjøringseffekter antas å komme som resultat av mikrobenes biokjemiske egenskaper, trengs det mer forskning på potensielle ringvirkninger av å blande ulike typer mikrober i ett produkt, og om dette kan ha synergistiske eller antagonistiske effekter. Vi trenger også mer kunnskap om mekanismene som bidrar til at mikrober i rengjøringsprodukter kan ekskludere uønskede mikroorganismer. Ikke minst

trengs det mer kunnskap om hvordan disse produktene kan påvirke dyre– og planteliv til lands og til vanns.

Sjekkliste for produktdokumentering

VKM foreslår at søkere leverer en standardisert liste med dokumentasjon for å gjøre det mulig å vurdere potensiell risiko for helse og miljø. Listen bør ha relevant informasjon om mikrobenes taksonomiske identitet og fenotypiske/genotypiske egenskaper, og om kvalitetskontroll og produktmerking.

Konklusjon

Det er fremdeles begrenset tilgjengelig kunnskap om effektivitet, sikkerhet og virkningsmekanismer, og lite informasjon om mikrobenes identitet til arts– eller stammenivå. Dette gjør det vanskelig å gjennomføre en konstruktiv vurdering av hvilke potensielle konsekvenser bruk av disse produktene har for miljø og helse.

Abbreviations and glossary

Abbreviations

ABSA	American Biological Safety Association
AMR	Antimicrobial resistance
BDS	Biological degreasing stations
CFU	Colony Forming Units
EC	European commission
EFSA	European Food Safety Authority
EU	European union
GM	Genetically modified
GMM	Genetically modified microorganism
GRAS	Generally recognized as safe
HAIs	Healthcare-acquired infections
MBCP	Microbial-based cleaning product
MLST	Multi-locus sequence typing
MLVA	Multiple-locus variable number tandem repeat analysis

NEA	Norwegian Environment Agency
NGS	Next generation sequencing
QA	Quality assurance
QC	Quality control
QPS	Qualified presumption of safety
RG	Risk group
VKM	Norwegian Scientific Committee for Food and Environment
WWTP	Waste water treatment plant

Glossary

Acquired resistance: Resistance to a particular antimicrobial agent to which the microorganism was previously susceptible. The change in resistance level is the result of genetic changes in a microorganism due to mutation(s), the acquisition of foreign genetic material, or a combination of both mechanisms.

Aerotolerant: An anaerobic organism capable of surviving or growing despite the presence of oxygen.

Antagonist: A substance that acts against and blocks the action of an active agent.

Antimicrobial resistance: A property of microorganisms that confers the capacity to inactivate or exclude antimicrobials, or a mechanism that blocks the inhibitory or killing effects of antimicrobials.

Biofilm: Microbial biofilms are populations of microorganisms that are concentrated at an interface (usually solid/liquid) and typically surrounded by an extracellular polymeric slime matrix.

Biocide/Biocidal products: Active substances and preparations containing one or more substances intended to destroy, deter, render harmless, prevent the action of, or otherwise exert a controlling effect on any harmful organism by chemical or biological means.

Disinfection: Use of physical procedures or chemical agents (disinfectants) to destroy most microbial forms (mainly on inanimate material, but also on skin surfaces). Disinfectants are often not effective against bacterial spores.

Intrinsic resistance: A natural property of an organism resulting in decreased susceptibility to a particular antimicrobial agent.

Microbiota: A community of microorganisms (such as bacteria, fungi, and viruses) that inhabit a particular environment.

Natural environment: In this report, the natural environment denotes the biotic and abiotic components of plants, soil, sediments, water, air etc. with which an individual, population, or species comes into contact.

Oligotrophic environment: Environments characterized by a low accumulation of dissolved nutrient salts, supporting but a sparse growth of algae and other organisms, and having a high oxygen content owing to the low organic content.

Opportunist: Denotes a microorganism that does not ordinarily cause disease but that, takes advantage under certain circumstances such as impaired immune responses resulting from other disease or drug treatment, and acts as a pathogen.

Pathogen: A bacterium, virus, or other microorganism that can cause disease/illness.

Probiotics: Live microorganisms, which when administered in adequate amounts may confer a health benefit on the host.

Quorum sensing: Mechanism by which bacteria regulate gene expression in accordance with population density through the use of signal molecules.

Sanitization: The process of reducing microbiological contamination.

Sludge hygienization: Most commonly a pasteurization step included in waste water treatment to reduce the amount of viable microorganisms in sludge, particularly that of thermostable faecal bacteria.

Spore: Highly resistant, dormant structure formed in response to adverse environmental conditions.

Synergist: A substance that enhances the effectiveness of an active agent.

Virulence: The degree of disease a pathogenic organism cause in a specific host species.

Background as provided by the Norwegian Environment Agency

Introduction

Cleaning products containing microorganisms as an active substance have in recent years become more common in housekeeping, institutions, industry and is also taken in use in hospitals abroad. Microbial cleaning products are considered as eco-friendly cleaning products and might contain viable bacteria and spores as active ingredients, but also enzymes and chemicals. The exact microbial composition in the products are often not identified in detail and the product labeling is often not given. To date there are no common international regulations or quality standards, regulating the production and use of microbial cleaners.

Today there is no uniform, international regulations that regulate the productions of these cleaning products. A lack of common quality standards might be a challenge for both industry and regulators. The Nordic ecolabel, the Swan, describes their requirements for documentation and information of microbial cleaners in their criteria document for cleaning products.

Regulatory background

In Norway microbiological products are regulated as any other product on the market under the Act relating to control of products and consumer services (the Product Control Act of 6 November 1976), and under a separate regulation of 22 January 1998 no. 93 relating to the declaration and labeling of microbiological products (Regulations on microbiological products).

The purpose of the regulation on microbiological products is to prevent microorganisms in microbiological products from causing damage to health or adverse environmental effects such as disruption of ecosystems, pollution, or waste.

According to the regulation any person that manufactures or imports microbiological products or places them on the market in Norway has a duty to declare any information necessary for an assessment of the risk the product poses to human health or possible negative environmental effects. The information is to be given in a declaration form (cf. appendix of the regulation) and amongst others include a description of the product and its composition, area of application, mode of use, and degradation products, antibiotic resistance and any pathogenic properties of the microorganisms. The guidelines to the regulations provide detailed description of the type of information and what documentation is required in order to satisfactorily declare a product. The information provided shall give the authorities a basis to assess the health and environmental risks associated with the use of the products.

There is limited access to general information about the content of cleaning products containing microorganisms from the developer, challenging the assessment of efficiency and safety of the products.

The Norwegian Environment Agency considers that there is a need to assess the most common microorganisms used in microbial cleaning products to assess health and environmental risk of these products.

Terms of reference as provided by the Norwegian Environment Agency

The Norwegian Environment Agency therefore requests VKM to:

1. Extend the VKM report from 2016; Health and environmental risk assessment of microbial cleaning product. The extended version shall contain:
    a. An updated overview of the microorganisms commonly used in microbial-based cleaning products based on publications in this field.
    b. An assessment of whether the microorganisms used pose environmental or health risk(s) by release into the environment.
    c. An assessment of the natural presence of the used microorganisms (species and strain) in the Norwegian environment.
    d. The potential of survival by release into the environment.
    e. An assessment of how the use and release of microbial-based cleaning products may affect the microbiological balance in the ecosystem.
    f. An overview over whether the microorganisms used contain antimicrobial resistance genes.
    g. An overview of the molecular mechanisms, which form the basis for the cleaning effect.
2. For risk assessment of microbial-based cleaning products, suggest a documentation checklist covering the information the declarant should specify in a declaration.

Assessment

1 Literature

1.1 Search strategy

Literature searches were undertaken January and April 2019 in Pubmed, Web of Science, Scopus, Medline, and Google scholar using Advanced Search Builder. Search terms used in Title/Abstract fields were "microbial", "microorganism", "bacterial", "fungal", "cleaner", "cleaning", "detergent" and for Google scholar in particular "microbial based cleaning" was the only term used due to numerous irrelevant hits obtained with the other search terms. Search strings were built using Boolean operators AND and OR. There were no restrictions on language, but the search was limited to the last 5 years for the initial search whereas the second search was limited to the last year or January – April 2019, where possible. The initial and second searches returned 246 and 144 hits respectively. After the removal of duplicates, the titles and abstracts of all search results were scanned for relevance to the terms of reference. Full texts for those of potential relevance were assessed to determine their relevance to this report. The reference lists in the selected articles formed the basis for identifying additional articles or reports within the topics listed in the terms of reference, overlooked by the searches. Additionally, individual searches were performed as needed on topics not directly related to microbial-based cleaning products (MBCPs).

1.1.1 Inclusion criteria

Relevant reviews as well as original articles published within the last 5 years relating to the terms of reference were included.

1.1.2 Exclusion criteria

Articles were excluded if they did not relate to the terms of reference. Particularly, articles based on genetically modified (GM) and synthetic microorganisms. Articles that were not in English, or a Scandinavian language (Swedish, Danish, and Norwegian) were also excluded.

2 Introduction

This document presents a scientific opinion prepared by the VKM Panel on Microbial Ecology in response to a request from the Norwegian Environment Agency. The Terms of Reference build upon the assessment that was performed by VKM in 2016. However, while the previous report focused on the information requirements laid down in national legislations the current report emphasizes the state of knowledge of the agents that are most commonly used in microbial-based cleaning products (MBCPs) and their potential health and environmental effects upon release into the environment. Furthermore, a "check list" of the minimum information required to perform a risk assessment is suggested, independent of current national legislations. The assessment is based upon available information on the microorganisms that are either claimed, or verified, to be active ingredients of the cleaning products. Products containing extracellular substances only, such as microbial derived enzymes, are not part of this assessment. Risk-benefit assessment was not part of the mandate for this report. Notably, the panel did not have access to data regarding usage of MBCPs in Norway, neither in terms of quantity nor types of products. However, a web-search using Norwegian translation of the terms "microbial", "bacterial" "biological" and "cleaning" generated several hits which revealed that 13 different MBCPs may be manufactured or distributed by Norwegian online stores.

2.1 Previous assessment

In 2015, the Norwegian Environment Agency requested, from the Norwegian Scientific Committee for Food and Environment (VKM), a scientific assessment of the information requirements laid down in the declaration for the regulation on microbial products and its guidelines (VKM, 2016). The VKM panel recommended that the information requirements should be revised to facilitate health and environmental risk assessment of the use of MBCPs in Norway. One of the important gaps identified by the panel was the insufficient accuracy in the information on the microbial content of the products. Furthermore, the insufficient emphasis on potential environmental impacts was highlighted.

3 Extension of the VKM report from 2016

3.1 Microorganisms most commonly used in MBCPs

Varying microorganisms (fungi, bacteria and bacteriophages) and combinations of these microbes are intentionally added as active ingredients in microbial-based cleaning products (MBCPs). According to literature, members of the genus *Bacillus* (spore-forming) are most

commonly used, followed by members of the genera *Bifidobacterium, Lactobacillus, Rhodopseudomonas and Saccharomyces* (Arvanitakis, Temmerman, & Spök, 2018; Spök, Arvanitakis, & McClung, 2018). Additionally, members of numerous other genera have been listed, including yeasts (Arvanitakis et al., 2018). This information is summarized in Table 2, Appendix I. In several of the products, the taxonomy of the microorganism was only specified to the genus level, and not to the species or strain level which challenges the assessment of their health and environmental impact. Confidentiality issues seem to be a challenge for obtaining the precise taxonomic identification of microorganisms added to MBCPs. Furthermore, inconsistencies in quality control and/or assurance during production leading to improper taxonomy and/or presence of unintentional contaminants in end-products is a concern (Spök et al., 2018). Subasinghe et al. (2018) examined the composition of five commercially available products in the US and Canadian markets. Metagenomics and culture-based methods revealed inconsistencies between product label and analysed content, and in some cases mixtures of bacteria and fungi were identified.

In most cases, the total concentration and form (e.g. spores versus viable cells) or information on whether the microorganism has been genetically modified or not, were not provided. The lack of third-party quality-assured and explicit product specification hampers comprehensive assessment of the environmental and health-related consequences of their use.

3.1.1 Environmental stability and survival

Microbial stability and survival during storage and use is a prerequisite for their activity on the intended site of action. A majority of the cleaning formulations employ spore-forming bacteria of genus *Bacillus.* Spore-forming bacteria are characterized by the ability to switch between two different life stages, the growing vegetative cell and the metabolically dormant spore. Dormant spores are formed in depletion of nutrients and are highly resistant towards outer stress such as heat, desiccation, high/low pH and disinfectants (Nicholson, Munakata, Horneck, Melosh, & Setlow, 2000). Thus, spores of microbial cleaning products may potentially survive for years during storage and upon application on surfaces, giving them a potential advantage over non-sporulating bacteria. Upon reintroduction of nutrients and subsequent "activation", spores may germinate and return to the actively growing vegetative stage. However, in MBCPs there seems to be a lack of documentation on the stability of spores in the final product as well as the mechanism and rate at which spores are eventually activated and their ensuing germination upon application on dirty surfaces.

Other bacteria commonly referred to in MBCPs are members of the genera *Lactobacillus, Bifidobacterium, Saccharomyces* and *Rhodopseudomonas.* Some members of these genera are popular in probiotics for humans and animals. In such products, storage stability is, often ensured by various drying and encapsulation methods (Dianawati, Mishra, & Shah, 2016). Notably, there seems to be limited access to information on the methods used to ensure their viability upon storage in MBCPs.

3.1.2 Antimicrobial resistance (AMR)

The spread of antimicrobial resistance (AMR) to pathogenic microbes is a major problem in global public health. The occurrence of AMR in pathogens is often associated with selective pressure caused by exposure of microbes to antimicrobial agents. However, genetic markers associated with AMR are also widespread in natural microbiomes such as those residing in the human gut (van Schaik, 2015), soils (Forsberg et al., 2014), or oceans (Hatosy & Martiny, 2015). Even in microbial communities living in remote and pristine arctic environments, large repertoires of AMR genes have been found (Van Goethem et al., 2018). There are still knowledge gaps regarding the role played by these natural reservoirs in spreading AMR to pathogens. Of particular concern are AMR genes carried on mobile genetic elements, as these genes have the greatest potential for being transmitted among species.

Extrachromosomal elements carrying macrolide (*erm*) and tetracycline (*tet)* resistance genes have been described in strains of *Bacillus subtilis,* being raised as a potential health risk if such strains are used in probiotics (Gueimonde, Sanchez, C, & Margolles, 2013; Jeżewska-Frąckowiak et al., 2018). There are several studies describing the resistance patterns in pathogenic and probiotic species and strains of *Bacillus.* However, information regarding resistance patterns in *Bacillus* species and strains used in MBCPs seems to be rather limited.

Information regarding antimicrobial resistance in *Lactobacillus* and *Bifidobacterium* are generally based on data from species and strains used as probiotics, used in humans or animals (Gueimonde et al., 2013).*Lactobacillus* and *Bifidobacterium* used in MBCPs, like other bacteria, may constitute a potential source of antimicrobial resistance determinants. These microbial populations represent reservoirs of antimicrobial resistance genes, with potential to be transferred to other bacteria; both pathogenic and non-pathogenic species.

After screening of all literature containing words "*Lactobacillus*" and/or "*Bifidobacterium"*, included in this assessment, we have not identified any study regarding antimicrobial resistance in *Lactobacillus* and *Bifidobacterium* species/strains, used in MBCPs (Al-Ghalith and Knights 2015; Arvanitakis et al., 2018; Caselli et al., 2016; Caselli et al., 2017; D'Accolti et al., 2019; De Cesare et al., 2019; Dural-Erem et al., 2019; Gupta et al., 2018; Jezewska-Frackowiak et al., 2018; La Fauci et al., 2015; Mora et al., 2016; Spök et al., 2018; Subasinghe et al., 2018; Vandini et al., 2014). There is a major lack of knowledge regarding antimicrobial resistance in *Lactobacillus* and *Bifidobacterium* used in MBCPs.

Antimicrobial resistance in these bacterial species/strains does not constitute a safety concern in itself, if point mutations or other intrinsic resistance mechanisms are responsible for the resistance phenotype, since such properties rarely transfer to other bacteria.

Identification of bacteria present in MBCPs to the species or strain level is important for regulatory purposes and risk assessments, particularly if virulence or antibiotic resistance genes are carried by certain strains within a species, but not others within the same species (Subasinghe 2018).

3.1.3 Pathogenic potential

A reliable taxonomic designation allows for the appropriate assessment of a microorganism's infectivity, virulence and pathogenicity towards humans, animals and plants. This includes but is not limited to its ability to produce toxic metabolites (toxigenic potential), allergens and potential effects on vulnerable populations and species. Notably, strains and species that are closely related may have completely different virulence profiles. High-throughput molecular tools such as next-generation sequencing (NGS) facilitate rapid screening and identification of genetic indicators of virulence (e.g. genes encoding toxins etc). Various classification schemes are commonly used to specify the potential health risk of microorganisms, such as the risk group (RG) categorization by the EU and the qualified presumption of safety (QPS) list published by EFSA and generally recognized as safe (GRAS) categorization by the FDA (EFSA, 2017; EU, 2000; WHO, 2004) +CBGS /FDA refs. Regarding the RG categorization, nation-specific systems exist, some of which are easily accessible on the web. One such example is the "ePATHogen" database run by the Public Health Agency of Canada (Public Health Agency of Canada, 2019), which assigns both human and animal risk groups. A similar online tool is hosted by the American Biological Safety Association (ABSA) (ABSA, 2019). It is therefore important to note that there might be some differences in RG categorization among nations.

Genus *Bacillus*

Genus *Bacillus* comprises a group of highly diverse species commonly found in soil, spanning from harmless agents with industrial and beneficial applications (RG 1) to those with pathogenic properties (RG 2-3). Importantly, members of genus *Bacillus* are able to produce highly resistant endospores under starvation, thus making them capable of withstanding a wide range of environmental stress.

Many potentially beneficial applications of members of genus *Bacillus* have been described in literature, such as the production of enzymes, detergents, antibiotics and vitamins by *B. licheniformis, B. amyloliquefaciens, B. subtilis* and *B. pumilus* (de Boer, Priest, & Diderichsen, 1994; Schallmey, Singh, & Ward, 2004). Another example is the insect pathogen *Bacillus thuringiensis* which since the 1950's has been used for insect pest control in agriculture (Bravo, Likitvivatanavong, Gill, & Soberon, 2011). Interestingly, non-toxin producing strains of different *Bacillus* species are widely used as probiotics in animals and humans and are included in the qualified presumption of safety (QPS) list by EFSA (Cutting, 2011; FAO, 2016).

On the other hand, some species are associated with severe human and animal disease such as anthrax (*Bacillus anthracis*) and foodborne gastrointestinal disease (*Bacillus cereus*). Importantly, the insect pathogen *Bacillus thuringiensis,* the enteropathogen *Bacillus cereus* and the highly pathogenic *Bacillus anthracis* are genetically almost identical, which challenges taxonomic discrimination.

The pathogenic potential of "non-anthrax" *Bacillus* species, in particular *B. cereus,* have been associated with the production of toxic peptides and proteins causing foodborne disease (Stenfors Arnesen, Fagerlund, & Granum, 2008).

Bacillus subtilis and its close relatives, e.g. *B. licheniformis, B. pumilus, B. amyloliquefaciens,* (commonly referred to as the "subtilis group") are generally considered harmless (RG 1) and are frequently referred to as ingredients of MBCPs. However, controversies exist regarding their safe use as probiotics in humans and animals. Although rarely reported, foodborne illness has been associated with members of this group (Logan, 2012; Salkinoja-Salonen et al., 1999). The production of cytotoxic lipopeptides with detergent properties (surfactants) has been proposed as possible virulence factors. Interestingly, these cyclic lipopeptides may also have antimicrobial effects which attracts their use in probiotics and perhaps also in MBCPs.

Other *Bacillus* species commonly used in industrial and probiotic applications are also referred to in MBCPs (e.g. *B. megaterium, B. coagulans)* as well as species that have been reclassified to other genera (e.g. *B. polymyxa, Paenibacills polymyxa* and *B. sphaericus-Lysinibacillus sphaericus*). These species are generally considered harmless although these may possess the ability to produce surfactants.

To summarize, the prediction of the pathogenic potential of *Bacillus* spp. remains difficult, given the great variation among strains within the same species, ranging from harmless probiotic strains to those that can cause severe human disease. Thus, their pathogenic potential must be assessed using a broad selection of genotypic (e.g. presence of toxin genes) and phenotypic (e.g. cytotoxicity assays) properties.

Genus *Lactobacillus*

Members of genus *Lactobacillus* are commonly used in probiotics and are generally considered harmless (RG 1) (Public Health Agency of Canada, 2019).

Lactobacillus is the largest genus within the group of lactic acid bacteria. Based on the 16S rRNA sequences, lactobacilli are phylogenetically distributed in seven groups: *Lactobacillus buchneri* group (bu), *Lactobacillus casei* group (ca), *Lactobacillus delbrueckii* group (de), *Lactobacillus plantarum* group (pl), *Lactobacillus reuteri* group (re), *Lactobacillus sakei* group (sa) and *Lactobacillus salivarius* group (sl) (De Angelis & Gobbetti, 2016). Bacteria belonging to this genus may be used in the manufacture of fermented dairy, sourdough, meat, vegetable foods, and used in microbial cleaning products. *Lactobacillus* are generally normal microbiota of the mammalian digestive system and integument. Although there is a lot of information regarding *Lactobacillus* strains used in fermented dairy, sourdough, meat, and vegetable food product, such knowledge about *Lactobacillus* strains used in microbial cleaning products is lacking.

Genus *Bifidobacterium*

Members of genus *Bifidobacterium* are commonly used in probiotics and are generally considered harmless (RG 1).

Bacteria belonging to this genus have been isolated from six different ecological niches, of which three are directly linked to the human and animal intestinal environment: e.g., the human gut, animal intestine (bovine, rabbit, murine, chicken and insect) and oral cavity, while isolation from other sources (sewage, blood and food) probably indicates fecal contamination (Ventura, Turroni, & van Sinderen, 2015).

While bacteria in genera *Lactobacillus* are aerotolerant, *Bifidobacterium* species are obligately anaerobic. Data regarding *Bifidobacterium* used in MBCPs are very sparse and it is not clear how anaerobe bacteria like *Bifidobacterium* can survive in aerobic conditions and suppress harmful microbial populations on surface areas.

Genus *Rhodopseudomonas*

Members of genus *Rhodopseudomonas* are commonly used in probiotics (*R. palustris)* in aquaculture and are generally considered harmless (RG 1) (Public Health Agency of Canada, 2019).

Genus *Saccharomyces*

Members of genus *Saccharomyces* are commonly used in probiotics and are generally considered harmless (RG 1) (Public Health Agency of Canada, 2019).

Other genera listed in MBCPs

In the product survey by Arvanitakis et al. (2018), several products are labelled only at the genus level, with genera containing species/strains of RG 2 and 3 pathogens, such as *Streptocccus, Pseudomonas, Bacillus, Aspergillus* and *Burkholderia.*

3.1.4 Natural presence (species and strain) in the Norwegian environment

Several of the genera and species referred to in MBCPs are commonly found in Norwegian environments, such as soil, water and air. However, in many cases accurate information on the identification of the microorganism to the species and strain level is missing. Related to the issue of reliable taxonomic designation method is the issue of consistency in quality control and quality assurance (QC/QA) methods applied during the productions of the microorganisms and/or the end products. Consistency in the application of these methods would help to ensure that the microbial strain is identified as the active ingredient in the product, thereby providing better information regarding possible side effects on humans, working environments, or outer environments (soil or water). Currently no broadly-recognized standards for the QC and QA of cleaning products exist. Furthermore, there are currently no regulatory requirements for specifically identifying microbial ingredients on the labels of MBCPs in Canada, the European Union or the United States (Arvanitakis et al.,

2018). Without specification of microorganisms in MBCP to the strain level, it is not possible to conclude on their natural presence in the Norwegian environment, neither terrestrial, freshwater nor marine. Safety aspects (assessment) of MBCPs is thus highly dependent on better taxonomical identification, which can be considered a major data gap.

Interestingly, Canada has generated a list of domestic substances (DSL) which includes microbial strains known to be used in MBCPs (Spök et al 2018). Microorganisms that are not included on the list need to be declared to the authorities to allow risk assessment before manufacture or import into the country.

3.1.5 Mechanism of cleaning effect

The mechanisms of action of MBCPs are rather complex compared to their chemical-based alternatives, and consequentially these mechanisms are poorly described in literature.

Generally, microbial-based cleaning products harness the capability of living microorganisms to produce extracellular enzymes, such as cellulases, proteases and ureases. These enzymes can degrade high molecular weight compounds often associated with dirt and effectively mitigate unfavourable odours by further metabolism of intermediates from these processes. Nitrifying and sulphur-oxidizing bacteria can convert ammonia and thiols (-SH containing compounds); often intermediates of degradation processes characterized by strong pungent or fouls smells, to odourless nitrate and sulphate alternatives, respectively (Friedrich, 1998; Kampschreur et al., 2006; Kuenen, Robertson, & Van Gemerden, 1985). Thus, microbial action usually aims at controlling odour in addition to supporting the cleaning action of detergents.

Most of the cleaning formulations employ spores or spore-forming bacteria, and as such it is not surprising that manufacturers claim long term effects. Some microorganisms are capable of inhibiting the growth of other unwanted microbes. Thus, spores employed in MBCPs deposited on surfaces may germinate and grow into vegetative cells, which could outcompete pre-existing pathogens for nutrients and outgrow/displace such pathogens (Dural-Erem, Wessman, Husmark, & Nierstrasz, 2019; Spök et al., 2018). MBCPs containing *Bacillus* spp. were shown to significantly reduce pathogens and antimicrobial resistance markers when used in hospital settings (Caselli et al., 2018; Caselli et al., 2016; Vandini et al., 2014).

While the main mechanism is considered to be based on competitive antagonism or exclusion, the ability to produce and release antimicrobial compounds may also play a significant role in inhibiting growth of microbial cells or causing death, possibly in combination with quorum sensing, which primarily involves the regulation of gene expression in response to fluctuations in cell-population density (D'Accolti, Soffritti, Mazzacane, & Caselli, 2019; Piewngam et al., 2018; Spök et al., 2018; Vandini et al., 2014). Multiple members of the genus *Bacillus* are well recognized for the capability to produce multiple compounds with antimicrobial properties (Jeżewska-Frąckowiak et al., 2018; Vandini et al.,

2014). For example, surfactins produced by various Bacillus species, have detergent-like properties and have been shown to exhibit antimicrobial properties by interacting with bio-membranes (Bartal et al., 2018; Vollenbroich, Pauli, Ozel, & Vater, 1997). Other microorganisms such as the lactic acid bacteria can inhibit growth by lowering the pH in its environment (CBSG 2016, Dural-Erem et al 2019). Such mechanisms are considered biocidal in nature (CBSG 2016). While disruption of biofilms is suggested to be a possible mechanism of the cleaning effect of MBCPs (Vandini et al., 2014), other studies report that it is the stabilization of biofilms that effectively counteract the proliferation of pathogenic microorganisms (La Fauci, Costa, Anastasi, Facciolà, & Grillo, 2015).

Apparently, an interplay of varying mechanisms could come into play when it comes to the cleaning effect of MBCPs (Spök et al., 2018). However, it is worth noting that evidence-based literature on specific mechanisms of action are lacking.

3.2 Impact of microorganisms on human health

The potential health risk of exposure to microorganisms in MBCPs can only be assessed on the basis of species and/or strain-specific knowledge of the pathogenic properties of the microorganisms employed. This should be combined with information on exposure scenarios and populations at risk, particularly immunocompromised individuals, infants, the elderly and pregnant women, when used in MBCPs.

3.2.1 Potential exposure

Human as well as animal exposure to the microbial content of MBCPs may occur by direct contact to the skin / eye, inhalation via bioaerosols or ingestion. The magnitude of exposure will be dependent on a number of factors such as follows:

- the composition/formulation of the product;
- the mode of application; spray, wipes, liquid or solid form
- the quantity of product employed per application and the frequency of application
- the environment of application; indoor or outdoor
- the surface area in question
- the stability and survival of the microorganisms on surfaces (indoor/outdoor) and in air (aerosols).

There are a number of studies on the application of MBCPs in hospital settings (Al-Ghalith & Knights, 2015; Al-Marzooq et al., 2018; Caselli et al., 2019; Caselli et al., 2018; Caselli et al., 2016; D'Accolti et al., 2018; Dural-Erem et al., 2019; Vandini et al., 2014) but rather few on occupational exposure assessments (Villeneuve et al., 2018), and limited information on household settings apparently exists. Notably, irrespective of the above-mentioned factors which may affect exposure, microorganisms that are not pathogenic and classified into risk group (RG) 1 or the QPS list are not of great concern and may cause minimal or no illness/injury to humans (adapted from EU directive 2000/54/EC; VKM 2018

Terminologiveilederen, Intern rapport fra Vitenskapskomiteen for mat og miljø). (Arvanitakis et al., 2018; Subasinghe et al., 2018)

3.2.1.1 Healthcare settings

The prevailing attitudes underlying practices of medical hygiene and sanitization is undergoing a paradigm shift. There is emergence of evidence linking cleaning/sanitization practices to mounting virulence and antimicrobial resistance in hospitals, clinics and other healthcare settings (Al-Ghalith & Knights, 2015; Caselli, 2017). Current aseptic strategies, while not selecting specifically for virulence, happen to constitute an evolutionary pressure for the development of antimicrobial resistance, which may potentiate virulence or other disease mechanisms under certain conditions (Al-Ghalith & Knights, 2015; Caselli, 2017).

The issue of healthcare-acquired infections (HAIs) is of global concern, in particular in view of the rapid spread of multi-resistant strains (Caselli et al., 2018; D'Accolti et al., 2019; Dural-Erem et al., 2019; La Fauci et al., 2015). The problem of eliminating these nosocomial infections has proven intractable despite continually emerging sanitization technologies and protocols (Dancer, 2016). A common theme underlying these observations, is that extensive emphasis on creating a completely sterile environment is not only impractical, but also results in the killing of beneficial as well as innocuous microorganisms, thereby paving the way for opportunistic bacteria, while imperfectly controlling the extremely pathogenic ones, thereby effectively selecting for resistance mechanisms and increased virulence (Al-Ghalith & Knights, 2015; La Fauci et al., 2015; Vandegrift et al., 2017).

Several studies have proven MBCPs more effective in limiting the proliferation, colonization and spreading of pathogenic microorganisms on surfaces, over a long period of time, and thereby prevent recontamination as opposed to their conventional counterparts (Al-Marzooq et al., 2018; Caselli et al., 2019; Caselli et al., 2018; Caselli et al., 2016; D'Accolti et al., 2018; Dural-Erem et al., 2019; Vandini et al., 2014). Some of these trial studies confirm the genetic stability of the microorganisms used, ruling out the potential risk of increasing/spreading infections regarding the use of MBCPs in healthcare environments (Caselli, 2017; Caselli et al., 2018; Caselli et al., 2016). Additionally, significant reduction in the incidence of HAI and selection of resistant bacteria related to the use of MBCPs have been observed (Caselli et al., 2019; Caselli et al., 2018; Caselli et al., 2016).

B. subtilis, for example, is well known for its ability to produce multiple compounds such as bacteriocins and lantibiotics/peptide antibiotics with antimicrobial properties. Notably, it is reported that despite the beneficial activity of certain *Bacillus* strains belonging to SG 1, a number of strains can pose health risks, carrying genes for various toxins or antibiotic resistance (Jeżewska-Frąckowiak et al., 2018). This emphasizes the need for QC/QA in taxonomic identification and control of the products to strain level. Genus and/or species identification will often not be enough.

Mandatory microbiological as well as molecular monitoring of the environmental microbiota or microbial community in healthcare facilities employing MBCPs are proposed to ensure human safety and effectiveness of these products (Caselli, 2017).

3.2.1.2 Industrial applications

As mentioned earlier, our literature search results returned relatively few publications relating to the use of MBCPs in occupational or industrial settings. Villeneuve et al. (2018) conducted a study, monitoring biological degreasing stations (BDSs) in five different mechanic workshops over a one-year period, using a combination of biochemical, microbiological and molecular assay techniques.

The authors observed that the bio-degreasing fluids of BDSs were rapidly colonized by various exogenous microorganisms such as *Pseudomonas aeruginosa*. Noting that the danger of skin contact is mainly related to the infection of open cuts or to ingestion through cross-contamination, the authors emphasized the absolute necessity of rigorous personal hygiene, wearing protective gloves and hand washing before and after the use of BDSs for employees. However, no respiratory protection was recommended as the study showed that workers using BDSs had very low respiratory exposure to bioaerosols ($\sim 10^2$ CFU/m^3)(Villeneuve et al., 2018). Exposure to some microbial proteins/enzymes, particularly among workers in detergent manufacturing facilities, has been reported to induce IgE-dependent respiratory sensitisation with the potential risk of the onset of allergic events (D. Basketter et al., 2012; D. A. Basketter et al., 2010; D. A. Basketter et al., 2015). A complex interplay exists between the level/duration/frequency of exposure as well as individual susceptibility for induction and eventual elicitation. Nevertheless, by the establishment of strict limits on airborne exposure with a defined minimal effect limit of 60 ng active enzyme protein/m^3, air and health monitoring, occupational safety is most probably achieved (D. Basketter et al., 2012; D. A. Basketter et al., 2010; D. A. Basketter et al., 2015).

The International Association for Soaps, Detergents and Maintenance Products has proposed individual surveillance measures for employees (A.I.S.E., 2017; SDA, 2005). These surveillance strategies include questionnaires and pulmonary function testing as well as strict preventive measures at the collective level such as air quality controls. Occupational health personnel are required to monitor workers in such working environments in order to detect the onset of sensitisation before symptoms of allergy occur. It has been observed that reliance on extensive experience and strict recommendations to avoid the onset of symptoms in workers by the detergent industry has proven effective in lowering the incidence of sensitisation (D. Basketter et al., 2012; D. A. Basketter et al., 2010; D. A. Basketter et al., 2015).

There is concern for association between the use of MBCPs and potential respiratory sensitization, in the event of chronic exposure most probably by the use of spray forms in indoor environments (Spök et al., 2018). However, a recent study suggests that the acellular filtrate of MBCPs, but not the microorganisms employed in the product, potentiated allergic

lung inflammation from house dust mites, a relevant allergen for residential settings (Tayabali, Zhang, Fine, Caldwell, & Navarro, 2018). This finding suggests that for allergic inflammation, the hazard from the microbial constituent is minor, compared to the potential hazard from the chemical and/or enzymatic constituents of MBCPs. Previous reports indicate similar findings and/or conclusions, with the exception of only a limited number of fungi (Martel, Nielsen, Mari, Licht, & Poulsen, 2010; OECD, 2017). Notably, these opinions are discussed in detail by Spök and colleagues (Spök et al., 2018). Presently, the major hurdle is that there is no harmonized or validated test for respiratory sensitization or allergy. Thus, a precautionary approach is generally advised until adequate methods or further guidance are available. In this regard, the European Chemical Agency (ECHA) specifies in its 2017 guidance that "generally, all microorganisms are considered to be potentially sensitizing and should be labelled with "Microorganisms may have the potential to provoke sensitising reactions.". If scientific evidence shows that microorganisms do not have sensitization potential, this warning phrase could be waived…" (ECHA, 2017).

3.2.1.3 Household applications

Whereas industrial/occupational exposure may be controlled and monitored, this is not so for the general consumer since they are not subject to control. Consumer exposure is apparently not comprehensively characterized for most cleaning products on the market. However, the same principles apply to consumer safety, particularly vulnerable individuals such as the immunocompromised, infants, pregnant women and the elderly.

Berg et al. (2018) investigated several safety aspects of the use of *Bacillus*-based cleaning products. Two such products composed of *Bacillus* spore blends were employed in model studies. The microorganisms contained in these commercially available products include *B, subtilis, B. amyloliquefaciens, B. megaterium and B. licheniformis*. Of particular interest is the formation of aerosols, due to the exposure to the human respiratory system. Two different cleaning procedures were investigated:

- Whole room carpet treatment
- Spot treatment (handheld trigger sprayer)

The data suggest that carpet cleaning products containing non-pathogenic *Bacillus* spores present a low potential for inhalation exposure and consequently minimal risk of adverse effects. Based on their experiments, as well as a literature review, the authors concluded that the *Bacillus*-based carpet cleaning products pose minimal risk for consumers. As stated in the previous section, the acellular filtrate of MBCPs, but not the microorganisms employed was reported to potentiate allergic lung inflammation from house dust mites, a relevant allergen for residential settings (Tayabali et al., 2018). Additionally, some producers claim that microbial cleaners reduce allergenic reactions by out-competing and hence mitigating mites, moulds and other allergenic agents. Notably, some mould species like *Aspergillus oryzae* employed in some microbial-based cleaning products may possess allergenic properties (Spök & Klade, 2009).

It should also be noted that the materials in surfaces close to patients or other humans exposed to harmful microbial environment is clearly influencing the survivability, not only of

beneficial bacteria, but also of pathogens. Gupta, Bisesi, and Lee (2017) stated that floor materials play a major role in preserving microbial contaminants in the indoor environment. Dural-Erem et al. (2019) studied biocontrol of solid surfaces in hospitals using microbial-based wipes, releasing beneficial bacteria from the wipes after wetting. Results suggest that such wipes can be loaded with *Bacillus* spores and used for cleaning in health care environments. However, no information was provided on aerosols or other exposure to humans or environment by this procedure.

3.3 Impact of microorganisms on animal health

The same considerations that apply to humans should, in principle, apply equally to the health of other animals that are exposed to MBCPs. However, as for humans, there is a paucity of information on the health consequences that exposure to MBCPs may have on wild and domesticated animals, both in the short and the long term. In a study with mice, Tayabali et al. (2018) found that the acellular filtrate of an MBCP potentiated an allergic reaction to house dust mites, indicating MBCPs may have a negative impact on animal health. On the other hand, treatment of chicken litter with a *Bacillus* preparation resulted in reduced abundances of *Escherichia* in the caecum of the animals, but no general loss of bacterial diversity, and the authors concluded that their results indicate a positive effect on the chickens (De Cesare et al., 2019). Consequently, it is clear that more data are required in order to assess the risk MBCPs may pose to animal health.

This would apply also to any other application of MBCPs in animal husbandry, terrestrial, freshwater as well as marine. No data was found on the impact by MBCPs in aquatic animals. As sewage is released into aquatic environments, freshwater or marine, this is identified as a major knowledge gap.

3.4 Impact of microorganisms on plant health

There seems to be a lack of information on the potential impact of MBCPs on agricultural plant health when released into the environment. Thus, more data are required in order to assess the risk MBCPs may pose to plant health. This is identified as a major knowledge gap.

3.5 Impact of microorganisms on the environment

3.5.1 Dissemination routes and the potential of survival by release into the environment

Microorganisms in MBCP can spread into the environment mainly through two routes: By disposal through sewage systems, and outdoor disposal with direct entry into soil and water. Minor dispersal routes also include transport (adherence, translocation and deposition) from treated surfaces to soil and water. One such scenario will be when some of the organisms adhere to shoes/skin of people or animals that walk upon or otherwise touch surfaces where

microorganisms from such products have been used, and subsequently transfer them to surfaces that have not been treated with MBCPs. The latter would represent a minor titer and have a minimal chance of survival and spread, while disposal of products or diluted products through sewage or directly into the environment has a substantial potential to reach natural environments.

Spore-forming bacteria of genus *Bacillus* are the most commonly used microorganisms in MBCPs. Generally, metabolically dormant *Bacillus* spores tend to possess a strong capability of survival upon release into the environment implicating higher long-term as well as long-distance dispersal potential.

Survival through the transportation process, from the location of disposal to the target environment, depends on time and condition encountered during transport. Apart from direct disposal of MBCPs or residues on soil or in water, MBCPs are likely to reach these environments mainly after passage through a sewage system and will then be part of either sludge spread on soil, cleaned water, or overflow water during high precipitation events. Transport through a sewage system involves multiple challenges for survival, including passage through channels lined with indigenous microorganisms and their biofilms, as well as multiple possibilities for predation or immobilization (adhesion to surfaces). Upon arrival at a waste water treatment plant (WWTP), organisms from MBCPs are challenged with adverse conditions during processes like anaerobic digestion, chemical precipitation, sludge hygienization and composting (Levantesi et al., 2015). MBCP organisms ending up in sewage systems may avoid WWTP during periods of high precipitation when overflow is circumventing the waste water treatment. In this case, untreated sewage is discharged to water recipients, typically deep marine (Martiny et al., 2006). Here, high salt concentrations, low oxygen availability and low temperature will prevent proliferation. Due to natural sedimentation processes, MBCP organisms are likely prone to immobilization by precipitation and burial in sediments. Results from experiments with decay rates of sewage-associated bacterial communities in marine and freshwater environments indicate that the decay rate is high (Ahmed et al., 2018).

The potential for survival of MBCP organisms depends on their physiological and genetic characteristics. Endospore-forming bacteria, such as *Bacillus* spp. that are most commonly used in MBCPs, will for example not be limited by dispersal potential, while successful colonization will depend on their capacity to germinate, live and proliferate on/in any given natural environment. Such environments are typically oligotrophic with high microbial diversity and niche differentiation, making proliferation of introduced organisms difficult, but not impossible. Data gaps mainly pertain to whether or not specific strains can survive and establish in the environment, but also whether or not they may displace equivalent indigenous microorganisms and ultimately modify ecosystem function.

3.5.2 How the use and release of microbial-based cleaning products may affect the microbiological balance in the ecosystem

Survival and proliferation to an extent that perturbs or otherwise affects natural environments like soil and water depend on a wide range of factors where individual species or even strains may differ widely in their success/potential of surviving and establishing in the environment upon release. It is generally presumed that foreign microorganisms will have a very low rate of success in establishing in environments with high titers and high diversity of indigenous microorganisms, like soils, waste and sewage, particularly when conditions pose severe selection pressures related to temperature, oxygen-availability, pH, competition for substrates. In such environments, biotic factors (resource competition, antagonism and predation/parasitism among others) will also have a high potential for determining proliferation (Hibbing, Fuqua, Parsek, & Peterson, 2010).

When the introduced microorganisms belong to the same species as the indigenous ones, microorganisms manage to persist, and they may simply carry out the same function. Different strains of the same organisms may however have different capacities or limitations to carry out the function they have within their niche, possibly rendering the rates of the processes they are involved in affected. Higher rates of e.g. organic matter degradation and mineralization can potentially affect carbon storage and carbon-dioxide fluxes to the atmosphere, though such processes are mainly limited by physical and chemical constraints rather than biological capacities. Such risks are thus very low.

4 Recommendations on the documentation required for assessing the risk of MBCPs

To date, there are no common international regulations or quality standards regulating the production and use of microbial cleaners. In Norway, microbial products are regulated under the Product Control Act as any other product on the market in Norway (VKM, 2016). Additionally, a separate regulation regarding the declaration and labelling of microbiological products (Regulations on microbiological products) is also in force, with the primary aim to prevent microorganisms in microbiological products and technologies from causing adverse effects on biological health, the physical environment and ecosystems. This regulation requires importers, distributors as well as manufacturers of microbiological products in Norway to declare any information necessary for assessing the risk the product poses to human health or the environment. The guidelines to the regulations provide detailed description of the required information and documentation needed to declare a product in a satisfactory manner. This information provides the authorities with the basis for assessing the health and environmental risks associated with the use of such products.

The Norwegian Environment Agency considers that there is limited access to general information on cleaning products containing microorganisms from the developer, challenging the assessment of efficiency and safety of the products. The following sections describe important documentations necessary for conducting effective health and environmental risk assessments. Based on this, a checklist covering the information the declarant should specify has been suggested (Appendix II).

4.1 Identification of species and strains

This section outlines guidelines regarding accurate taxonomic identification of microbes intentionally added to MBCPs.

It is essential that accurate taxonomic information on the microorganisms used in the product is provided to the strain level. Strains are sub-populations within a microbial species, usually distinguished by an alphanumeric identifier added to the species name, and in some species, strains can differ dramatically in their phylogenetic properties. For example, the relationship of *Escherichia coli* strains to human hosts ranges from probiotic through commensal to pathogenic (Tenaillon, Skurnik, Picard, & Denamur, 2010). A second example is the genus *Bacillus* which includes innocuous species like *B. subtilis*, a frequent component of MBCPs, as well as pathogens such as those found within the *B. cereus* group (Bottone, 2010). The identity of the species should be determined by recognized phenotypic (morphological and biochemical) and genotypic means (e.g. 16S rRNA gene sequence). For strain level typing, a recognized method should be used, e.g. multi-locus sequence typing (MLST) (Maiden, 2006) or multiple-locus variable number tandem repeat analysis (MLVA) is often an appropriate means of strain identification (Lindstedt, 2005; Maiden, 2006). If the

whole genome sequence is available, this would be the ideal means of identifying the strain. If a microbe used in a MBCP has been acquired from a recognized culture collection (e.g. ATCC or DSMZ), strain level taxonomic information is available from the provider of the culture and this information should be declared with the product along with the origin (source) of the strain.

4.2 Characterization of species and strains

This section highlights the need for documentation on important characteristics of microorganisms employed in MBCPs. In addition to species/strain level taxonomic information, the specific function of a microbe in a cleaning product should be stated as well as the presence/absence of potential virulence factors. Thus, the applicant should declare the properties of a microbe (biochemical, genetic, physiological, toxicological etc.) that motivates its inclusion in the MBCP.

4.2.1 Cleaning effect

The MBCPs that work by an antagonistic principle should state the proposed mechanism through which the effects are obtained.

4.2.2 Pathogenic (human, animal, plant) potential

A cytotoxin production profile of any microbe included in an MBCP should be provided as well as the genomic screening results for known species-specific virulence genes. Microorganisms belonging to risk groups 2 or higher should not be used. Documentation on the allergenic properties of the strain should be provided.

4.2.3 Resistance profile

The antimicrobial resistance profile (intrinsic or acquired) of any microbe that is part of a MBCP should be provided along with the product. Furthermore, information on any mobile genetic elements that may be related to the transfer of resistance, such as plasmids, transposons, IS-elements, integrons, should be made available. Finally, if the complete genome sequence of a microbe has been published an accession number should be provided.

4.2.4 Genetic modifications and alterations

According to a literature survey of MBCPs little or no information is provided regarding the presence or absence of strains that have been genetically modified (Arvanitakis et al., 2018). Furthermore, unintentional genetic alterations may arise over time after repeated passages in the laboratory and during long-term storage. Thus, the applicant should declare that no

genetically modified strains have been added to the product, and provide information on how the cultures are maintained (VKM, 2014).

4.2.5 Synergistic and antagonistic effects

If an MBCP consists of a combination of several microbial species ("cocktail"), as is often the case (Arvanitakis et al. 2018, Subasinghe et al. 2018), the rationale behind the specific combination should be provided. In particular, antagonistic interactions among component species may render the mixture unstable (Hibbing et al., 2010), leading to the loss of species from the mixture and reduced efficacy of the product. Thus, some documentation of the stability of the "cocktail", under normal usage conditions, should be provided.

4.2.6 Environmental dispersion and survival

The ability of microorganisms used in MBCPs should be characterized with respect to dispersal potential and survival in different environments (fresh water, salt water, soil).

4.3 Quality control

Producers of MBCPs should provide documentation that commercial MBCPs meet certain quality standards. This is important to ensure that consumers receive products with predictable properties, produced using standardized processes subject to strict control routines. It is also important that the producer provide instructions on optimal usage.

4.3.1 Composition of the product

An MBCP may consist of both viable microbes and chemical components like surfactants. All ingredients should be listed, and any safety concern relating to any component should be addressed. For microbes, the number of viable cells of each species in the product within the time frame of its shelf life should be clearly given including a proviso that recommended storage conditions have been upheld. Viable cell numbers may be expressed as log Colony Forming Units (CFU) per weight or volume unit of the product. The state of microbial cells during storage (e.g. spores or vegetative cells) should be declared. Furthermore, information on the routines and methods used for quality proofing of the product in terms of the viability and stability of the microbes contained therein should be stated.

MBCPs may be subject to contamination by unwanted microbes during production, and there are studies suggesting this might have happened (Subasinghe et al., 2018; Teasdale & Kademi, 2018). Therefore, it is important that good routines for preventing contamination during the production process are in place, and that appropriate documentation of product purity is provided.

4.3.2 Storage conditions and shelf life

The storage conditions for maximizing viability of microbial species in an MBCP should be clearly described. Storage conditions will normally include factors like temperature, exposure to air and relative humidity. The expected shelf life of the product should be provided, including information documenting the survival of component microbial species during normal storage within the expected shelf life of the product. If the expected shelf life is affected by opening the product packaging, thus allowing access of oxygen, moisture, contamination etc., then information regarding the shelf life after opening should also be given.

4.3.3 Areas of usage/application

MBCPs have a number of different areas of usage, including surface cleaning, deodorizing, degreasing etc. (Arvanitakis et al., 2018). For a given product the intended usage should be clearly stated. This information should include the amount of product used for specific purposes and cleaning tasks of various magnitudes. Furthermore, if the product needs to be applied over a sustained amount of time in order to achieve the desired cleaning effects, the recommended time of action should be stated.

4.3.4 Dosage during usage

The appropriate amount of an MBCP that should be used for optimal efficacy in typical cleaning tasks should be clearly stated, as well as how the applicant established that this dosage is appropriate (experimental studies, dose finding studies etc).

4.3.5 Batch

During industrial production of microbial cells, there is always a risk that the production system may become contaminated by unwanted species. It is therefore imperative that stringent control routines for ensuring production batch integrity are in place and that all methods are validated and documented. Furthermore, the microbes being produced may change over time due to selective processes. Thus, even in the absence of contamination there should be routines for ensuring that key properties of the microbial species/strains being produced, including target enzyme production and antimicrobial resistance profile, are maintained over time.

4.3.6 Safety precautions for use and disposal

If there are specific safety precautions that should be taken during usage of a MBCP, e.g. protection of the eyes, airways and skin should be clearly stated. Also, recommended practices for disposing of used or expired product should be provided.

5 Uncertainties

EFSA recommends that assessments identify areas of uncertainties and state clearly their subsequent impact on the overall assessment outcome for the purpose of clarity and transparency in risk assessment processes. Additionally, this is critical in the subsequent selection of risk management options (EFSA Scientific Committee, 2018).

The degree of confidence in the final estimation of risk depends on the variability, uncertainty, and assumptions identified in all the previous steps. Discrimination between uncertainty and variability is important in the subsequent selection of risk management options. Biological variation includes for instance the differences in resistance levels that exist in microbiological populations over time, and between hosts and environments, including random fluctuations (FAO, 1999).

A number of uncertainties have been identified in this assessment. For the majority of commercial products there is limited or no information on the potential effects on the environment, including natural microbial communities and plants, as well as human/animal health hazards related to exposure to MBCPs in the workplace, households or farms. Most of these uncertainties are qualitative and may overlap with data gaps (see chapter 7).

- Insufficient knowledge on the environmental or health risks posed by the release of microorganisms employed in MBCPs.
- Insufficient taxonomic characterization of the microorganisms employed in MBCPs to the strain level.
- Uncertainty on the potential of survival by release into the environment and the subsequent effect on the microbiological balance in the ecosystem, as well as potential virulence factors in the microorganisms employed in MBCPs.

Notably, some degree of uncertainty may always exist when it comes to the ecological impact resulting from the use of MBCPs. Ecosystems are highly complex, and our understanding of underlying processes, such as invasion by foreign organisms and resilience to disturbance, is still rudimentary. This is especially true for microbial systems where we have only a cursory understanding of biotic interactions.

6 Conclusions with answers to the terms of reference

The Norwegian Environment Agency requested VKM to extend the VKM report from 2016; Health and environmental risk assessment of microbial cleaning products (VKM, 2016). The answers to the terms of reference (p.17) are provided below.

1. **Extension of the VKM report from 2016**
    1. The most commonly used microorganisms utilized in microbial-based cleaning products

According to literature, the most commonly used microorganisms in MBCPs are members of the genus *Bacillus*, followed by members of the genera *Bifidobacterium, Lactobacillus, Rhodopseudomonas* and *Saccharomyces*. A wide variety of other microorganisms are also found, including fungi and genera with potential risk group (RG) 2 and 3 agents. Overall, there seems to be lack of accurate and detailed information on the microbial composition of the MBCPs to the species and strain level.

2. Assessment of whether the microorganisms pose environmental or health risk upon release into the environment

The assessment of environmental or health risk upon release requires taxonomic classification to the species or strain level, as well as information about relevant release and exposure scenarios. Additionally, information on vulnerable groups such as the immunocompromised, infants, the elderly and pregnant women must be taken into consideration. Possible risks include spread of pathogens (human, animal, and plant), transmission and spread of antimicrobial resistance genes, production of toxins and allergenic properties. Non-toxigenic and non-pathogenic members of the genera *Bacillus, Bifidobacterium, Lactobacilllus, Rhodospeudomonas* and *Saccharomyces* are generally regarded harmless to humans and animals (RG 1). However, there is a paucity of information on the formulation of MBCPs, as well as the potential health consequences of MBCPs for humans, animals and plants, and effects on the environment, in both the short and the long term. Due to the lack of detailed information, the release of potentially pathogenic microorganisms cannot be excluded. Thus, the VKM panel is of the opinion that critical data gaps exist as specified in chapter 7, and that more information (Chapter 7; Appendix II) is required to assess the environmental or health risk upon release.

3. Assessment of the microorganism's natural presence of the used microorganisms in the Norwegian environment.

Several of the genera and species referred to in MBCPs are commonly present in Norwegian environments, such as soil, water and air. However, there is lack of accurate QA/QC-based

information on the identification of the microorganism to the species and strain level. Thus, more information is required to assess their natural presence in Norway.

4. Assessment of their potential survival by release into the Norwegian environment

A majority of the cleaning formulations employ spore-forming bacteria of the genus Bacillus. Endospores are highly resistant to environmental impact and may survive for an extremely long period of time (years) upon release.

5. Assessment of how use and release of MBCPs may impact the microbial balance in the ecosystem

The VKM panel is of the opinion that critical data gaps exist (e.g. on the identity of the microorganism and chemical ingredients), and that more information is required to assess the potential impact of MBCPs on microbial ecosystems.

6. Assessment of whether the microorganisms carry antimicrobial resistance genes

Based on the information available in literature, the microorganisms employed in MBCPs may carry antimicrobial resistance genes. However, taxonomic information of the microorganisms to the species and strain level, as well as genomic information on the respective organism(s), are needed to specifically address this question.

7. Overview of the molecular mechanisms which form the basis for the cleaning effect

A general presentation of the presumed cleaning effect has been provided in Chapter 3.1.5 and is generally based on fundamental knowledge on biochemical and physical properties of the most commonly used microbial species. The VKM Panel concludes that more studies are needed to document the biochemical basis for the cleaning effects, especially when it comes to potential biocidal effects.

2. Suggested documentation check list for declaration

VKM has suggested a list of important documentation necessary for conducting effective health and environmental risk assessments in Appendix II. The list outlines information an applicant should specify in an application for declaration.

7 Data gaps

In this chapter, insufficient knowledge and/or data related to the topic covered in the assessment is described. All data gaps described were uncovered during the assessment process. It is important to note that the panel did not have access to data regarding usage of MBCPs in Norway, neither in terms of quantity nor types of products.

Table 1: Knowledge and/or data uncovered in the current assessment and the impact upon provision of the knowledge and/or data that is missing.

Data gaps	**Expected impact in the event of filled data gaps** **(for VKM, the assigner, and/or the society)**
***In-vitro* as well as animal studies prior to testing for general usage of MBCPs in different settings seem to be lacking. In addition, data from long-term quantitative studies are lacking, particularly in household and industrial settings. More data are needed on specific exposure scenarios (inhalation, ingestion, dermal).**	Data collection as well as development of standard methods in this regard will enable the proper characterization of the potential hazard that the microorganisms employed in MBCPs pose to human health as well as the environment. This will in turn enable risk managers to make informed decisions in future measures and regulations.
Published research on the efficacy of MBCPs is limited. More studies are needed to document the biochemical basis for the cleaning effects, particularly when it comes to potential biocidal effects.	Such knowledge is key, especially for risk managers in decision-making regarding approval processes. Additionally, this will enable end-users to make informed decisions on their choice of cleaning products. Research on potential synergistic and antagonistic cleaning effects of mixtures of species/strains as well as the mechanism of potential inhibitory effects on unwanted microorganisms, including any biocidal

	effects will be highly beneficial to risk assessors in quantitative estimations.
There is little or no information on potential effects of MBCPs on the environment, including plants and natural microbial communities.	Mapping short-term/long-term effects of MBCPs in the various ecosystems via comprehensive studies will enable risk assessors to estimate their potential impact on these systems in an orderly manner.
Information on numerical data on usage as well as composition of MBCPs is scarce.	Availability of such information is important for use in the attainment of holistic risk assessments.
Regarding environmental risks, the lack of information on the species and strains used in MBCP, including their origin and abilities to persist in the environment upon use and disposal, constitute the main data gap. Apart from such information, which should be provided by the producer/applicant, there is a general lack of knowledge on transport, survival, establishment and proliferation of foreign microorganisms in soil and water, and existing knowledge may only be relevant for specific conditions (for soils; soil pH, ecosystem type, climate, etc., for water; content of suspended material, DOC, hardness/salinity, O_2-levels, etc).	Provision of the necessary information on species and strain will enable the potential immediate risks to be adequately assessed. Whereas surveillance and other forms of data collection mapping out the subsequent survival and establishment of the microorganisms employed will contribute to assessing long-term effects of MBCPs. Collection of data on specific exposure scenarios will enable risk assessors to perform qualitative/quantitative exposure assessments. This will in turn lead to proper characterization of the risk posed by MBCPs and risk managers will have access to concrete conclusions to assessments for implementation in decision-making processes.

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Appendix I

Table 2. Characteristics of selected microorganisms employed in microbial-based cleaning products

Microorganism (genus)[1]	Microorganism (species)[1]	Risk group[2] (human)	Risk group[2] (animal)	References
Bacillus	*Bacillus subtilis*	RG 1	RG 1	(Arvanitakis et al., 2018; Berg et al., 2018; D'Accolti et al., 2019; Public Health Agency of Canada, 2019; Subasinghe et al., 2018; Tayabali & Ashby, 2018; Vandini et al., 2014; Villeneuve et al., 2018)
	Bacillus pumilus	RG 1	RG 1	(Arvanitakis et al., 2018; D'Accolti et al., 2019; Public Health Agency of Canada, 2019; Subasinghe et al., 2018; Vandini et al., 2014)
	Bacillus megaterium	RG 1	RG 1	(Arvanitakis et al., 2018; Berg et al., 2018; D'Accolti et al., 2019; Public Health Agency of Canada, 2019; Subasinghe et al., 2018; Vandini et al., 2014)
	Bacillus amyloliquefaciens	RG 1	RG 1	(Arvanitakis et al., 2018; Berg et al., 2018; Public Health Agency of Canada, 2019; Subasinghe et al., 2018)
	Bacillus licheniformis	RG 1	RG 1	(Arvanitakis et al., 2018; Berg et al., 2018; Public Health Agency of Canada, 2019; Subasinghe et al., 2018; Tayabali & Ashby, 2018)
	Bacillus circulans	RG 1	RG 1	(Arvanitakis et al., 2018; Public Health Agency of Canada, 2019)

Microorganism (genus)[1]	Microorganism (species)[1]	Risk group[2] (human)	Risk group[2] (animal)	References
	Bacillus thuringiensis	RG 1	RG 1	(Public Health Agency of Canada, 2019; Subasinghe et al., 2018)
Rhodopseudo-monas	*Rhodopseudo-monas palustris*	RG 1		(Arvanitakis et al., 2018; Public Health Agency of Canada, 2019)
Lactobacillus	*Lactobacillus plantarum*	RG 1	RG 1	(Arvanitakis et al., 2018; Public Health Agency of Canada, 2019)
	Lactobacillus lactis	RG 1	RG 1	(Arvanitakis et al., 2018; Public Health Agency of Canada, 2019)
	Lactobacillus casei	RG 1	RG 1	(Arvanitakis et al., 2018; Public Health Agency of Canada, 2019)
	Lactobacillus acidophilus	RG 1	RG 1	(Arvanitakis et al., 2018; Public Health Agency of Canada, 2019)
	Lactobacillus fermentum	RG 1	RG 1	(Arvanitakis et al., 2018; Public Health Agency of Canada, 2019)
	Lactobacillus helveticus	RG 1	RG 1	(Arvanitakis et al., 2018; Public Health Agency of Canada, 2019)
	Lactobacillus bulgaricus	RG 1	RG 1	(Arvanitakis et al., 2018; Public Health Agency of Canada, 2019)
	Lactobacillus delbrueckii	RG 1	RG 1	(Arvanitakis et al., 2018; Public Health Agency of Canada, 2019)
Bifidobacterium	*Bifidobacterium bifidum*	RG 1	RG 1	(Arvanitakis et al., 2018; Public Health Agency of Canada, 2019)
Saccharomyces	*Saccharomyces cervisiae*	RG 1	RG 1	(Arvanitakis et al., 2018; Public Health Agency of Canada, 2019)
Lysinibacillus	*Lysinibacillus (former Bacillus) sphaericus*	RG 1	RG 1	(Arvanitakis et al., 2018; Public Health Agency of Canada, 2019)
Paenibacillus	*Paenibacillus (former Bacillus) polymyxa*	RG 1	RG 1	(Arvanitakis et al., 2018; Public Health Agency of Canada, 2019)
Pseudomonas	*Pseudomonas fluorescens*	RG 1	RG 1	(Arvanitakis et al., 2018; Public Health Agency of Canada, 2019)

Microorganism (genus)[1]	Microorganism (species)[1]	Risk group[2] (human)	Risk group[2] (animal)	References
Aspergillus	*Aspergillus oryzae*	RG 1	RG 1	(Arvanitakis et al., 2018; Public Health Agency of Canada, 2019)
Candida	*Candida utilis*	RG 2		(Arvanitakis et al., 2018; Public Health Agency of Canada, 2019)
Rhodobacter	*Rhodobacter sphaeroides*	RG 1		(Arvanitakis et al., 2018; Public Health Agency of Canada, 2019)
Streptomyces	*Streptomyces griseus*	RG 1	RG 1	(Arvanitakis et al., 2018; Public Health Agency of Canada, 2019)
	Streptomyces albus	RG 1	RG 1	(Arvanitakis et al., 2018; Public Health Agency of Canada, 2019)
Mucor	*Mucor hiemalis*	RG 1	RG 1	(Arvanitakis et al., 2018; Public Health Agency of Canada, 2019)

1: Only microorganisms defined to the species level have been included in this table. Products may contain a mixture of different species.

2: Only non-pathogenic microorganisms belonging to risk group 1 should be used in MBCPs.

Notably, various risk classification schemes exist. However, for the purpose of table 2 in this report, the scheme below was employed.

Definitions and risk classification scheme from the Canadian Biosafety Handbook (CBSG, 2016):

Risk Group 1 (RG1; low individual and community risk)

A microorganism, nucleic acid, or protein that is either a) not capable of causing human or animal disease; or b) capable of causing human or animal disease, but unlikely to do so. Those capable of causing disease are considered pathogens that pose a low risk to the health of individuals or animals, and a low risk to public health or animal population. RG1 pathogens can be opportunistic and may pose a threat to immunocompromised individuals. Due to the low risk to public health and animal population associated with RG1 material, there are no physical or operational requirements for handling them. Nonetheless, due care should be exercised and safe work practices (e.g., good microbiological laboratory practices) should be followed when handling these materials.

Risk Group 2 (RG2; moderate individual risk, low community risk)

A pathogen or toxin that poses a moderate risk to the health of individuals or animals, and a low risk to public health and the animal population. These pathogens are able to cause serious disease in a

human or animal but are unlikely to do so. Effective treatment and preventive measures are available and the risk of spread of diseases caused by these pathogens is low.

Risk Group 3 (RG3; high individual risk, low community risk)

A pathogen that poses a high risk to the health of individuals or animals, and a low risk to public health. These pathogens are likely to cause serious disease in a human or animal. Effective treatment and preventive measures are usually available and the risk of spread of disease caused by these pathogens is low for the public. The risk of spread to the animal population, however, can range from low to high depending on the pathogen.

Risk Group 4 (RG4; high individual risk, high community risk)

A pathogen that poses a high risk to the health of individuals or animals and a high risk to public health. These pathogens are likely to cause serious disease in a human or animal, which can often lead to death. Effective treatment and preventive measures are not usually available and the risk of spread of disease caused by these pathogens is high for the public. The risk of spread of disease to the animal population, however, ranges from low to high, depending on the pathogen.

Appendix II

Table 3. Proposed documentation check list covering information an applicant should specify in an application for declaration regarding risk assessment of microbial-based cleaning products

	Yes/No	References/Comments
Identification of microorganisms		
All microorganisms in the product belong to a certified international culture collection (e.g. ATCC, DSMZ) and have been identified to the species and strain level using internationally recognized methods		
The identity of all microorganisms in the product has been verified by a third-party accredited laboratory		
All microorganisms in the product are considered unlikely to/not capable of causing human or animal disease (Risk group I, QPS, GRAS)		
The product shall not contain genetically modified microorganisms (GMMs)		
Characterization of microorganisms		
Presence of genes associated with virulence		
Presence of genes associated with antimicrobial resistance		

	Yes/No	References/Comments
Resistance profile (specify method, third-party accredited laboratory): All intentionally added microorganisms shall be, with the exception of intrinsic resistance, sensitive to each of the major antibiotic classes, e.g. aminoglycosides, macrolides, beta-lactams, tetracyclines, fluoroquinolones, glycopeptides, in accordance with reference method		
Cytotoxic activity (specify method, third-party laboratory)		
Allergenic properties		
Documented cleaning effect		
Environmental dispersion and survival of all intentionally-added microorganisms, relating to their recommended use and dispersion routes		
Quality control		
The final cleaning product has been tested for the absence of microbial contaminants (purity check, by third party accredited laboratory)		
The final cleaning product has been tested for the viability and concentration of microorganisms (incl. spores) by third party accredited laboratory		

	Yes/No	References/Comments
The concentration of viable microorganisms shall not decrease by more than xx% every 12 months (ISO 4833-1:2014)		
Product information		
Composition		
Storage		
Shelf life		
Areas of usage		
Concentration (incl. spores)		
Batch		
Safety precaution		
Information related to user groups (suitability of product in food/feed/healthcare facilities) / vulnerable individuals (immunocompromised, infants, the elderly and pregnant women)		

The checklist is based on the following information sources: i) the current assessment of health and environmental risks of MBCPs (Chapters 3 & 4 in this report), ii) VKM (2016), iii) Commission Decision (EU) 2017/1217 of 23 June 2017, establishing the EU Ecolabel criteria for hard surface cleaning products (EU, 2017), iv) Nordisk Miljömärkning v.6.0 (2018).

Contact information (name, address, telephone and website of the Company) should be stated on the packaging.

NEA's evaluation of the documentation received	
Application accepted	
Application rejected	
Need for further documentation	
Need for risk assessment – request to VKM	

Comments: