Probiotics In Progress – The PIP principle

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A broad range of pathogenic (= disease causing) micro-organisms cause numerous health problems to humans and animals. Some examples are for instance *Camphylobacter, Candida, Clostridium, E. coli, Legionella*, *Listeria* (Fig 1), *Salmonella*, *Staphylococcus* (MRSA, hospital bacterium) 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 hold in hand during the past decennia. 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.

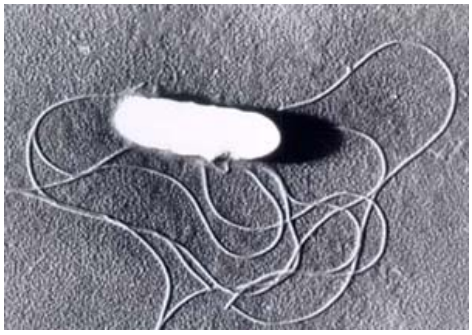

Fig 1. A pathogenic *Listeria.*

The probiotic concept

By the creation of the PIP – *Probiotics In Progress* – products, Chrisal offers an innovative and sustainable solution to the 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

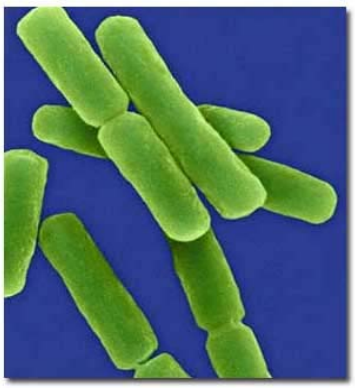

Fig 2.
Probiotic PIP bacteria

and health care industry because of their health promoting properties to man and animal. Inside our intestinal tract they occupy empty spaces and consume all of the food before detrimental or dangerous bacteria do so. By means of extensive research and validation tests in collaboration with Ghent University, Chrisal succeeded in applying this probiotic concept to environmental applications, in companies and our personal environment.

In contrast to the rather stable and protective situation in the gut, environmental conditions may strongly fluctuate, thereby imposing strict survival demands to the applied bacteria. All PIP products contain probiotic bacteria as a crucial ingredient, which possess the unique property of sporulation (Fig 3). 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 apply probiotics successfully for environmental or industrial process applications.

Fig 3. Red cells are active probiotic PIP bacteria. Gray dots represent PIP spores.

Mechanism 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 PIP 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 a surface free of organisms, making it readily available to any remaining pathogen in order to bloom within a very limited timeframe. Each

disinfection procedure leaves sufficient organic matter behind that may serve as carbohydrate and protein source to sustain a fast recolonisation of the surface. Using these remaining energy sources one pathogenic cell will multiply to a population of one million cells within 8 hours (Fig 4).

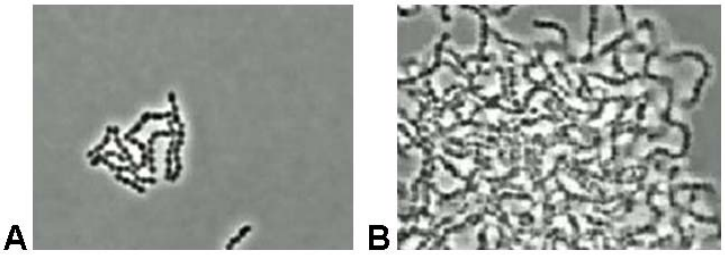

Fig 4. Bacterial growth (Cells Alive)

Hence, disinfection results in a fast reduction of the number of micro-organisms of which the effect is very short and unstable. Because of the current resistance problems, continuously increasing concentrations and frequencies of disinfectant have to be applied, which is detrimental to man and the environment due to their aggressive chemical nature.

Why are these problems not relevant to the probiotic PIP 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, leaving nothing behind for potential pathogenic invaders looking for space and food. The probiotic PIP bacteria are much more active in this competition and outdo all other bacteria.

In heavily polluted environments the competitive exclusion itself will not be sufficient to induce a fast and strong effect. This is counteracted by means of the **quorum sensing** between pathogenic bacteria. This is an extremely fast way of communication between bacteria, making use of numerous signal molecules. When the probiotic PIP bacteria are applied to a surface, a competition for space, food and moisture is established (see above). This immediately results in the fact that pathogenic bacteria, by means of quorum sensing, will inform each other about these unfavourable conditions, leading to a dormant phase and their subsequent removal.

The PIP approach has two main advantages: it provides a stable solution to problems with pathogens and no resistance can be developed. The only demand set by this method is that the frequency of cleaning is kept constant,

which is already evident in most cases. Important as well is to realize that the total number of micro-organisms will not or only slightly rise after PIP treatment; **the good bacteria simply replace the bad ones**.

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

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

The following part of this information brochure presents a short overview of the different PIP products that are currently successfully applied. More detailed information on each of the products is available on our website or by contacting us.

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