Chapter 9

Microbial-based cleaning products in use and the potential role of transgenic micro-organisms

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This chapter provides a survey of the currently known uses of micro-organisms in different types of cleaning products based on searches conducted of publicly available information sources such as the scientific literature, patent databases and commercial websites. Examples of microbial species known to be used in different types of cleaning applications will also be given as well as potential human health and environmental issues associated with their use. A brief summary of Canadian regulatory experiences with these products, in particular those of the New Substances Program of Health Canada and Environment Canada, will be provided as well.

Introduction

Cleaning products are familiar to virtually everyone who lives or works in any kind of domestic residential setting, commercial place of business or institutional setting such as hospitals or daycare centres. Because of their widespread use, they are a large industry in many countries, including the United Kingdom (>GBP 3 billion in 2011) and the United States (USD 30 billion in 2010) (UK Cleaning Products Industry Association, 2011; American Cleaning Institute, 2012). Exact figures for sales of cleaning products in Canada could not be found, but it appears that a significant portion of the CAD 20 billion industry on consumer specialty products consists of soaps, detergents, disinfectants, sanitizers and air care products (i.e. deodorisers) (Canadian Consumer Specialty Products Association, 2012a; 2012b).

Cleaning products are mostly liquid formulations (although many come in powder form) used by consumers, typically in domestic settings, or by cleaning professionals in larger business or institutional settings. Any visit to a local supermarket, hardware or home renovation store indicates that the vast majority of cleaning products currently on the market in North America and Europe continue to contain chemical substances that tend to be reactive or corrosive in nature. Examples of these include solutions of sodium hypochlorite (household bleach), sodium hydroxide (found in many detergents and drain cleaners) and ammonium hydroxide (used in hard surface cleaners). Because of their reactive nature and their widespread use, these substances are very often a concern for human health effects as well as environmental impacts. In some cases, inappropriate mixing of some of these products have produced toxic chlorine and ammonia gases leading to acute poisoning and illness as well as more chronic effects (Nazaroff and Weschler, 2004).

In recent years, cleaning products containing various strains of micro-organisms as active ingredients have become increasingly prevalent in many countries as an alternative to chemically based cleaning products. These products appear to be increasingly sold for use in many of the domestic, commercial and institutional settings mentioned above, as well as for a variety of cleaning activities (hard surface cleaning, odour control, degreasing, septic tank treatments, etc.) where chemically based cleaning products have traditionally been used. Many of these products are very often advertised and described as "environmentally friendly", "biodegradable" and "non-toxic". These products are part of the larger category of "green cleaning products" that are available in supermarkets and hardware stores, and are very often advertised and sold online (an Internet search using a few relevant key words such as "bacteria" + "cleaning" + "green" + "enzyme", etc. produces many examples of these). Although microbial-based cleaning products are likely a relatively small portion of this market, it has been projected that the overall global market for green cleaning products may reach USD 9.32 billion by 2017 (PR Web, 2011).

The purpose of this chapter is to provide a survey of the currently known uses of micro-organisms in different types of cleaning products based on searches conducted of publicly available information sources such as the scientific literature, patent databases and commercial websites. Examples of microbial species known to be used in different types of cleaning applications will also be given as well as potential human health and environmental issues associated with their use. A brief summary of Canadian regulatory experiences with these products, in particular those of the New Substances Program of Health Canada and Environment Canada, will be provided as well as a proposal for a workshop to be hosted in Canada to further examine and discuss these and other issues.

Survey of microbes currently used in cleaning products

Known uses of these products

Table 9.1 provides a broad sample of what has been found through a search of publicly available information (scientific literature, patent databases, commercial websites, etc.) on current uses of microbial-based cleaning products and the types of micro-organisms they contain.

It thus appears that microbes (both as vegetative cells and as spores) are found in a wide variety of cleaning products and treatment applications where chemical agents have traditionally been applied for the same end uses. It should be noted that a large number of additional commercial websites were found advertising the sale of such products but without providing any specific details on the formulation of their products.

Although it is not within the scope of this chapter, there appears to be little publicly available information (aside from anecdotal evidence such as product testimonials) on the effectiveness of these products.

Microbial species used in these products

This section provides brief summaries of some of the microbial species that have been identified as being the active ingredients in these products.

Bacillus *spp.*

The most prevalent microbial species contained in these products appear to be those from the genus *Bacillus*. Most *Bacillus* species are commonly found soil micro-organisms which have the ability to form endospores in response to extreme environmental conditions. Of these, *B. subtilis* appears to be the one the most commonly identified. It is generally considered to be non-pathogenic and has been used as a probiotic and in the production of fermented foods (Hong et al., 2008) as well as a production organism for enzymes in detergents (Adisesh et al., 2011). *B. licheniformis* and *B. amyloliquefaciens* strains have also been used for this purpose (Adisesh et al., 2011). *B. polymyxa* strains have also been used as production organisms for topical antibiotics (Gelmetti, 2008).

Other bacterial genera

A variety of other bacterial genera appear to be represented in these products, many of which are not identified to the species level. These include *Achromobacter*, *Actinobacter*, *Alcaligenes*, *Arthrobacter*, *Rhodopseudomonas*, *Rhodobacter* and *Lactobacillus*. Of these, *Lactobacillus* is perhaps the best known, various species of which have been used as probiotics and in food production, and are generally considered non-pathogenic (Wassenaar and Klein, 2008). *Achromobacter* species are commonly found in fresh water and marine environments and are considered, among other things, as "beneficial bacteria" for use in aquaculture operations (Zhou et al., 2009). Various literature was found describing how species of some of these genera have been found to degrade various xenobiotic compounds (for example, see Perez-Pantoja et al., 2009). Other examples of this include various species of *Alcaligenes*, *Arthrobacter* and *Rhodopseudomonas* that have been found to degrade textile azo dyes (Xingzu et al. 2008; Pearce et al., 2003).

Table 9.1. **Micro-organisms used in cleaning products**

Product/process	Use	Micro-organisms in formulation	Number of micro-organisms in formulation	Information source/website
Liquid cleaner	Improve surface cleaning	Primarily *Bacillus subtilis*. Other potential organisms: *Pseudomonas*, *Arthrobacter*, *Enterobacter*, *Citrobacter* and *Corynebacter*	$1x10^6$ – $1x10^9$ /ml	Sybron Chemical Industries WIPO: EP0245560
Detergent	Soap	Lactic acid bacteria, yeast and photosynthetic bacteria	Yeast: $1×10^5$ /ml Lactic acid bacteria: $1.5×10^5$ / ml	EM Res Organization Inc. WIPO: EP1717301
Detackifying organic solvent	Degradation of water-borne paint containing high levels of organic solvent	Primarily *Bacillus subtilis, Pseudomonas fluorescens*. Other organisms used include: *Bacillus* sp., *Citrobarobacter* sp., *Aeromonas* sp., *Shewanella* sp., *Pseudomonas* sp., *Corynebacterium* sp., *Rhodococcus* sp. or a mixture thereof.	Not provided	Atotech Deutschland GMBHDEUTSCHLAND GMBH WIPO: WO2008094872
Cleaning solution	Removal of hydrocarbons	*Achromobacter*, *Actinobacter*, *Alcaligenes*, *Arthrobacter, Bacillus, Flavobacterium* and *Pseudomonas* species, and mixtures thereof. Preferred are those naturally occurring of the genus *Bacillus*, particularly *B. subtilis, B. licheniformis* and *B. polymyxa*	Not provided	Earth Alive Resources Inc. WIPO: WO2002033031
Cleaning agent	Enhance fermentation in soap production process	*Lactobacillus* group, yeast group and photosynthetic bacterium group	Not provided	EM Research Organization WIPO: KR1020070003956
Stable antimicrobial composition including spores, bacteria, fungi and/or enzymes	Various cleaning applications	Suitable spores (bacterial or fungal), vegetative bacteria, fungi or enzymes from *Bacillus*, *Pseudomonas*, *Arthrobacter, Enterobacter, Citrobacter, Corynebacter, Nitrobacter* species, mixtures thereof or the like; *Acinetobacter, Aspergillus, Azospirillum, Burkholderia, Ceriporiopsis, Escherichia, Lactobacillus, Paenebacillus, Paracoccus, Rhodococcus, Syphingomonas, Streptococcus, Thiobacillus, Trichoderma, Xanthomonas, Lactobacillus, Nitrosomonas, Alcaligenes, Klebsiella* species, mixtures thereof or the like.	$1x10^3$ – $1x10^9$ cfu/ml	Ecolab USA Inc. USPTO: 8211849 20110207649
Composition comprising organic matter and micro-organisms	Deodorizer, degreaser, mould inhibitor	Phototrophic bacteria belonging to: *Rhodopseudomonas, Rhodobacter, Rhodospirillum, Chromatium and Chlorobium*; lactic acid bacteria belonging to: *Lactobacillus, Propionibacterium, Pediococcus* and *Streptococcus* and yeasts: *Saccharomyces* and *Candida*	Not provided	USPTO: 20080085546
Probiotic micro-organism for the reduction of manure odour	Odour control	Micro-organisms belonging to the genus *Lactobacillus* or yeast belonging to the genera *Cryptococcus*, *Kluyveromyces*, *Candida* or *Metschnikowia*	Yeast content: 1.4×10^8 cfu *Lactobacillus* content: 5.4×10^9 cfu	OrganoBalance GmbH USPTO: 20110117068
Multi-action drain cleaning composition and method	Drain cleaning	*Bacillus subtilis* ATCC 6051, ATCC 14415 and ATCC 35946, *Bacillus licheniformis* ATCC 6598 and ATCC 1194, and *Bacillus polymyxa* ATCC 12060	Not provided	Organica Biotech USPTO: 20090263884

Table 9.1. **Micro-organisms used in cleaning products** (*cont.*)

Product/process	Use	Micro-organisms in formulation	Number of micro-organisms in formulation	Information source/website
Enz-Odor® 4, Enz-Odor® 4-2XFF , Nu-Bind 1* etc	Odour control agents	Liquid products containing exclusively spore-forming *Bacillus* species with preservatives, with or without additional ingredients	On average 2 x 10^9 cfu / g	Alken Murrary Corp: www.alken-murray.com/Odorindex.html; United States
EM-1 microbial inoculant	Aquarium/fish pond treatment	*Rhodopseudomonas palustris* and other unidentified phototropic and lactic acid bacteria, yeast	Not provided	Emerald Earth: www.emearth.com/NewFiles/Aquariums.html; United States
Clear-Flo (various products)	Surface cleaners	*Bacillus subtilis* strains	Various concs.; link provides extensive list of products	Alken Murrary Corp: www.alken-murray.com/CleaningIndex.html; United States
Drainbo	Drain cleaner	Several strains of *Bacillus*	Not provided	Drainbo The Natural Solution: www.drainbo.com/news.html; United States
Bio Source Zymo Super Concentrated Drain Cleaner, Green Bin Deodorizer	Drain cleaner and deodorizer	*Bacillus* cultures and substrains	Not provided	BioSource Solutions Inc.: www.biosourcesolutionsinc.com/site/products; Canada
Various cleaning products	Household drain cleaners and degreasers, septic tank additives and general cleaning products	*Pseudomonas aeruginosa* ATCC 31480, 700370 and 700371	Not provided	www.chemicalsubstanceschimiques.gc.ca/fact-fait/glance-bref/pseudomonas-eng.php; Canada
EcoWorks (various cleaning products)	Washroom cleaner, multi-surface cleaner, odour eliminator	*Bacillus* strains	5.25 x 10^7 /ml, equivalent to 200 billion/5 litres	www.eco-works.co.uk/probiotic-cleaning-liquids.html; United Kingdom
BioPure	Household cleaner	*Lactobacillus* cultures	Not provided	Pure Alternatives: www.purealternatives.net/biopure.html; Australia
EM-1 septic treatment	Septic tank additives/ treatment	Mixture of *Lactobacillus plantarum, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus delbrueckii, Bacillus subtilis, Saccharomyces cerevisiae, Rhodopseudomonas palustris*	1 million cfu/ml	Teraganix: www.teraganix.com/32oz-EM-1-Septic-Treatment-p/1022.htm; United States
Sporyzme BCC	Degrade organic waste and eliminate associate odours	Blend of *Bacillus* spores	5.0 x 10^{10} cfu/gm	NovoZymes: www.waterguru.net.au/pdfs/Sporzyme%20BCC%20PDS-1.pdf; United States

Sources: World Intellectual Property Organization (WIPO), www.wipo.int/portal/index.html.en; United States Patent and Trademark Office (USPO), www.uspto.gov.

Fungal species

Some of the cleaning products found in the literature were declared to contain *Saccharomyces* and *Candida* species. It is common knowledge that a number of *Saccharomyces* species (such as *S. cerevisiae*) have a long history of safe use in the baking, brewing and winemaking industries. In recent years it has also been recognised that a number of yeast species, including some belonging to *Saccharomyces* and *Candida*, have the potential to be effectively used in the biodegradation of a variety of hazardous chemicals (Xiuyan et al., 2011; Harms et al., 2011).

Potential targets of gene modification

No information was found indicating that any of the micro-organisms contained in the above-mentioned cleaning products were genetically modified in any way. However, there are indications in the literature that some of the genes involved in producing enzymes or biosurfactants and bioemulsifiers whose mode of action involves the increased solubilisation and breakdown of organic substances could be modified to enhance some of their properties. Thus, it is at least possible that genetically modified micro-organisms could find their way into cleaning products in the future, although it is questionable whether such products would continue to be regarded as "green".

Enzymes

Some of the main targets for gene modification have been those coding for the production of various amylases and proteases used in detergent products, mainly with the aim of improving their activity at lower water temperatures and more alkaline pH levels (Kirk et al., 2002). For example, *B. subtilis* strains have been engineered to express some of these modified genes (Ness et al., 1999). As well, a number of recombinant lipase enzymes have been produced using engineered *Bacillus* and *Aspergillus* species (Hasan et al., 2010).

Biosurfactants/bio-emulsifiers

Much research has been conducted recently towards engineering improved versions of various biosurfactant and bio-emulsifying substances (such as surfactin, rhamnolipids and emulsans) for use in detergent and other cleaning product applications. For the most part, the aim of the research has been to increase yields of these substances when expressed in various bacterial species (mostly *Bacillus*, but also in a number of *Acinetobacter*, *Pseudomonas* and *Serratia* species as well). A thorough review of this research is provided in Satpute et al. (2010).

Potential human health and environmental issues

A number of potential human health issues related to the use of microbial-based cleaning products have previously been described in a recent report on the use of such products, mainly in Europe (Spok and Klade, 2009). Environmental issues may also potentially exist because of the widespread use of such products and releases into the environment that may result. These issues can be categorised as issues: *i)* related to the micro-organism itself; and *ii)* related to formulation/use of the product.

Issues related to the micro-organism itself

Likely the single most important issue related to the micro-organisms themselves is the reliability of their taxonomic designation. Many of the micro-organisms found in these products were identified only to the genus level. For those identified to the species level, little to no information is provided as to what methods or tests were used to arrive at their identification. Some of the products do appear to have used micro-organisms from well-known culture collections (such as the ATCC), thus providing somewhat increased confidence in their taxonomic designation. From an overall risk assessment perspective, reliable taxonomic designation of a given micro-organism is the most important determinant of its potential hazard to human health and environment (Environment Canada and Health Canada, 2011a). A reliable taxonomic designation allows for the appropriate assessment of a micro-organism's infectivity, virulence and overall pathogenicity. This includes its ability to produce toxins, toxic metabolites and allergens as well as potential effects on sensitive populations (e.g. the immunocompromised, children/elderly, pregnant women, etc.) (Spok and Klade, 2009; Environment Canada and Health Canada, 2011a).

Based on the micro-organisms identified as being contained in the products listed in Table 9.1, even a cursory survey of the scientific literature reveals that it is possible that some of these products may contain pathogens. For example, some toxin-producing strains of *B. licheniformis* have been identified in outbreaks of food poisoning (Mikkola et al., 2000). Another example is *Acinetobacter baumanii*, which has recently emerged as a cause of healthcare associated infections (Fournier and Richet, 2006). A third example is several *Candida* species, including *C. albicans*, considered to be opportunistic pathogens for which a number of different virulence factors have been identified (Yang, 2003). In cases like these, proper taxonomic designation of a micro-organism to at least the species level (and in some cases, the sub-species or strain level) becomes very important, since it can help to distinguish between pathogenic and non-pathogenic strains.

Issues related to formulation/use of product

As far as the products themselves are concerned, a number of issues have become apparent. Somewhat related to the issue of reliable taxonomic designation mentioned above is the issue of consistency in quality control (QC) and quality assurance (QA) methods applied during the production of the micro-organisms and/or the end products. There are indications from previous studies (Spok and Klade, 2009), as well as from past experiences of the New Substances Program in Canada, that there is a wide variation in how QC/QA methods are applied in the production of these products. This includes procedures in place to monitor for potential contaminants. Currently, no broadly recognised standards for the QC and QA of cleaning products exist. However, in Canada, the EcoLogo Program, a voluntary third-party certification programme for environmentally preferable products, requires that all biologically based cleaning and degreasing products be manufactured in a facility that has a documented QC/QA system (EcoLogo, 2011).

As well, there are currently no regulatory requirements for specifically identifying microbial ingredients in these products in Canada. Since many of these types of products appear to be imported into Canada, and because the active ingredients are very often considered confidential business information, importers, distributors and end users very often do not know what micro-organisms are present in these products. There also do not

appear to be any specific labelling requirements for these products in the European Union or in the United States. However, as of April 2011, the United States Environmental Protection Agency's voluntary programme "Design for the Environment" requires that all non-trade secret ingredients be listed for all products that carry the Design for the Environment label, including cleaning products. Non-trade secret ingredients also need to be described as specifically as possible without revealing trade secret information (United States Environmental Protection Agency, 2011).

Considering the way in which microbial-based cleaning products would typically be used, human exposure to the micro-organisms contained within them is likely to some extent. Dermal exposure is the most obvious route; however, spray applications and powders can create aerosols leading to inhalational exposure as well. To a lesser extent, oral ingestion may also be possible, particularly if these products are applied anywhere near surfaces used for food preparation. Long-term exposures may also be possible since many of these products appear to contain spores that can remain viable for long periods of time. All of these exposures may also be enhanced by the fact that many of these products will be used in indoor settings where proper ventilation may not always be in place. There currently appears to be a significant lack of information in the scientific literature on the nature and magnitude of potential human exposures to micro-organisms through their use in these products, thus making any attempt to more precisely assess human health risks from such products somewhat difficult.

Regulatory experiences in Canada with these products

In terms of systematically assessing any potential risks to human health and the environment from the use of such micro-organisms in cleaning products in Canada, only one legislative authority currently exists: the Canadian Environmental Protection Act, 1999 (CEPA1999; Department of Justice Canada, 2012a). Information and data required from manufacturers or importers of new micro-organisms subject to CEPA1999 that are contained in cleaning products are outlined in the New Substance Notification Regulations (Organisms) (NSNR(Organisms); Department of Justice Canada, 2012b). Screening assessments are also currently being conducted on "existing" microbial strains found on the Canadian Domestic Substances List (DSL) (Environment Canada, 2012).

Assessments of "new" micro-organisms in cleaning products in Canada

Since 2000, four new (i.e. not on the DSL) micro-organisms intended for use in various types of cleaning applications were notified and assessed for potential risks to human health and the environment under CEPA1999. These applications included drain cleaning, carpet cleaning, in grease traps and in odour control. All four notified micro-organisms were *Bacillus* species, including strains of *B. subtilis, B. megaterium* and *B. pumilus*. None of these strains were genetically modified. Three of these strains were obtained from or have been deposited into well-known culture collections such as the American Type Culture Collection (ATCC) (American Type Culture Collection, 2012) or that of the United States' Department of Agriculture's Agricultural Research Service, also known as the NRRL collection (United States Department of Agriculture, 2011). The fourth was an environmental isolate.

Information substantiating the taxonomic designation of the notified micro-organism is the cornerstone of these assessments. A "polyphasic" approach is usually recommended, which typically involves any combination of information/data on cell and colony morphology, nutrient requirements, biochemical/metabolic testing (e.g. substrate

utilisation) and molecular and/or genotypic testing (e.g. fatty acid methyl ester – FAME, 16S rRNA, etc.). Typically, a taxonomic designation to the species level is expected. However, the primary goal of this approach for the purposes of conducting a CEPA1999 assessment would be to distinguish between potentially pathogenic and non-pathogenic strains. The assessment outcome in all four cases was "no suspicion of toxic" according to the definition of "toxic" found in Section 64 of CEPA1999.

DSL micro-organisms in cleaning products in Canada

The DSL is a list of all substances (chemicals, polymers and living organisms) that were: *i)* in Canadian commerce between 1 January 1984 and 31 December 1986; or *ii)* added to the list following notification and risk assessment, in accordance with CEPA1999. The list currently contains 67 microbial strains and 2 complex microbial cultures. Sixty-eight micro-organisms currently on the list were nominated based on the in commerce provisions described above. One complex microbial culture was added to the DSL following notification and risk assessment as a "new" substance, in accordance with the NSNR (Organisms). The current list of DSL micro-organisms can be viewed at Environment Canada (2011). All micro-organisms nominated to the DSL that have the potential to cause harm to human health or the environment must undergo a screening assessment as required under paragraph 74*(b)* of CEPA1999.

To establish whether micro-organisms on the DSL continue to be manufactured in or imported into Canada, a notice pursuant to paragraph 71(1)*(a)* of CEPA1999 was published in Part I of the *Canada Gazette* on 3 October 2009 for the 45 micro-organisms that were on the list in October 2009. Since then, 23 strains have been added to the DSL and these were not subject to this notice.

Based on information submitted by manufacturers and importers as part of the DSL nomination process as well as on the survey conducted as part of the CEPA1999 §71 notice mentioned above, 14 strains were found to be used in various types of cleaning products. These products included drain cleaners, degreasers, deodorizers/odour control, septic tank additives and aquarium/pond treatments. Several strains considered to be risk group 2 pathogens are among them. This information is based on activities that have occurred since 1984, so in almost all of these cases it is not clear whether these risk group 2 pathogens continue to be used in these products today. For example, there is no available information indicating that any of the three strains of *Pseudomonas aeruginosa* on the DSL are currently used in any type of cleaning products in Canada. However, a search of publically available information (Internet, patent databases) suggests that *P. aeruginosa* strains may possibly be found as active ingredients in commercial and household drain cleaners and degreasers, septic tank additives and general cleaning and odour-control products (Environment Canada and Health Canada, 2011b).

Knowledge gap in the use of Microbial-based cleaning products

Considering the current state of knowledge of the use of microbial-based cleaning products in Canada, the United States and Europe, it has become evident that there are significant gaps in terms of what is known about the extent of commercial and domestic use of these types of products as well as the specific strains of micro-organisms used as the active ingredients. These and other issues are to be the focus of a proposed international workshop on the subject of microbial-based cleaning products which will attempt to assemble stakeholders from government, industry, academia and public advocacy groups. Some of the more specific issues can include:

- Information gathering to fill in knowledge gaps: this includes information on the portion of the "green cleaners" market made up of microbial-based products, the specific microbial strains used in the different types of products, the extent of commercial and domestic uses of these products, their effectiveness compared to chemically based cleaners, etc.
- Industry stakeholder engagement: many of the information gaps identified above as well as other issues related to these types of products may not reliably be addressed unless there is engagement with industry stakeholders who could potentially benefit in the long-term by being more publically transparent about their products and thus gain greater public confidence in the safe use of these products.
- Human exposure scenarios: another significant knowledge gap which will need to be addressed in cases where more comprehensive risk assessments of the micro-organisms involved are deemed necessary.
- Environmental impacts: although environmental impacts are not expected as a direct result of their use, issues may arise should microbial-based cleaning products be manufactured, imported and/or used in exponentially greater quantities than what is currently known. These could result in significant environmental releases that may warrant greater scrutiny from a regulatory oversight perspective.
- Evaluation of current regulatory/policy frameworks: a re-evaluation of current regulatory and policy frameworks may be necessary once the above-mentioned issues are more thoroughly examined. This can include an evaluation of the most appropriate instruments (e.g. regulations, standards, codes of practice, etc.) to use for strengthening these frameworks to mitigate risks to human health and the environment without undue burden on the industry manufacturing and/or importing these products.

Conclusion

Based on the currently available scientific literature and information on microbial-based cleaning products, it appears that genetically modified micro-organisms could potentially play a significant role in the production of modified enzymes with enhanced properties for use as active ingredients in cleaning products for a variety of applications. However, currently known use patterns for these products may involve significant human exposure. As well, public perceptions regarding genetically modified organisms continue to be generally unfavourable. Thus, there is little indication at the present time that genetically modified micro-organisms themselves will find their way into commercially available microbial-based cleaning products as active ingredients in the foreseeable future.

References

Adisesh, A., et al. (2011) "Occupational asthma and rhinitis die to detergent enzymes in healthcare", *Occupational Medicine*, No. 61, pp. 364-369.

American Cleaning Institute (2012), "Windows, blinds, curtains & carpets: Top targets for spring cleaners", in: *For Immedeate Release on March 14, 2012*, website News Center, www.cleaninginstitute.org/2012_spring_cleaning_survey (accessed on 24 April 2012).

American Type Culture Collection (2012), www.atcc.org (accessed on 30 April 2012).

Canadian Consumer Specialty Products Association (2012a), "Who we are", www.ccspa.org/who/index.html (accessed on 23 April 2012).

Canadian Consumer Specialty Products Association (2012b), "Questions and answers about our products", www.healthycleaning101.org/english/QA.html (accessed on 23 April 2012).

Department of Justice Canada (2012a), Canadian Environmental Protection Act 1999, http://laws-lois.justice.gc.ca/eng/acts/C-15.31 (accessed on 30 April 2012).

Department of Justice Canada (2012b), New Substance Notification Regulations (Organisms), http://laws-lois.justice.gc.ca/eng/regulations/SOR-2005-248/index.html (accessed 30 April 2012).

EcoLogo (2011), "Environmental Standard – Certification Criteria Document CCD110: Cleaning and Degreasing Compounds: Biologically-Based", available at: http://services.ul.com/service/ecologo-certification/?ind=Environment.

Environment Canada (2012), "Substances list", Government of Canada, Gatineau, Quebec, Canada, www.ec.gc.ca/subsnouvelles-newsubs/default.asp?lang=En&n=47F768FE-1 (accessed on 30 April 2012).

Environment Canada (2011), "List of organisms on the Domestic Substances List (DSL) Liste des organismes sur la Liste intérieure des substances (LIS) Updated/Mise à jour 2012-07-16", Government of Canada, Gatineau, Quebec, Canada, www.ec.gc.ca/subsnouvelles-newsubs/C4E09AE7-28F3-4531-A771-632DE54CD01D/bioDSL_B.pdf (accessed on 23 July 2012).

Environment Canada and Health Canada (2011a), "Framework for science-based risk assessment of micro-organisms regulated under the Canadian Environmental Protection Act, 1999", Government of Canada, www.ec.gc.ca/subsnouvelles-newsubs/default.asp?lang=En&n=120842D5-1.

Environment Canada and Health Canada (2011b), "Screening assessment for *Pseudomonas aeruginosa* (ATCC 31480) *Pseudomonas aeruginosa* (ATCC 700370) and *Pseudomonas aeruginosa* (ATCC 700371)", Government of Canada, www.ec.gc.ca/ese-ees/FC109F45-68C6-4D65-9F60-9DF6B33B3C97/FSAR%20-%20P%20aeruginosa%20-%20EN.pdf.

Fournier, P.E. and H. Richet (2006), "The epidemiology and control of *Acinetobacter baumannii* in health care facilities", *Clinical Infectious Diseases*, No. 42, pp. 692-699.

Gelmetti, C. (2008), "Local antibiotics in dermatology", *Dermatologic Therapy*, No. 21, pp. 187-195.

Harms, H., D. Schlosser and L.Y. Wick (2011), "Untapped potential: Exploiting fungi in bioremediation of hazardous chemicals", *Nature Reviews Microbiology*, No. 9, pp. 177-192.

Hasan, F., et al. (2010), "Enzymes used in detergents: Lipases", *African Journal of Biotechnology*, Vol. 9, No. 31, pp. 4 836-4 844.

Hong, H.A., et al. (2008) "The safety of *Bacillus subtilis* and *Bacillus indicus* as food probiotics", *Journal of Applied Microbiology*, No. 105, pp. 510-520.

Kirk, O., T.V. Borchert and C.C. Fuglsang (2002), "Industrial enzyme applications", *Current Opinion in Biotechnology*, No. 13, pp. 345-351.

Mikkola, R., et al. (2000), "Toxic lactonic lipopeptide from food poisoning isolates of *Bacillus licheniformis*", *European Journal of Biochemistry*, No. 267, pp. 4 068-4 074.

Nazaroff, W.W. and C.J. Weschler (2004), "Cleaning products and air fresheners: Exposure to primary and secondary air pollutants", *Atmospheric Environment*, No. 38, pp. 2 841-2 865.

Ness, J.E., et al. (1999), "DNA shuffling of subgenomic sequences of subtilisin", *Nature Biotechnology*, No. 17, pp. 893-896.

Pearce, C.I., J.R. Lloyd and J.T. Guthrie (2003), "The removal of colour from textile wastewater using whole bacterial cells: A review", *Dyes and Pigments*, No. 58, pp. 179-196.

Perez-Pantoja, D., et al. (2009) "Chapter 94: Phylogenomics of aerobic bacterial degradation of aromatics", in: K. Timmins et al. (eds), *Handbook of Hydrocarbon and Lipid Microbiology*, Springer-Verlag, Berlin Heidelberg.

PRWeb.com (2011), "Global household green cleaning products market to reach US$9.32 billion by 2017, according to a new report by Global Industry Analysts, Inc" http://www.prweb.com/releases/household_green_cleaning/non_toxic_products/prweb8857227.htm.

Satpute, S.K., et al. (2010), "Chapter 2: Molecular genetics of biosurfactant synthesis in micro-organisms" in: *Biosurfactants*, Ramkrishna Sen (ed.), Landes Bioscience and Springer Science+Business Media.

Spok, A. and M. Klade (2009), "Environmental, health and legal aspects of cleaners containing living microbes as active ingredients", *IFZ-Electric Working Papers*, No. 3-2010, available at: www.ifz.tugraz.at/Publikationen/Electronic-Working-Papers.

UK Cleaning Products Industry Association (2011), *Review 2011*, UKCPI, Chester, United Kingdom, www.ukcpi.org/PDFs/AnnReport-2011c.pdf.

United States Department of Agriculture (2011), *ARS Culture Collection (NRRL)*, Agricultural Research Service, Washington, DC, http://nrrl.ncaur.usda.gov (accessed on 30 April 2012).

United States Environmental Protection Agency (2011), "EPA's DfE Standard for Safer Cleaning Products (SSCP)", US EPA, Washington, DC, www.epa.gov/dfe/pubs/projects/gfcp/standard-for-safer-cleaning-products.pdf.

Wassenaar, T.M., and G. Klein (2008), "Safety aspects and implications of regulation of probiotic bacteria in food and food supplements", *Journal of Food Protection*, No. 71, pp. 1 734-1 741.

Xingzu, W., et al. (2008) "Biodecolorization and partial mineralization of reactive black 5 by a strain of *Rhodopseudomonas palustris*", *Journal of Environmental Sciences*, No. 20, pp. 1 218-1 225.

Xiuyan, L., et al. (2011) "Biochemical degradation pathway of reactive blue 13 by *Candida Rugopelliculosa* HXL-2", *International Biodeterioration & Biodegradation* , No. 65, pp. 135-141.

Yang, Y.-L. (2003), "Virulence factors in *Candida* species", *Journal of Microbiology, Immunology and Infection*, No. 36, pp. 223-228.

Zhou, Q., et al. (2009) "Role and functions of beneficial micro-organisms in sustainable aquaculture", *Bioresource Technology*, No. 100, pp. 3 780-3 786.