REPORT FOR PHARMACIES.

Intensive use of disinfectants and antibiotics has led to the emergence of many resistant microorganisms that are increasingly becoming the cause of various infections. Especially Methicillin Resistant Staphylococcus Aureus (MRSA) and Clostridium is a serious problem even for hospitals now causing severe illnesses and death among patients.

Currently used processing / purification and disinfection products are no longer effective to remove these and other (moderate) pathogens in the premises. An experienced company in the manufacture of products for microbiological treatment / purification, has developed a new line of cleaning products / agents, based on the use of probiotic bacteria (Bacterial Active Concentrate - BAC). After the application and testing in public areas in hospitals this large scale achievement showed that these new washing probiotics of BAC series are really able to solve the problems with pathogens, particularly MRSA and Clostridium.

The concept of BAC - in microbial management (management of microbes), in the quest for healthy and stable microbial community, rather than absolute and complete sterility.
This report provides information on the prevalence of several bacterial groups in a hospital environment and clearly demonstrates the high efficiency of BAC Healthcare products to manage pathogenic hospital bacteria.

PROJECT DESCRIPTION.

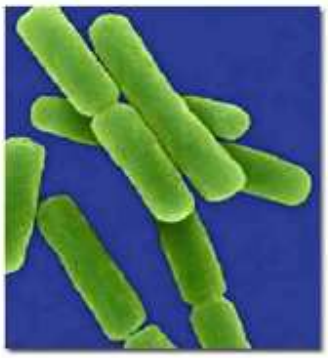

Fig. 1

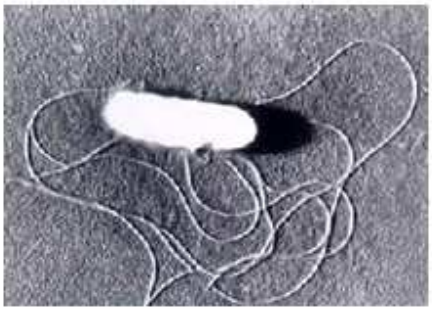

Fig. 2

Product information.
This part of the report provides a brief overview of the general concept of microbiological cleaning, as developed by the company. The mode of action and safety of the BAC products, their advantages over disinfection, as well as an overview of the products used during this study is presented.
Concept.

A broad range of pathogenic (= disease causing) micro-organisms cause numerous health problems to humans and animals. Some examples are for instance Campylobacter, Candida, Clostridium, E. coli, Legionella, Listeria (Fig 1), Salmonella, Staphylococcus (MRSA) and Streptococcus. In addition to the dangers induced by these organisms in each of our personal environment, they are also responsible for a large number of economic losses due to increased animal mortality (breeding programs), reduced productivity (food industry) and increased health care costs (hospital bacterium, dust mite). Using antibiotics and disinfectants, these problems could easily be managed during the past decades. However, the past years a rapidly increasing resistance against these 'miracle agents' has been noticed in all sectors, to such an extent that a radical new approach is eminent.

By the **creation of the BAC (Bacterial Active Concentrate) products**, offers an innovative and sustainable solution to resistance problems. These products rely on the concept of 'microbial management', in which no longer complete sterile environments are desired, but a stable and healthy microbial community is created. This can be achieved by means of probiotic micro-organisms (Fig. 2). These are safe and useful bacteria or yeasts that are already known and exploited for years in food and healthcare industry because of their health promoting properties to humans and animals. By means of extensive research and validation tests, company succeeded in applying this probiotic concept to environmental applications. All BAC products contain probiotic bacteria as a crucial ingredient, which possess the unique property of sporulation. This process makes it possible for these bacteria to survive harsh conditions and regain their activity as soon as environmental parameters improve. Without this feature it would be impossible to implement probiotics into cleaning products for environmental or industrial process applications.

Mode of action: Competitive exclusion and quorum sensing.
Bacteria, especially pathogens, have a strong tendency to develop resistance to any substance that might be detrimental or lethal to them. This phenomenon is currently flagrant in case of antibiotics and disinfectants. In order to avoid such resistance development, none of the BAC products has any direct biocidal action towards other organisms. The mechanism of action is based on the principle

of '**competitive exclusion**', combined with an influence on the '**quorum sensing**' communication between pathogenic organisms. Especially in case of disinfectants, an important disadvantage is the unspecific action of these agents, killing both good and bad micro-organisms. This results in an open surface, subject to fast recolonisation by (pathogenic) bacteria. Hence, disinfection results in a fast but short and unstable reduction of the number of micro-organisms. Because of the current resistance problems, continuously increasing concentrations and frequencies of disinfectant have to be applied, which is very detrimental to humans and the environment because of their aggressive chemical nature.

Why are these problems not relevant to the probiotic BAC products?

The idea behind **competitive exclusion** is that during the cleaning procedure a layer of probiotic bacteria is placed on the treated surface, immediately occupying the 'field' by good bacteria. They will consume all remaining food sources (incl. dead organic matter by means of necrotrophy), leaving nothing behind for potential pathogenic invaders looking for space and food. The probiotic BAC bacteria are extremely efficient and outdo all other (pathogenic) bacteria. Additional to competitive exclusion, also **quorum sensing** between pathogenic bacteria is influenced. This is an extremely fast way of communication between bacteria, making use of signal molecules. When the probiotic BAC bacteria are applied to a surface, this immediately results in the fact that pathogenic bacteria, by means of quorum sensing, will inform each other about these unfavourable condition, turning them into an inactive metabolic state. The BAC approach has the main advantage that it provides a stable solution to problems with pathogens, without any resistance build-up. The only demand set by this method is that the frequency of cleaning is kept constant, which is already evident for any hospital environment. After BAC cleaning, the total number of micro-organisms on the surface will not necessarily be higher; **the good bacteria**
simply replace the bad ones.

The following table presents a conclusive comparison between disinfection and BAC cleaning:

Disinfection	**BAC cleaning**
- 50/50 ratio of good/bad bacteria	+ 50/50 ratio of good/bad bacteria + 95/5 ratio of good/bad bacteria
- short effect (unstable effect)	+ long lasting effect (stable effect)
- resistance problems	+ no resistance possible
- detrimental / unsafe products	+ harmless / safe products
- chemical / environment unfriendly	+ biological / environment friendly
- aggressive	+ neutral

BAC products are completely safe to use for several reasons:

The probiotic bacteria used in the BAC products are members of the genus *Bacillus* and belong to **biosafety class 1**, as listed by the American Type Culture Collection (ATCC). The following table presents all four biosafety classes:

Class	Description	Risk
1	Non-pathogenic micro-organisms	None
2	Micro-organisms and parasites that may cause disease, but with an unlikely spread and for which efficient prophylaxis or treatment exists.	Low
3	Micro-organisms and parasites that are able to spread and cause disease, but subjective to efficient prophylaxis or treatment.	Average
4	Micro-organisms and parasietes with large scale spreading and serious illness, for which no prophylaxis or treatment exists.	High

- A number of probiotic Bacillus species have been granted the GRAS (Generally Recognized As Safe) label by the Food and Drug Administration (FDA) and can as such be used for human purposes without any hazard.
- The BAC bacteria belong to the group of sporulating probiotics, of which over hundred commercial pharmaceutical and nutritional products are available for human oral consumption. A regular dose of these preparations is 10 billion bacteria per day, which is about 10.000x more concentrated than the BAC products.
- Additional to the safety classification by ATCC, the producer of our bacterial strains performed a large number of toxicity tests to guarantee the safety of our bacteria. No single toxic effect from our Bacillus strains was measured.
- Company itself performed multiple safety tests in collaboration with external and accredited laboratories. All BAC products are certified as safe to use.

- In view of antibiotic resistance, Bacillus strains are Gram-positive organisms, which have much less tendency to develop, acquire or transfer antibiotic resistance. Although certain Bacillus strains are intrinsically resistant to certain cephalosporin, macrolide and quinolone antibiotics, from scientific literature, it can be concluded that at this moment, no Bacillus strains are known to transfer this antibiotic resistance to other organisms, neither in vitro nor in vivo.
- Members of the genus Bacillus are used intensively in different kinds of industries because of their high enzyme production capacity. Examples are washing powders, waste water treatment, food preservation…

In conclusion, the probiotic BAC bacteria are perfectly safe to use. These organisms have been officially classified as save organisms and have been used for decades without any negative effect.

Product range

During the course of this study, the following BAC Healthcare products were used:

- BAC Floor Cleaner NFG: This floor cleaner is a probiotic bacteria containing product, with a neutral composition suitable for all kinds of floors. The chemical composition is consultable in the MSDS file on demand; the number of probiotic bacteria is 30 million CFU/ml, with a dilution factor depending on the type of application (average of 2%). Dilution has to be done using water of approximately 40°C.
- BAC Universal Cleaner: This product has a neutral composition, making it suitable for all kinds of materials and surfaces. The chemical composition is again available through the MSDS file on demand; the bacterial composition is equal to the above mentioned Floor Cleaner.
- BAC Sanitary Cleaner: This cleaner is suitable for all kinds of sanitary installations and contains a higher concentration of probiotic BAC bacteria. This in order to compensate for the increased washout because of the running water in the installation. The bacterial concentration of the sanitary cleaner totals 50 million CFU/ml.
- BAC Allergy Free: This product has been developed to render any kind of textile free of pathogenic bacteria, as well as dust mite allergens. The product contains 50 million CFU/ml of BAC bacteria and has to be sprayed on the textile during 3 seconds.

The effectiveness of BAC Allergy Free was tested. A piece of cloth was taken, inhabited by ticks as well as the average bed. One half of tissue was treated with BAC Allergy Free, while others simply washed. The number of ticks on each half of the tissue was measured regularly in the laboratory. Total number of ticks for some time remained the same as before, but the mites from the half processed BAC Allergy Free. Switched to half, where just a wash was made. This suggests that close to the BAC bacterium mites feel badly. After 10 days of regular treatment the total count of mites also began to decrease, because in the "crowded" environment mites also cannot multiply. This means that BAC AIIergv Free lowers the number of allergens, absorb excrements, the remains spitted food, because the BAC bacteria eat this. Nothing is left fot mites to eat. Ticks chew food and spit it, and the bacteria eat it and thus remove moisture from the environment. In spray bacteria are in condition of spores.

Conclusions

Results obtained by using BAC series infected with MRSA, indicate that daily treatment with BAC inhibits the growth and spread of pathogenic bacteria, which leads to improving the environment indoors and reduced risk of various infections. In addition, BAC series washing agents are good as it cleans and disinfects at the same time. Its work does not end after drying of the surface. Enzymes break down organic matter during 5-6 days, and the bacteria can continue to work while remaining on the surface up to two months. The use of BIORAIN series washing agents provides an answer to a question what is more convenient to control infection with short-term chemical attack that does not solve all our problems, or to fight with their weapons of microbes for which there is no difference who displace through domination.