Microbial interest for cleaning

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Introduction

Almost everyone is using cleaning products in order to clean their house, their pets or even themselves.

Cleaning products are mainly found in liquid or powder form. A visit to the local supermarket is constantly indicating that the majority of the cleaning products available on the market contain chemical products with a tendency of being reactive or corrosive. As an example, cleaning products can contain solutions of sodium hypochlorite (bleach), sodium hydroxide (found in many detergents and drain cleaning products) as well as ammonium hydroxide (used in hard surface cleaners).

Due to their chemical property combined with a widespread utilization, these chemical substances are cause for concern related to the human health and the environment. Inappropriate mixing of some of these chemicals produces chlorine and ammonia toxic gases, leading to acute intoxications and diseases, as well as chronic effects [1]. In 2018, one study from Brussel's University showed how dangerous these chemicals can be for people being often exposed to them [2]. Indeed, the mortality rate due to cardiovascular and pulmonary diseases is considerably much higher for people regularly using these type of products (Figure 1).

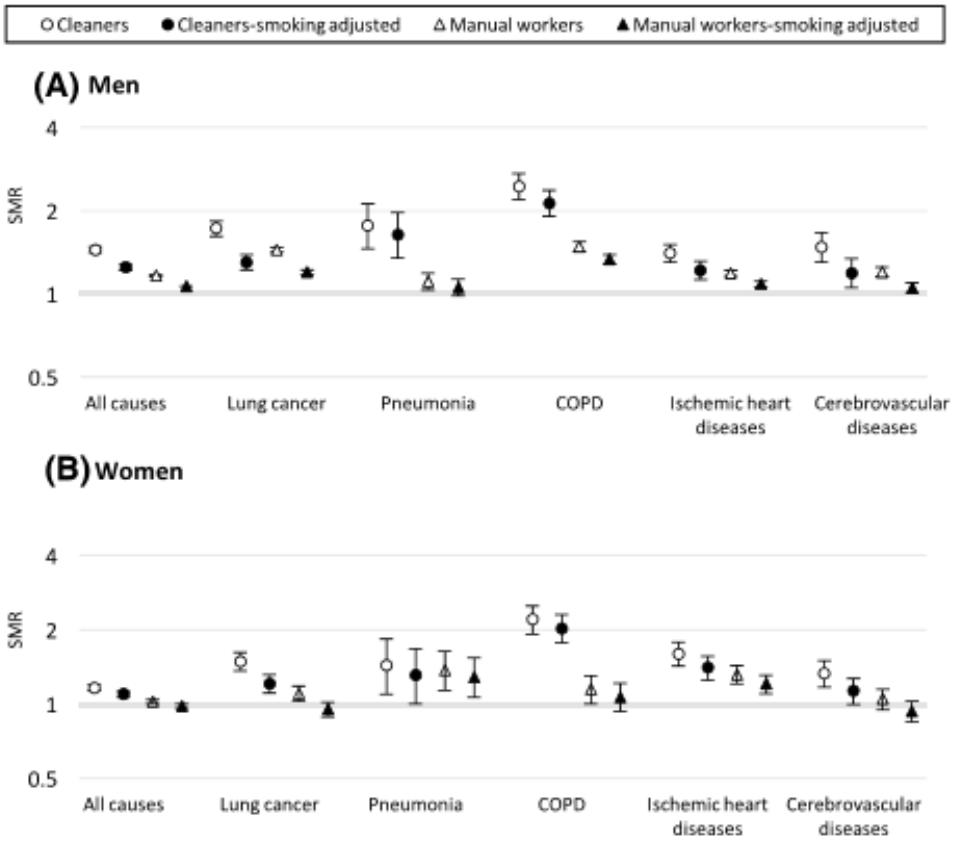


Figure 1: Standardized mortality ratios with confidence interval of 95% for cleaners as well as manual workers. Regrouped by by sex. Study realized between 2001 and 2011.

Biological competition vs Disinfectant

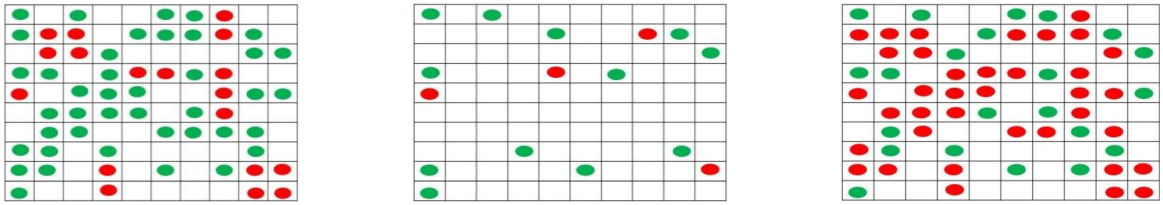
Probiotic Group Luxembourg (PBGL) is a company offering healthy and microbial-based cleaning, hygiene and care products using microorganisms to increase the efficiency of the products.

Unlike the conventional disinfection technique, the PBGL cleaning method exploits the "biological competition principle" [3] of microorganisms. They colonize the surfaces on which they are applied, neutralizing the proliferation of unwanted bacteria on the basis of the principle of exclusion competition [4]. Different species (bacteria and/or fungi) trying to dominate the same ecological micro-space cannot coexist in equilibrium. Competition for access to nutrients will favor the strongest and least nutrient-demanding bacteria [5]. The strongest bacteria then become supernumerary and therefore dominant compared to the others which undergo extinction.

Comparison of cleaning systems (classic vs with probiotics)

For these tests, the good bacteria and the pathogenic bacteria were sampled on a given small surface, in the case of a conventional cleaning on the one hand and of cleaning with products enriched with probiotics on the other hand.

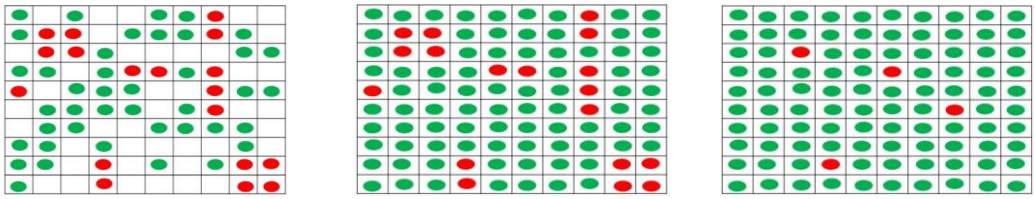
When using conventional cleaning solutions, most bacteria, good and bad, are eliminated by disinfection. The fact is that a few minutes later, the bad bacteria quickly recolonize the cleaned surface (Figure 2), faster than the good bacteria.



Start situation= Natural microflora | *After disinfection* | *Few minutes later*

Figure 2: impact of disinfection on the colonization of pathogenic bacteria from a surface over time. GREEN = good bacteria / RED = bad bacteria

During cleaning with probiotic products, bad bacteria are not impacted at the first glance. However good bacteria (probiotics) progressively colonize unoccupied zones. After a few minutes, the supernumerary of good bacteria repulses the bad ones (Figure 3).



Start situation = Natural microflora | *After the use of probiotics* | *Few minutes later*

Figure 3: Biological competition utilization to avoid pathogenic bacterial colonization. GREEN = good bacteria / RED = bad bacteria

Additionally, microorganisms capacity to clean surfaces is another advantage. Indeed, dirties are usually organic matters that bacteria can digest. Thanks to the auto production of specific enzymes [6], they are able to destroy dirties particles before their ingestion [7]. Therefore, dirties are eliminated during and after cleaning, as probiotics colonizing the area will continue to act during few days (Figure 4).

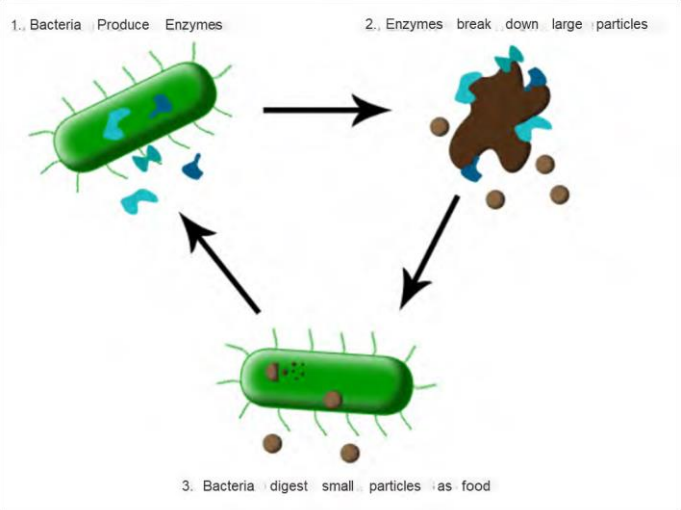


Figure 4: Bacterial influence on dirties particles metabolization

Efficacity measurement of microbial competitive solutions

Seeking for efficacity approaches, recently it has been demonstrated that an eco-sustainable cleaning system based on probiotics stably reduces pathogenic surface agents, without selecting antibiotic resistant species [8]. The aim of this study was to establish if a probiotic application would have an impact on nosocomial infections incidence. From 1st of January 2016 to 20th of June 2017, a pre-post intervention multicentre study was realized for 18 months in internal medicine services of six public hospitals. The intervention consisted in the replacement of conventional sanitation by probiotic based products, without changing any other procedure influencing nosocomial infections control.

Result measurement was set as the nosocomial infections incidence during pre and post intervention period. In total, 11 842 patients and 24 875 environmental samples were studied. The probiotic based cleaning product was associated to a significant diminution of cumulative nosocomial infections incidence, from 4,8% (284 patients out of 5 930 in total) to 2,3% (128 patients out of 5 531 in total) (OR = 0,44, CI 95% 0,35-0,54) (P<0,0001).

In parallel, the use of probiotics was associated to a stable decrease of pathogenic surface agents, compared to conventional sanitation (average decrease of 83%), together with a simultaneous fall up to 2 log of surface microbiota drug resistant genes (P<0,0001 ; Pc = 0,008) [9]. The results of this study confirm the impact of a sanitation procedure on nosocomial infections incidence, pointing out a decrease of the risk to contract nosocomial infections potentially associated to probiotic based environmental intervention in hospitals.

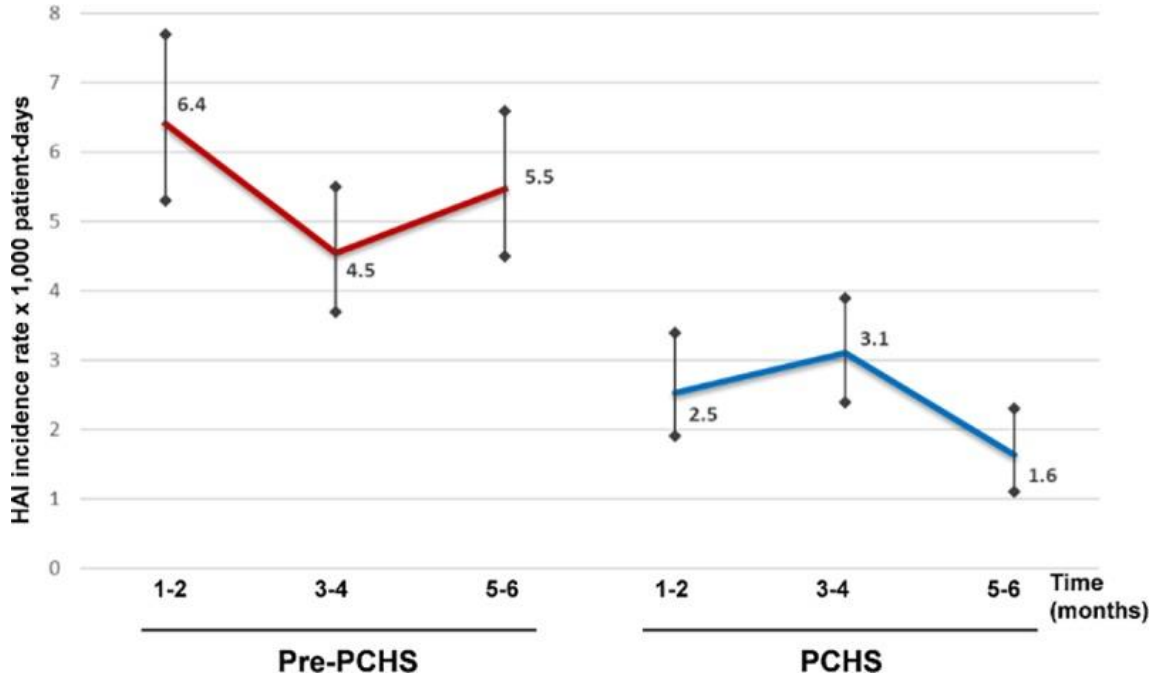


Figure 5: Results are expressed as bimonthly value of incidence rate per 1,000 patient-days, respectively in the pre-PCHS (red) and PCHS periods (blue). 95% CI intervals are also reported.

Bacillus species influence on *Staphylococcus aureus* growth

Although bacterial effect is established in the literature, it appears essential for Probiotic Group to prove the efficacity of their probiotics.

In this sense, we collaborated with Dr. Henry Michel Cauchie team from LIST (Luxembourg Institute of Science and Technology), who studied the impact of LUCAA+ Wound Care product to fight against one of the main bacteria responsible of infections in hospitals : Staphylococcus aureus [10, 11]. Volumes from 100 to 300 µL (corresponding to 1 and 3 sprays, respectively) of LUCAA+ were sprayed on Petry dishes containing a dose of pathogenic *S. aureus* bacterium. The use of the spray help being as close as possible to the real conditions of the product use.

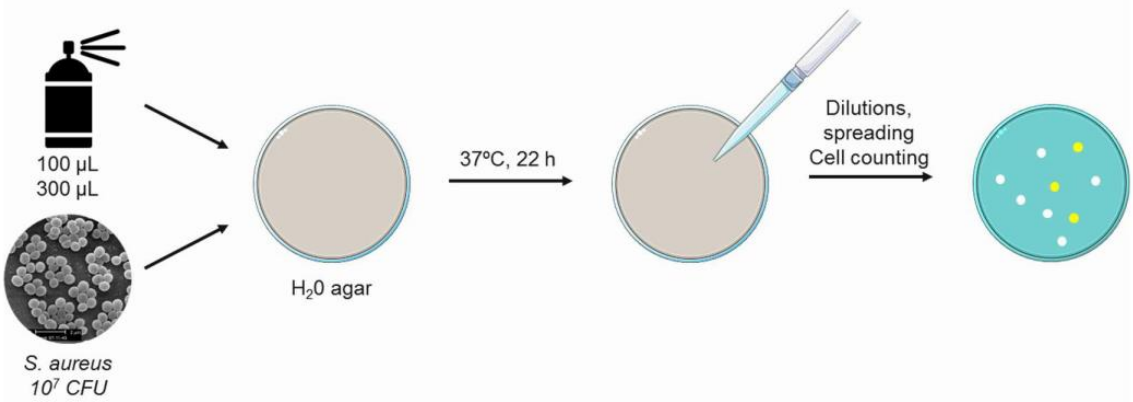


Figure 6: Experimental design for testing the inhibitory properties of probiotics.

Figure 7 is showing the results of inhibitory testing of commercial LUCAA+ product and LUCAA+ ingredients only (without probiotics) towards *Staphylococcus aureus* (Figure 7). Initial concentration of *S. aureus* at the beginning of the experiment is represented by a dotted line. The red line represents the limit of detection (LOD) above which it is not possible to count S. aureus.

The commercial LUCAA+ product yields to an important decrease of *S. aureus* (> 3 units log CFU/mL). Additionally, the low effect of LUCAA+ ingredients only is an indicator showing that the inhibitory effect is related to the presence of the probiotics rather than the other ingredients.

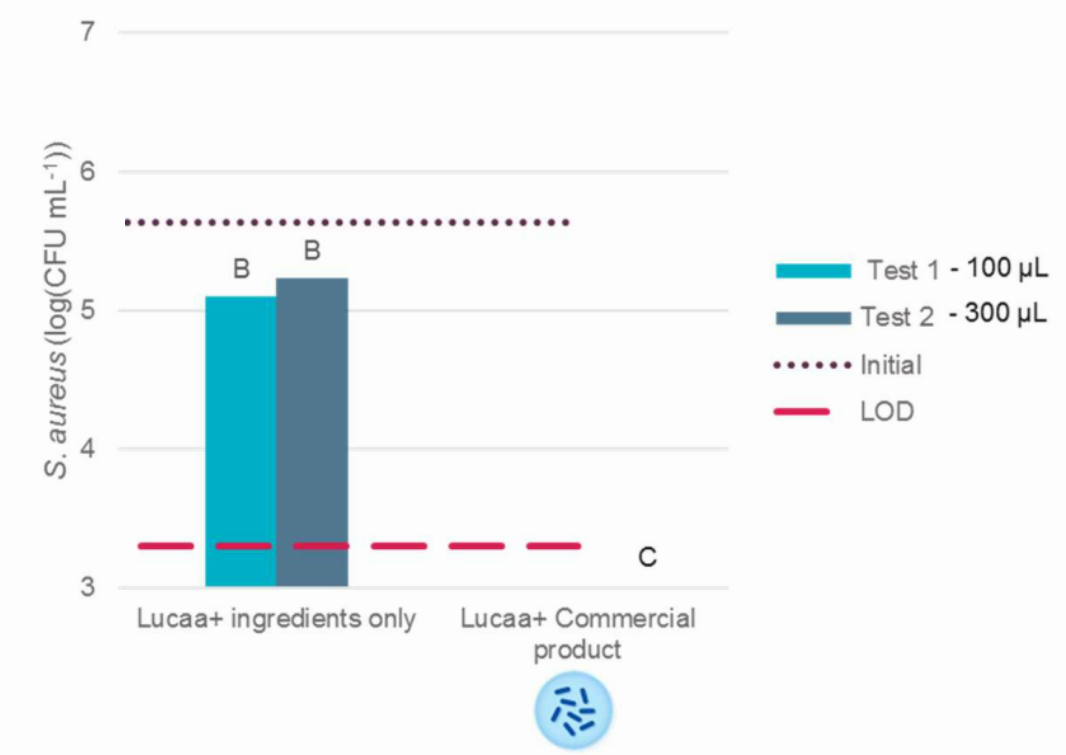


Figure 7: – Variation of the density of Staphylococcus aureus exposed to LUCAA+ commercial product and LUCAA+ ingredients (without probiotic agents). Colors are showing the number of sprays (1 or 3) used on the Petry dishes.

Therefore, bacteria used in the LUCAA+ products are efficient strivers towards pathogenic bacteria such as *Staphylococcus aureus*.

Microbial competition and air quality

Safety of Probiotic Group products is as important as their efficiency. Indeed, a lot of products are able to eliminate pathogenic bacteria. Unfortunately, they are not safe for humans as well as the environment [12, 13].

Thus, it is essential to check the products impact on the air quality. Allergy free product from PBGL was sprayed in an area equivalent to the bathroom volume (2,5m^3). The air of this area was analyzed and the concentration of 20 pollutants was quantified. The product was again sprayed within the area to be as close as possible to the real conditions of use of the product.

The application is done based on the standard NF EN ISO 16000-11 (adapted to cleaning products): determination of the emission of volatile organic compounds from products – Sampling, samples preservation and preparation for assay (AFNOR, 2006).

Packaging is done base on the standard NF EN ISO 16000-9: determination of the emission of volatile organic compounds from products – Emission test chamber method (AFNOR, 2006)

Sampling and analysis were realized according to the following standards:

- NF ISO 16000-3: dosage of formaldehyde and other carbonyl compounds – Active sampling method (AFNOR, 2011)
- NF ISO 16000-6: dosage of volatile organic compounds in the indoor air of areas and test chambers by active sampling on Tenax TA absorbent, thermal desorption and gas chromatography using MS of MS-FID (AFNOR, 2012)

The results are extremely encouraging since after the application of Allergy Free, the residual concentrations of the 20 toxic substances are extremely low: on average, 200 times under the most stringent European standards. Indeed, for each substance, Figure 8 presents the concentration of each measured compound expressed in% compared to the limit of the lowest category. The 100% therefore represents the threshold beyond which the limits of category A + are exceeded. These results show that the concentrations detected in each substance is far below this 100% threshold, specific to each substance. Our products therefore fall into category A + with regard to air quality.

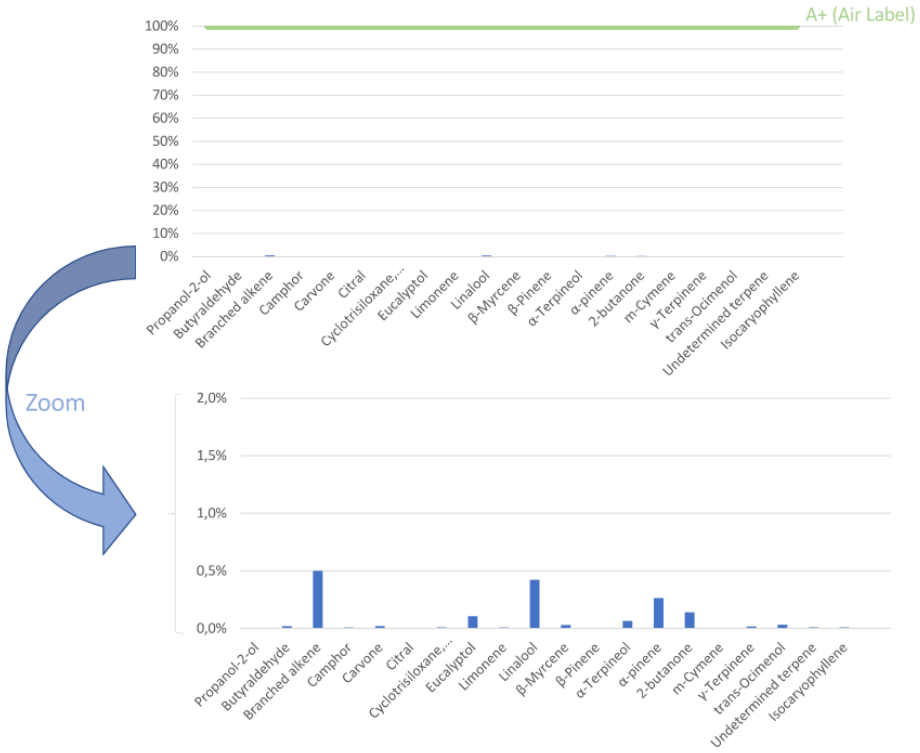


Figure 8: Residual concentration of 20 toxic substances after using Allergy Free compared to their A + limits (green line). A zoom on the first two percent of the first graph is provided in order to visualize the final results

Innovations in perspective

Probiotic group recently started a collaborative research project with the LIST and LCSB, two state-of-the-art laboratories in Luxembourg. The aim of this research project is to discover and characterize new and efficient bacterial species. This 3 years project is cofounded by the Luxembourg State.

The team behind the research project

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Partners

In order to study the products, probiotic group works together with few renowned laboratories. The LIST (Luxembourg Institute of Science and Technology), the Laboratoires Réunis, the Luxembourg university (Luxembourg Centre for Systems Biomedicine) as well as Air Intérieur Contrôlé contributed to the results of this report.

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