



Safety aspects of the Chrisal probiotic bacteria

Chrisal pays the utmost attention to the safety of all of its products, both on the chemical and biological level.

For chemical ingredients, a lot of general safety documentation is available and knowledge among customers and the public is large. For the probiotics used in the Chrisal products, however, less knowledge and safety documentation is known among our (potential) customers. As such, we often get questions regarding the safety of our probiotic strains and bacteria in general.

For that purpose, we have drafted this document, to demonstrate the absolute safety of our probiotics. Safety documentation consists of common scientific knowledge about probiotic bacteria, and tests performed specifically on Chrisal products and probiotics.

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1. Origin of the Chrisal probiotics

All bacteria used by Chrisal in its products are natural probiotics, also used in food industry as dietary supplements. As such, all Chrisal probiotics are food grade (meaning allowed for human consumption).

Definition of a probiotic: *‘Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host.’*

This definition states that probiotics are bacteria that improve your health, which of course, automatically implies **they are absolutely safe**. Also, it states that high enough numbers of probiotic bacteria are needed in order to reach the health benefits.

Chrisal has selected a wide range of probiotic bacteria, Bacillus species, that are classified and recognized as probiotic food supplements. This classification is officially done by two institutions:

a) ATCC (American Type Culture Collection)

All bacteria that were ever discovered are listed in the official international collection of bacteria. This collection is called the American Type Culture Collection (ATCC) and maintains samples of all bacteria, together with all of the scientific data available about them.

www.atcc.org for all information.

ATCC classifies every existing micro-organism in a biosafety level, level 1 being safe micro-organisms and level 4 being very contagious pathogens.

This biosafety classification is based on U.S. Public Health Service Guidelines (<http://www.cdc.gov/biosafety/publications/bmbl5/index.htm>)

All Chrisal probiotics classify under Biosafety level 1 and as such, are safe!

b) EFSA (European Food Safety Authority)

The second part of the safety classification is done by the European Food Safety Authority (EFSA) who publish a list of micro-organisms that are allowed for human consumption. This list is called the QPS list (Qualified Presumption of Safety) and can be found at this link:

(<https://www.efsa.europa.eu/en/topics/topic/qps>)

All probiotic bacteria used in any of the Chrisal products are on the QPS list and as such, are safe for human consumption!



All Chrisal probiotics are of natural origin and not GMO (Genetically Modified Organisms). They are classified in Biosafety Level 1 (ATCC) and are on the QPS list (EFSA). These official classifications guarantee that Chrisal uses only 100% safe probiotics.

2. Specific safety experiments on Chrisal probiotics

Despite the existing official scientific information and classification of our probiotics as being safe; Chrisal has invested significantly in additional safety tests on our probiotics and the products they are used in. We hereby provide an overview:

a) OECD tests

An external accredited lab in France was appointed to perform a range of safety trials according to OECD (Organisation for Economic Cooperation and Development) standard normations, including:

OECD423 - acute orale toxiciteit: A test to screen the probiotics for possible toxic effects.

OECD404 - skin irritation: A test to screen whether the probiotics irritate skin.

OECD405 - eye irritation: A test to screen whether the probiotics irritate the eye.

OECD406 – skin sensitation: A test to screen whether the probiotics affect skin structure



All Chrisal probiotics passed the above tests and did not demonstrate any negative effect.

b) Cosmetic registration tests

Chrisal has a number of probiotic cosmetic products and in Europe such products can not enter the market if they are not registered at the Cosmetic Product Notification Portal, CPNP. In order to register, a wide range of safety documentation has to be provided for which independent safety trials are mandatory.

The following tests were performed by a specialised cosmetics unit of the University of Ferrara (Italy) on the probiotic cosmetic products of Chrisal:

- **Cytotoxicity UNI EN ISO 10993-5** to assess "in vitro" cytotoxic effects (interleukins IL-6 IL-8) and possible irritative nature of the product.
- **In Use Test:** in vivo test with 20 volunteers that use the test product for a month, subjected to microbiological analysis at time intervals to check the microbiological stability during use.
- **PATCH Test:** in vivo dermatological test with 40 volunteers to screen for phenomena of acute irritation.
- **Safety Test:** in vivo dermatological test during 6 months with 40 volunteers dermatological test for phenomena of long term irritation and evaluate the safety of the formulation.



All Chrisal probiotics passed the above tests and did not demonstrate any negative effect.

3. Quality certifications

For a broad range of certifications, safety of the product and its production processes are very important. Chrisal has several international certifications on several of its probiotic products, of which we present the most relevant:

a) ISO9001:2008

Besides the actual formulation, the safety of a product is largely depending on the quality of the production process and quality control protocols. Since 1999 Chrisal has the ISO9001:2008 certificate, given after annual external audit.

The Chrisal ISO quality management system involves protocols to manage and improve the quality of all operations within the company, including production, R&D, quality control, sales, administration, general management...

b) NSF (National Sanitation Foundation – USA)

Several probiotic products from Chrisal have received the NSF certification in the USA. The NSF certification mark on a product means that the product complies with all standard requirements. NSF conducts periodic unannounced inspections and product testing to verify that the product continues to comply with the standard.

c) Green Seal (USA)

Green Seal provides science-based environmental certification standards that are credible, transparent and essential to helping manufacturers, purchasers and consumers make responsible choices that positively impact business behavior and improve quality of life.

A number of probiotic products by Chrisal have been granted the Green Seal mark after thorough inspections of the product, its performance and the production process, including all safety aspects.



4. User experience

Perhaps the most important safety guarantee is the long history of safe use of our probiotic products in many markets. Certainly the most demanding market in terms of safety is healthcare. Already for 10 years, Chrisal has a large number of hospitals that use the probiotic products for optimal infection control. These hospitals conducted very long and thorough tests in order to verify the efficacy and safety of the products. Some of the test results were officially published in scientific papers.

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Hard Surface Biocontrol in Hospitals Using Microbial-Based Cleaning Products

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Abstract

Background: Healthcare-Associated Infections (HAIs) are one of the most frequent complications occurring in healthcare facilities. Contaminated environmental surfaces provide an important potential source for transmission of many healthcare-associated pathogens, thus indicating the need for new and sustainable strategies.

Aim: This study aims to evaluate the effect of a novel cleaning procedure based on the mechanism of biocontrol, on the presence and survival of several microorganisms responsible for HAIs (i.e. coliforms, *Staphylococcus aureus*, *Clostridium difficile*, and *Candida albicans*) on hard surfaces in a hospital setting.

Methods: The effect of microbial cleaning, containing spores of food grade *Bacillus subtilis*, *Bacillus pumilus* and *Bacillus megaterium*, in comparison with conventional cleaning protocols, was evaluated for 24 weeks in three independent hospitals (one in Belgium and two in Italy) and approximately 20000 microbial surface samples were collected.

Results: Microbial cleaning, as part of the daily cleaning protocol, resulted in a reduction of HAI-related pathogens by 50 to 80%. This effect was achieved after 3-4 weeks and the reduction in the pathogen load was stable over time. Moreover, by using microbial or conventional cleaning alternatively, we found that this effect was directly related to the new procedure, as indicated by the rise in CFU/m² when microbial cleaning was replaced by the conventional procedure. Although many questions remain regarding the actual mechanisms involved, this study demonstrates that microbial cleaning is a more effective and sustainable alternative to chemical cleaning and non-specific disinfection in healthcare facilities.

Conclusions: This study indicates microbial cleaning as an effective strategy in continuously lowering the number of HAI-related microorganisms on surfaces. The first indications on the actual level of HAIs in the trial hospitals monitored on a continuous basis are very promising, and may pave the way for a novel and cost-effective strategy to contract or biocontrol healthcare-associated pathogens.

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Introduction

Healthcare-Associated Infections (HAIs) are one of the most frequent complications occurring in healthcare facilities and represent a problematic concern regarding the safety and quality of healthcare worldwide [1], as also stated in a recent report by the World Health Organization estimating hospital-wide prevalence in high-income countries at 6% [2]. The European Center for Disease Control point prevalence study confirmed that healthcare-associated infections are a major public health problem in Europe

with a prevalence of 5.7% (4.5-7.4%) which means 81,889 (61,624-103,895) patients with one HAI for each day in European acute care hospitals [3]. In particular, this European survey reported a similar incidence of nosocomial infections for Italy and Belgium, where the percentage of patients with HAI has been calculated as 6.3% (5.4-7.4%) and 7.1% (6.1-8.3%), respectively [1]. Based on this study, the estimated total annual number of patients with an HAI in European acute care hospitals in 2011-2012 was 3.2 million, albeit with a wide confidence interval from

RESEARCH ARTICLE

Impact of a Probiotic-Based Cleaning Intervention on the Microbiota Ecosystem of the Hospital Surfaces: Focus on the Resistome Remodulation

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Abstract

Background: Contamination of hospital surfaces by clinically-relevant pathogens represents a major concern in healthcare facilities, due to its impact on transmission of healthcare-associated infections (HAIs) and to the growing drug resistance of HAI-associated pathogens. Routinely used chemical disinfectants show limitations in controlling pathogen contamination, due to their inefficacy in preventing recontamination and selection of resistant strains. Recently we observed that an innovative approach, based on a cleanser added with spores of non-pathogenic probiotic *Bacilli*, was effective in stably counteracting the growth of several pathogens contaminating hospital surfaces.

Methods: Here, we wanted to study the impact of the *Bacillus*-based cleanser on the drug-resistance features of the healthcare pathogens population. In parallel, the ability of cleanser-derived *Bacilli* to infect hospitalized patients was also investigated.

Results: Collected data showed that *Bacilli* spores can germinate on dry inanimate surfaces, generating the bacterial vegetative forms which counteract the growth of pathogens and effectively substitute for them on treated surfaces. Strikingly, this procedure did not select resistant species, but conversely induced an evident decrease of antibiotic resistance genes in the contaminating microbial population. Also importantly, all the six HAI-positive

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5. Conclusion

Based on general scientific information and specific safety research by Chrisal, the above information should demonstrate that the probiotic products from Chrisal are absolutely safe to use. Furthermore, the various quality certifications guarantee a high quality production process.

For all additional questions, please go to www.chrisal.com for contact details in your region. We will be happy to provide you with profound answers.

All the best from the global Chrisal family!

