Enzymatic Activity of PBGL probiotic cocktails

Date de remise du rapport : **22/04/22**

Rapport rédigé par **D. Rentmeister**

Rapport approuvé par **E. Bouhajja**

probiotic Group

probiotic Group

Contents

probiotic Group

Probiotic cleaner

What is a probiotic cleaner?

Probiotic cleaners are sustainable detergents enriched with “good” bacteria. When you spray your product on any kind of surface, probiotics will stick to it and attack the dirt even in the invisible and unreachable pores and cracks. The cleaning action is continuous, after the first application, the cleaning army keeps destroying the dirt components for hours.

What is the key of bacterial cleaning?

A probiotic bacterium, like any living cell, produces diverse enzymes. These catalysts are the key players in breaking down organic dirt into smaller molecules which will be used afterwards by the producer itself and other bacteria on the surface to grow. They have promising cleaning properties and have been used for years, especially in laundry detergents and other multipurpose detergents. The action of enzymes upon a substance (called substrate) is very specific. Scientists have been working on the listing of these catalysts and grouped them into families depending on the substrate specificity. The major classes of enzymes used in detergents include cellulases, proteases, lipases and amylases (Niyonzima & More, 2015, Gürkök, 2019). Proteases can cleave protein stains from food such as blood, egg, milk, etc. into polypeptides or free amino acids. Lipases increase the hydrolysis of fats into glycerol and free fatty acids. Amylases split starch molecules into simple sugars. In this context, a single probiotic bacterium can produce many different enzymes that could attack dirt components such as oil, carbohydrates and proteins, all at once. The efficacy of degradation depends on the producer probiotic because not all probiotics produce the same enzymes, and not all enzymes from the same family have similar degradation efficacy. Therefore, it is more interesting and effective to use a mixture of probiotics instead of pure strains in cleaning products.

What happens when you use Provilan products?

In Provilan products, there are two different probiotic cocktails depending on the product range. The cocktail used in pets and animal care products referred to as probiotic cocktail 1 (UB2) and another used in detergents referred to as probiotic cocktail 2 (BEBS2). Both cocktails contain food-grade Bacillus bacteria and are safe for the user. They are dormant in the product (spore form) to ensure the stability of the formulation. When used on a surface, the contact of dormant probiotics with water and organic substances induces the activation of spores and the probiotics become active. They will multiply around the dirt stain and start to produce degradative enzymes. The efficacy of our probiotics was proven in the laboratory before being added to the final product. They were able to remove organic dirt which could be found on inanimate surfaces used in everyday life either in the domestic or in the professional environment (e.g. countertop, tables, floor...) and on animals' skins.

A glance at the laboratory efficacy testing

To assess the efficacy of our probiotics to tackle the main dirt components, we tested their ability to produce different degradative enzymes before testing the final product according to cleaning standards. The test consists of spreading one million (10^6) probiotic bacteria on the center of an agar culture media supplemented with specific substrates for each enzymatic activity. Media used are starch agar, egg yolk agar, skim milk agar, and Columbia sheep blood agar to test the degradation of starch, fat, protein and blood hemolysis. The incubation is carried out at room temperature for 7 days to be as close as possible to the conditions in which our products are used. Positive activities could be detected by the appearance of a clearing zone surrounding the bacteria. All activities can be detected on the media without adding an indicator, except for the starch degradation, where Lugol’s reagent is

added at the end of incubation to reveal the degradation halo. The reagent turns blue-black as it reacts with non-degraded starch (Valls et al., 2012).

Potential of enzymes degradation in our probiotic cocktails

Starch degradation

Starch is a complex sugar commonly found in wheat (e. g. pasta), rice, potatoes, maize, etc. It is composed of multiple linked subunits of glucose (Figure 1, a) (de Souza, 2010). Our probiotic cocktails produce amylases enzymes to degrade starch at room temperature as shown in Figure 1 b. and c. The starch agar medium contains mainly starch and other nutrients required for the growth of bacteria. Amylases produced by bacteria degrade the starch into simple sugars such as tri-, di- and glucose (de Souza, 2010). Resulting glucose is then metabolized by bacteria to survive and to grow.

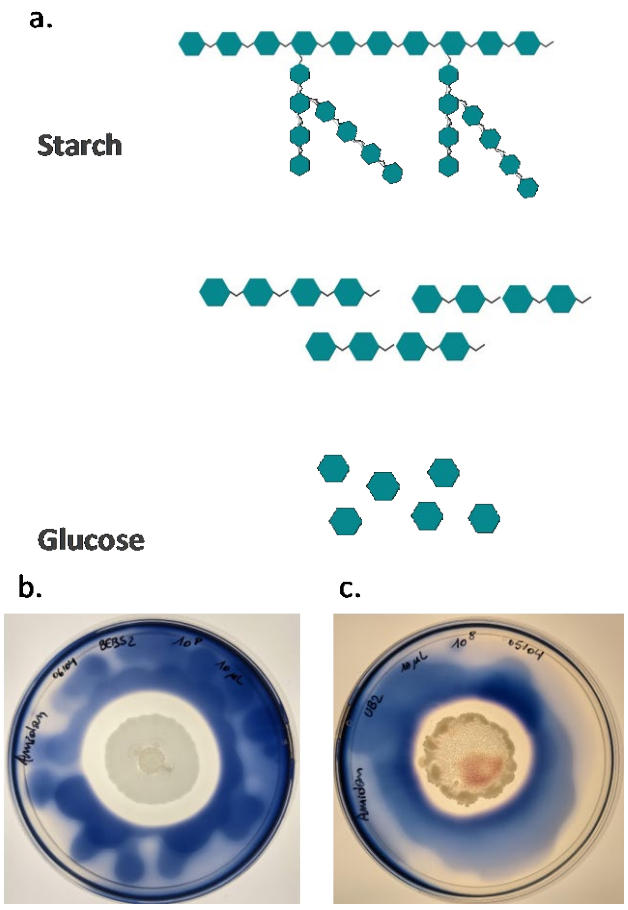

Figure 1: a. Starch structure. b. Amylase activity of probiotic cocktail 2 (BEBS2) after 7 days c. Amylase activity of probiotic cocktail 1 (UB2) after 7 days. A clear halo around the growing probiotics indicates that starch has been hydrolyzed by amylases produced.

Degradation of egg yolk

Egg yolk contains a mixture, among others, of different proteins and lipids. The egg yolk agar media contains egg yolk emulsion and essential nutrients for the growth of bacteria. The egg yolk emulsion gives to the media an opaque yellow-white color. Bacteria which produce proteases and lipases could catalyze the degradation of proteins and lipids in the egg yolk (Aryal, 2022).

Our probiotic cocktails were tested positive to proteases, lecithinases and other lipases active on egg yolk. Degradation of lecithin (a functional lipid in egg yolk) by lecithinase enzymes can be seen by the appearance of a narrow white opaque zone of precipitation that spreads beyond the edge of the growing bacteria (Figure 2 a.). At the same time, a wider clear halo appears surrounding probiotics due to the degradation of the egg yolk's proteins as shown in Figure 2 a. . Other lipases activity can be detected by the appearance of a sheen (oily layer on water) on top of bacteria colonies because these enzymes cannot diffuse easily through the agar and they locally hydrolyze the free fats present in the medium to glycerol and free fatty acids (Figure 2 b.) (Aryal, 2022).

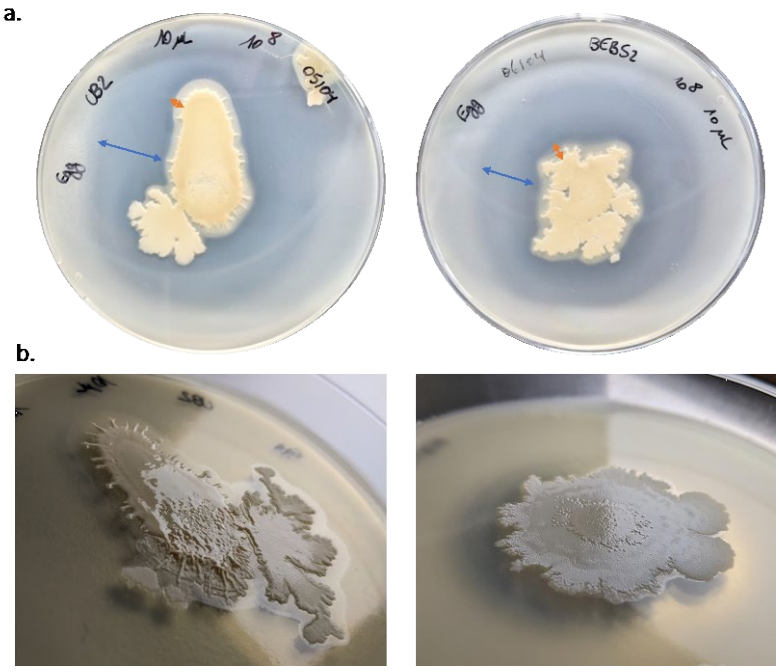

Figure 2: Proteases, lipases and Lecithinases activities of probiotics after 7 days at room temperature. a. Proteases (Blue arrow) and lecithinases (orange arrow) activities of probiotics cocktails. b. Lipase activity of probiotics cocktails on egg yolk as revealed by the shiny layer on the growing bacteria.

Degradation of skim milk

The skim milk media contains nutrients essential to the growth of bacteria and skim milk powder. The media has a white color due to the milk's casein protein. Many bacteria can produce different proteases which can hydrolyze proteins contained in milk, including casein. Degradation products are subsequently used in the metabolism of bacteria to ensure their multiplication (Aryal, 2021).

Probiotics cocktails can degrade casein as shown in figure 3. The initial off-white color of the media disappears as the casein in the skim milk powder is hydrolyzed by probiotics' proteases leading to a clear halo. The enzymatic activity starts to be visible after 2 days and becomes stronger as probiotics are growing. It is noticeable that the two different cocktails don't have the same activity as the halo size is different after 7 days. Bacteria of mixture 1 have higher activity than bacteria in mixture 2. In fact, both cocktails have different bacteria strains with different enzymatic potential.

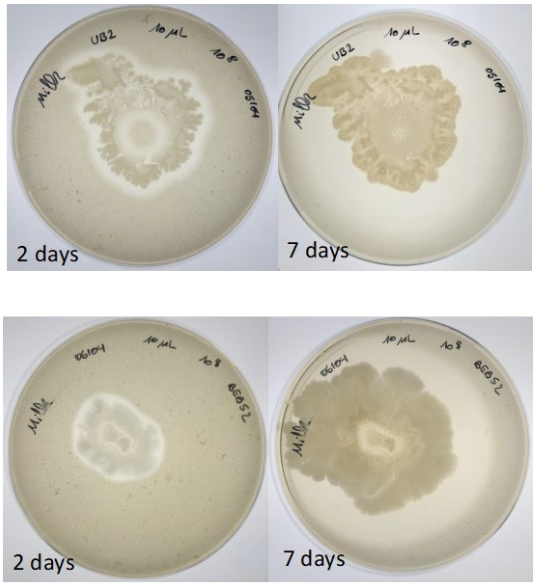

Figure 1: Protease activity of probiotics on skim milk agar. Production of proteases by probiotic cocktail 1 (top) and 2 (below) after 2 and 7 days of incubation at room temperature. The degradation halo around the growing bacteria becomes bigger as bacteria are growing. around the colony grows between day 2 and 7 which indicates that more proteases have been produced and that the proteases can diffuse through the media

Hemolysis of sheep blood

The red color of red blood cells is attributed to hemoglobin which is an iron containing protein responsible for carrying oxygen from the respiratory organs to the other tissues in the body. Some bacteria, notably those of Bacillus group, can dissociate hemoglobin and use some of the released products as an iron source (Sato et al., 1999).

The Columbia sheep blood agar is the medium used to detect enzymes involved in blood hemolysis. It cotains sheep blood and nutrients necessary for the growth of bacteria. Probiotics cocktails 1 and 2 can produce hemolytic enzymes within 7 days at room temperature. These enzymes break down red blood cells and the denaturation of hemoglobin to form a coloourless product (Figure 7).

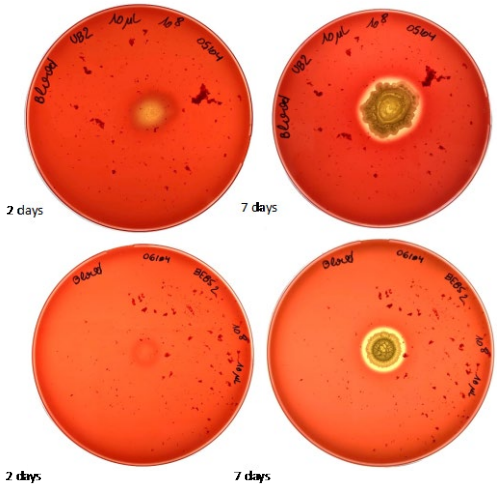

Figure 7: Hemolysis induced by probiotic cocktail 1 (Top) and 2 (below) after 2 and 7 days. A clear halo appears around the growing bacteria where hemolysis has taken place.

Conclusion

Probiotic cocktails used in our range of products can produce a handful of enzymes which are able to break down main components of organic dirt such as starch, egg yolk proteins and lipids, milk proteins and blood. Probiotics can use the nutrients resulting from degradation of complex substances on a surface to multiply and maintain the microbial diversity of surfaces and skin.

References

Aryal, S. (2021, August). Casein Hydrolysis Test- Objectives, Principle, Media, Procedure, Results. *Microbes Notes*. https://microbenotes.com/casein-hydrolysis-test/

Aryal, S. (2022, January). Egg Yolk Agar- Composition, Principle, Preparation, Results, Uses. *Microbe Notes*. https://microbenotes.com/egg-yolk-agar/?msclkid=55965705c15611ec8b3faf181fc92676

de Souza, P. M. (2010). APPLICATION OF MICROBIAL ALPHA-AMYLASE IN INDUSTRY – A REVIEW. *Brazilian Journal of Microbiology*, *41*, 850–861.

Gürkök, S. (2019). *Microbial Enzymes in Detergents: A Review*. *10*(9), 8.

Niyonzima, F. N., & More, S. S. (2015). Coproduction of detergent compatible bacterial enzymes and stain removal evaluation. *Journal of Basic Microbiology*, *55*(10), 1149–1158. https://doi.org/10.1002/jobm.201500112

Sato, N., Ikeda, S., Mikami, S., & Matsumoto, T. (1999). Bacillus cereus dissociates hemoglobin and uses released heme as an iron source. *Biol Pharm Bull .*, *10*, 1118–1121. https://doi.org/doi: 10.1248/bpb.22.1118.

Valls, C., Rojas, C., Pujadas, G., Garcia-Vallve, S., & Mulero, M. (2012). Characterization of the activity and stability of amylase from saliva and detergent: Laboratory practicals for studying the activity and stability of amylase from saliva and various commercial detergents. *Biochemistry and Molecular Biology Education*, *40*(4), 254–265. https://doi.org/10.1002/bmb.20612