Food and Chemical Toxicology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

# Bacterial and fungal composition profiling of microbial based cleaning products

R.M. Subasinghe[a], A.D. Samarajeewa[a], M. Meier[a], G. Coleman[b], H. Clouthier[b], J. Crosthwait[b], A.F. Tayabali[b], R. Scroggins[a], P.S. Shwed[b], L.A. Beaudette[a,*]

[a] Biological Assessment and Standardization Section, Environment and Climate Change Canada, 335 River Road, Ottawa, Ontario K1V 1C7, Canada
[b] Mechanistic Studies Division, Environmental Health Science and Research Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Canada, Ottawa, ON, Canada

ARTICLE INFO

*Keywords:*
Microbial based cleaning products
Next generation sequencing
16S rDNA gene sequencing
Fatty acid methyl ester phenotyping
Metagenomic DNA
16S rDNA survey analysis

ABSTRACT

Microbial based cleaning products (MBCPs) are a new generation of cleaning products that are gaining greater use in household, institutional, and industrial settings. Little is known about the exact microbial composition of these products because they are not identified in detail on product labels and formulations are often proprietary. To gain a better understanding of their microbial and fungal composition towards risk assessment, the cultivable microorganisms and rDNA was surveyed for microbial content in five different MBCPs manufactured and sold in North America. Individual bacterial and fungal colonies were identified by ribosequencing and fatty acid methyl ester (FAME) gas chromatography. Metagenomic DNA (mDNA) corresponding to each of the products was subjected to amplification and short read sequencing of seven of the variable regions of the bacterial 16S ribosomal DNA. Taken together, the cultivable microorganism and rDNA survey analyses showed that three of the products were simple mixtures of *Bacillus* species. The two other products featured a mixture of cultivable fungi with *Bacilli*, and by rDNA survey analysis, they featured greater microbial complexity. This study improves our understanding of the microbial composition of several MBCPs towards a more comprehensive risk assessment.

## 1. Introduction

Microbial based cleaning products (MBCPs) are a type of eco-friendly cleaning product that contains viable bacteria and spores as the active ingredients and may also include enzymes and/or chemicals. MBCPs are formulated for use in domestic, industrial, and institutional settings with applications including: septic tank treatments, drain cleaners, odor controllers, stain removers and hard surface cleaners. The bacteria present in MBCPs collectively produce a range of extracellular biosurfactants and enzymes (e.g., proteases, cellulases, amylases, ureases) that can degrade organic substances. Many of the biosurfactant producing microorganisms are useful as hydrocarbon degraders (Volkering et al., 1997; Willumsen et al., 2001). Biosurfactants facilitate the cleaning process by reducing the interfacial surface tension and increasing the biodegradability at lower toxicity levels than chemical surfactants (Vijayakumar and Saravanan, 2015; Cameotra and Makkar, 2004). Another advantage of bacterial surfactants is stability levels at extremes of pH, temperature, and salinity (Vijayakumar and Saravanan, 2015; Desai and Banat, 1997).

The microbial composition of contemporary formulations of MBCPs is not well understood. Generally, the strain details and contents are not stated on products, so the assessment of microbial compositions are based on available methodologies performed by third parties. The risk assessment of MBCPs on human health and the environment is considered an important issue by many governing bodies (VKM, 2016; OECD, 2015a; OECD, 2015b). The EU Directive 2000/54/EC (European Union, 2000) regulates the minimum requirement for the protection of workers from risks related to biological agents for MBCPs including the classification of microorganisms in cleaning products. The Canadian Environment Protection Act, 1999 (CEPA-1999) regulates the notification under the New Substance Notification Regulations (NSNR) of microorganisms that are not included in the Domestic Substances List (DSL). However, risk assessments are yet to be established for NSNR of microorganisms in cleaning products. According to the health and environment risk assessment of MBCPs published by the Norwegian Scientific Committee for Food Safety in 2016 (VKM, 2016), the composition of microbes present in the products and their concentrations within the formulations are important in improving the guidelines for the regulation of MBCPs (VKM, 2016). According to a survey report on risk assessment of MBCPs, a cultivation-dependent study of MBCPs carried

* Corresponding author.
*E-mail address:* lee.beaudette@canada.ca (L.A. Beaudette).

https://doi.org/10.1016/j.fct.2017.12.006
Received 31 August 2017; Received in revised form 1 December 2017; Accepted 2 December 2017

out in the late 1990s revealed variations among the formulations in terms of viability of the active microorganisms and the presence of unspecified microbial species that raised concerns with their quality control (VKM, 2016; OECD, 2015b; WVA - Voedsel en Waren Autoriteit, 2004). Many of the MBCPs profiled in this early report are no longer manufactured, and there have since been technical advances that permit parallel culture-dependent and culture-independent analyses of microbial mixtures (OECD, 2015b). A study based on a product survey by literature review and consultation identified 30 different genera in MBCPs, including both bacteria and yeasts (e.g. *Bacillus, Bifidobacterium, Lactobacillus, Rhodopseudomonas*, and *Saccharomyces*) (Spök and Klade, 2009). To date, few studies have described the microbial composition of modern MBCPs.

The objective of this study was to profile the microbial compositions of five commonly available North American MBCPs (purchased in the Canadian marketplace) by the taxonomic characterization of culture isolates (FAME, ribosequencing) and the analysis of 16S rDNA surveys. Considering that, to date, no surveys of the microbial composition of MBCPs manufactured in North America have taken place, such data would be helpful in risk assessment. Here, we show that the combined use of culture-dependent and independent methods provides a complementary perspective for profiling of the microbial composition of MBCPs.

## 2. Materials and methods

### 2.1. Selection of MBCPs

Five different commercially available MBCPs (hereafter Products 1, 2, 3, 4 and 5) were purchased from anonymous suppliers. Collectively, they encompassed different physical states, compositions, and functions (Table 1). The products were stored at room temperature, according to manufacturer's recommendations.

### 2.2. Cultivation of bacteria and fungi

Cultivable colony forming units (CFUs) in each MBCP were enumerated on either trypticase soy agar (TSA; Teknova, California, USA) with and without 5% sheep blood. Selective media included Cetrimide agar for *Pseudomonas* selection, and Sabouraud dextrose or Yeast peptone dextrose supplemented with 50 μg $mL^{-1}$ gentamycin for fungal selection. For liquid formulations, 100 μl of each MBCP was diluted in 10 mL of water and then serially diluted, spread-plated on to 100 mm TSA petri dishes, and incubated for 24–48 h at various temperatures before enumeration of colonies. Solid formulations were diluted to 1% (w/v) suspensions in distilled water (i.e., 100 mg per 10 mL) and plated after serial dilution. Products were regularly monitored for viability. New lots of Products 3 and 5 were obtained when a reduction in viability was observed.

### 2.3. Identification of cultivable microorganisms and fungi

Microbial fatty acid phenotyping was carried out using the MIDI-Sherlock® fatty acid methyl ester (FAME) microbial identification system (MIDI Inc., DE) as described in previous studies (Tayabali et al., 2017). Top similarity indexes were selected by comparisons to three libraries: the “traditional” aerobic environmental library (RTSBA6 version 6.21), the clinical library (RTCLIN6 version 6.21) and an in-house prepared custom Canadian Domestic Species library. Genetic identification of bacterial colonies was carried out as described earlier (Tayabali et al., 2017) with the following exceptions: Amplitaq™ 360 DNA Polymerase (ThermoFisher, ON) was used for full length 16S ribosomal DNA gene amplification and amplicons were sequenced using an Applied Biosystems™ 3500 Series Genetic Analyzer. Genetic identification of fungal colonies was carried out by amplification of the fungal internal transcribed spacer region of the ribosomal DNA operon using primers ITS1 and LR7 described earlier (White et al., 1990; Vilgalys and Hester, 1990).

### 2.4. Metagenomic DNA extraction and amplification by PCR

Metagenomic DNA (mDNA) was extracted from the liquid MBCPs using the PowerWater® DNA Isolation Kit (MoBio Laboratories) and from the solid MBCPs using the PowerSoil® DNA Isolation Kit (MoBio Laboratories Inc.). 100 mL of liquid cleaning product was filtered through a 0.2 μm nitrocellulose membrane using a vacuum filter and then the genomic DNA was removed from the membrane according to the manufacturer's instructions. One half gram of solid cleaning product was used per sample to extract mDNA. Three samples were taken from each cleaning product and the final eluted volume was 20 μL for all samples. The quality and quantity of mDNA were measured on a NanoDrop 2000c UV–Vis spectrophotometer.

The mDNA from three replicates of each cleaning product were combined and the polymerase chain reaction (PCR) was performed to amplify the V2, V3, V4, V6/7, V8 and V9 hypervariable regions (V regions) of the 16S ribosomal DNA (rDNA) using the Ion 16S™ Metagenomics Kit (Life Technologies) following the manufacturer's instructions. mDNA was also screened for fungal/eukaryotic DNA using the primers ITS1 and ITS4 for the internal transcribed spacer region of the ribosomal DNA operon, as described previously (WVA - Voedsel en Waren Autoriteit, 2004). Amplified products were sequenced directly as with the cultivable isolates, or the amplicons were used to create a library as described previously (Tayabali et al., 2017).

### 2.5. Metagenomic library preparation and sequencing

Library preparation was performed following the manufacturer's instructions for the Ion Plus Fragment Library Preparation Kit (Life Technologies). The PCR products were purified using an Agentcourt® AMPure XP Kit (Beckman Coulter Genomics, MA, USA) and quantified using an Agilent 2100 bioanalyzer with DNA 1000 reagents. Following end- and nick-repair of the amplicons, an IonXpress barcode and P1 adaptor were ligated for each cleaning product. The libraries were pooled for sequencing at a final concentration of 26 pM. The emulsion PCR to prepare the sequencing template was done using the Ion OneTouch 400 Template Kit (Life Technologies), and enrichment of the template-positive Ion Sphere Particles (ISPs) was carried out on the Ion OneTouch™ ES (Life Technologies) instrument following the

**Table 1**
Characteristics of the commercially available MBCPs used in this study.

| Product | Country of Manufacture | Physical State | Listed Ingredients | Specificity of cleaning |
|---|---|---|---|---|
| Product 1 | USA | Solid | Bacteria, Nutrients | Digests organic and sludge buildup in septic tanks and drains |
| Product 2 | USA | Solid | Bacteria, Yeast, Organic and Inorganic components | Similar to Product 1 |
| Product 3 | CAN | Liquid | Bacteria | Eliminates odour from sports equipment, home environment |
| Product 4 | USA | Liquid | Bacteria, enzymes | Removes pet odours and stains |
| Product 5 | CAN | Liquid | Bacteria, surfactants | Biodegrades starches, carbohydrates, cellulose, fats, greases and oil in drain lines, septic tanks |