

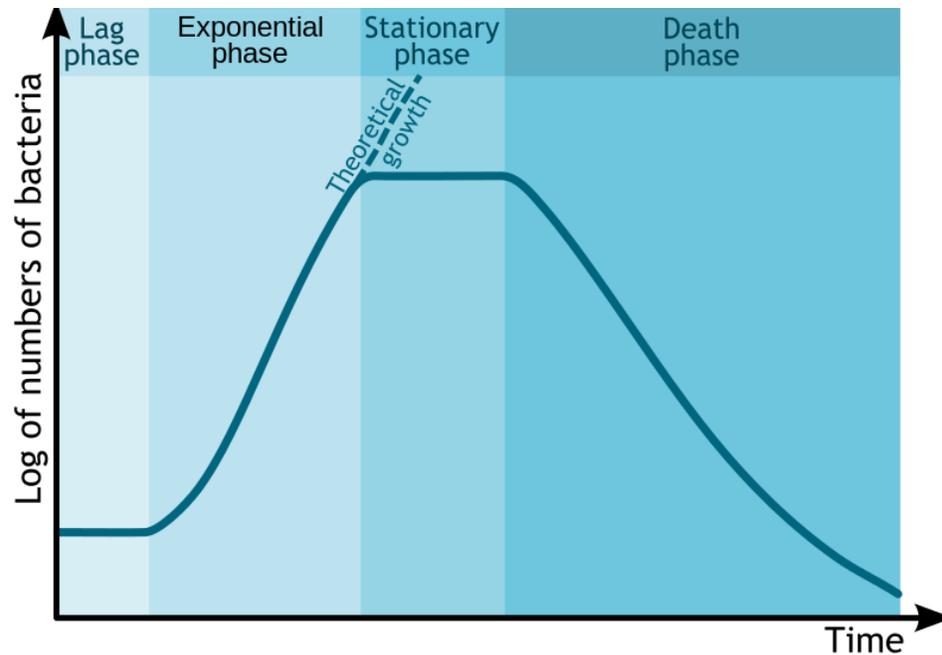
Probonix, the state-of-the-art probiotic line created and developed by Humarian Research Lab is certainly not an ordinary probiotic. Based on years of scientific research, university studies, and field trials, Humarian has developed a proprietary process yielding 4 separate and distinct characteristics to all their probiotic products, which no other probiotic in production contains. These four components consist of: ***Lag Phase Bacteria (LPB)***, ***Acid Resilient Strains (ARS)***, ***Toxin-Scavenging Lactobacillus (TSL)***, and ***Probiotic Bacterial Lysates (PBL)***.

Lag Phase Bacteria (LPB)

The growth process of bacteria occurs in four distinct phases: **lag phase, exponential phase/log phase, stationary phase, and death phase**.¹

1. During **lag phase**, Bacteria adapt themselves to new growth conditions. During this phase, individual bacteria mature (grow in size) but they do not divide nor do they grow in population or numbers.
2. The **exponential phase** (sometimes called the log phase or logarithmic phase) is a period characterized by cell doubling. The number of new bacteria appearing per unit time is proportional to the present population. It is during this phase that cells are most susceptible to adverse environmental conditions, which affect the frequency of cellular division and the probability of both daughter cells surviving. It is important to note that exponential growth cannot continue indefinitely because this growth depletes the medium of nutrients while simultaneously increasing metabolic waste.
3. The **stationary phase** is often due to a growth-limiting factor such as the depletion of an essential nutrient, or due to the build-up of metabolic waste material from cellular division or the formation of an inhibitory product such as an organic acid. The stationary phase is a result of a situation wherein the growth rate and death rate are equal. The number of new cells created is limited by the growth factor and, as a result, the rate of cell growth matches the rate of cell death.
4. At **death phase** (decline phase), bacteria die. This could be caused due to the lack of nutrients, build-up of waste material, environmental temperature above or below the tolerance band for the species, or other injurious conditions.

¹ Fankhauser, David B. (17 July 2004). "[Bacterial Growth Curve](#)". University of Cincinnati Clermont College. Retrieved 29 December 2015.



The thorough understanding of each of the bacterial growth phases has laid the foundation for Humarian to utilize an exclusive proprietary process and create **Lag Phase Bacteria (LPB)** and **Acid Resilient Strains (ARS)** of probiotics ensuring survivability in low pH (highly acidic) environments, such as transit through stomach acid.

The probiotics in Probonix are saturated in a proprietary poly-matrix protection and immuration system to preserve and ensure stability, without the need for refrigeration or prebiotics such as fructooligosaccharides and arabinogalactans. This poly-matrix and immuration system places the individual bacteria in a temporary lag phase where the bacterial strains are able to adapt to the acidic conditions in which they are bathed in and it is this adaptation to the environment that ultimately protects the probiotics against the stomach acid, allowing them to reach the intestines. Once the organisms in this product reach the intestines, the environment changes, allowing bacterial growth to shift from the **lag phase** to the **exponential phase** where they **multiply in number and proliferate throughout the entire intestinal tract** providing their many health benefits to the user of the product. This process **dramatically enhances the survival and growth of bacteria** over traditional probiotic supplements, since most other probiotic products rely on traditional encapsulation, refrigeration and prebiotic food for their live and growing **exponential phase bacteria**.

Research indicates that anywhere from 80-99% of traditional, unprotected, live probiotic cells will be killed off by stomach acid before reaching the intestine.² In order for the good bacteria to provide their beneficial effects, they must be able to withstand processing conditions and also be viable in sufficient numbers during storage.³ As stated earlier exponential phase bacteria can quickly move to the stationary and death phase while on the shelf, when depletion of their essential nutrient (prebiotic) occurs, or build up of metabolic waste material from cellular division occurs, inhibiting its growth due to injurious conditions. **Traditional probiotic supplements will claim billions of active cells per dose on their labels, but they do not promise whether these will stay alive and healthy once they enter the intestines.** Some companies claim to be superior with a process called, “microencapsulation,” that is supposed to shield the probiotics from the damaging effects of the stomach acid. However, research indicates that none of these reported methods have resulted in shelf-stable, viable probiotics.⁴

To validate their products; Humarian Research Lab retained TNO Triskelion to perform a non-biased, independent validation study. This study aimed at determining whether Probonix was superior to the top physician probiotic, in its ability to obtain a higher percentage of live probiotic bacteria after passage through an in-vitro model, simulating the upper gastro-intestinal tract (TIM-1 system).

Table 2 (below) shows the theoretical and viable amount of probiotic cells brought into the TIM-1 system for each of the three test products. It is interesting to note that Probonix contained half as much viable lactobacilli as compared to the Top Physician Brand, but had over 5.5 times more bifidobacteria.

Table 2. Theoretical and viable amount of probiotic cells as brought into TIM-1 for lactobacilli, bifidobacteria and the total of lactobacilli plus bifidobacteria in the three test products, measured by using qPCR-PMA analysis (converted to CFU, mean, n=2).

<i>test product</i>	<i>lactobacilli</i>		<i>bifidobacteria</i>		<i>total</i>	
	<i>theoretical</i>	<i>viable</i>	<i>theoretical</i>	<i>viable</i>	<i>theoretical</i>	<i>viable</i>
	<i>CFU</i>	<i>CFU</i>	<i>CFU</i>	<i>CFU</i>	<i>CFU</i>	<i>CFU</i>
#1 Probonix	7.00E+09	3.40E+09	2.80E+09	7.48E+06	9.80E+09	3.40E+09
#2 Probonix Plus	5.00E+09	1.33E+10	5.00E+09	2.83E+07	1.00E+10	1.34E+10
#3 Top Physician Brand	5.00E+09	6.85E+09	5.00E+09	1.34E+08	1.00E+10	6.98E+09

² Cook MT, Tzortzis G, Charalampopoulos D, Khutoryanskiy VV. Microencapsulation of probiotics for gastrointestinal delivery. J Control Release. 2012 Aug 20;162(1):56-67.

³ Del Piano M, Morelli L, Strozzi GP, Allesina S, Barba M, Deidda F, Lorenzini P, Ballaré M, Montino F, Orsello M, Sartori M, Garello E, Carmagnola S, Pagliarulo M, Capurso L. Probiotics: from research to consumer; Dig Liver Dis. 2006 Dec;38 Suppl 2:S248-55.

⁴ Kailasapathy K. Microencapsulation of probiotic bacteria: technology and potential applications. Curr Issues Intest Microbiol. 2002 Sep;3(2):39-48.

Furthermore, the average absolute number of lactobacilli that survived passage through TIM-1 was highest for Probonix ($3.1 \times 10^8 \pm 1.2 \times 10^8$ CFU) vs. the Top Physician Brand ($6.4 \times 10^7 \pm 2.9 \times 10^7$ CFU). Note the difference of one whole log between Probonix and the Top Physician Brand. These results are displayed below, Figure 5.

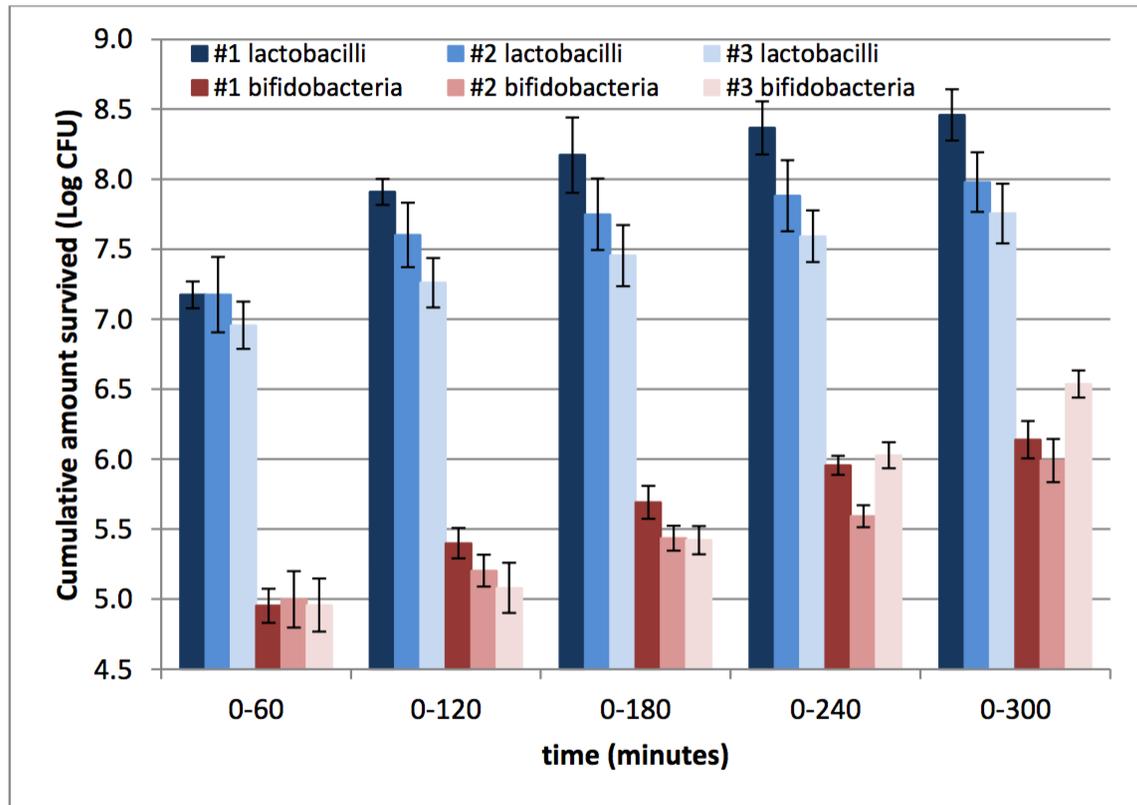


Figure 5. Cumulative amount of lactobacilli (blue) and bifidobacteria (red) in #1 Probonix, #2 Probonix Plus and #3 Top Physician Brand, surviving passage through TIM-1 as measured by using qPCR-PMA analysis converted to CFU (expressed as log CFU, mean \pm range, n=2).

Due to the significant differences between viable amount of probiotic cells as brought into TIM-1 for lactobacilli and bifidobacteria, the most important highlight from the study is the expressed percent survival after passage through TIM-1. This is visually represented below, in Figure 6. The lactobacilli survival (% of intake) was highest for Probonix ($9.2 \pm 3.7\%$) as compared to the Top Physician Brand ($0.9 \pm 0.4\%$). **This indicates that Probonix has 10.2 times (1020%) more lactobacilli that survive passage in the upper gastrointestinal system than the Top Physician brand.** For the bifidobacteria, the ranking in survival (% of intake) was highest for Probonix with $19.3 \pm 5.8\%$, as compared to $2.6 \pm 0.6\%$ for Top Physician Brand. **This indicates that**

Probonix has 7.4 times (740%) more bifidobacteria that survive passage in the upper gastrointestinal system than the Top Physician brand.

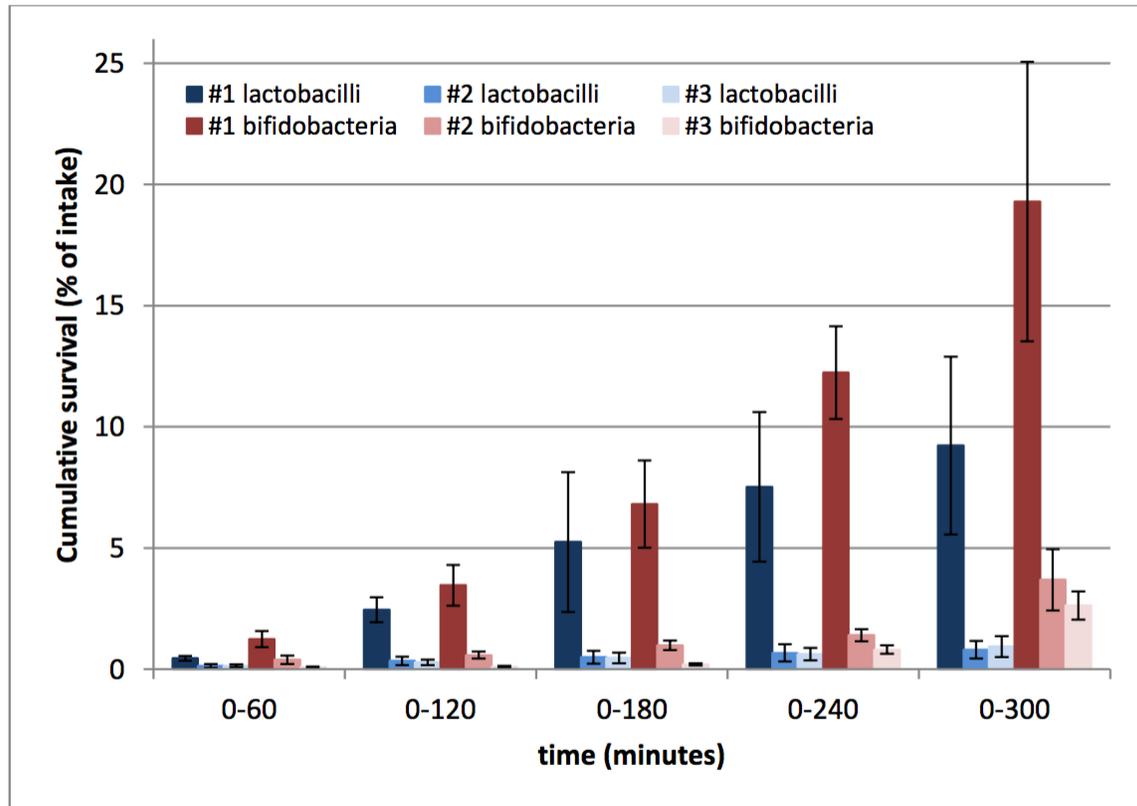


Figure 6. Cumulative survival of lactobacilli (blue) and bifidobacteria (red) in #1 Probonix, #2 Probonix Plus and #3 Top Physician Brand, during 5 hours through TIM-1 as measured by using qPCR-PMA analysis converted to CFU (% of intake, mean ± range, n=2).

These results indicate that Humarian Research Lab’s proprietary process of creating **Lag Phase Bacteria (LPB)** and **Acid Resilient Strains (ARS)** of probiotics ensures survivability in low pH (high acidic) environments, with superior shelf life and without the need for refrigeration.

Toxin-Scavenging Lactobacillus (TSL)

In addition to creating an industry-first in maximizing bacterial colonization in the gut, Humarian Research Lab has pioneered the nutraceutical industry’s **first Toxin-Scavenging Lactobacillus**. The exclusive proprietary formula uses a unique, but naturally occurring fermentation process that allows natural and beneficial modifications to the bacterium’s cell wall. During this process the **bacterium are trained by repetitive passages of increased doses of mixed toxins from mold (mycotoxins) and other toxic substances bound to primarily gram-negative bacterial cell walls, called endotoxins; also known as Lipopolysaccharides (LPS), that are released when the bad bacterium ruptures or disintegrates.**

The strains ultimately selected for the Humarian products thrive in a toxin-challenged environment, thus they inherently seek and destroy toxins. These effects are part of the Toxin-Scavenging Lactobacilli's innate biological capacities. This exclusive proprietary process allows the Toxin-Scavenging Lactobacillus to easily affect the tiniest and hardest-to-reach spaces without affecting its functional capacity.

Probiotic Bacterial Lysates (PBL)

In general, the term "lysis" refers to the breaking down of a cell. Scientists use one of many procedures to carry out this process in order to "lyse" the cell. Scientific literature demonstrates **gentle lysis of beneficial bacteria provides more immunogenic lysate components because no denaturation of proteins occurs during disruption of the cells.**⁵ The resulting fluid containing the contents of the lysed cells is referred to as a "lysate". The components of the probiotic lysates, in our system, activate receptors of the immune system, which in turn, start events that lead to the creation of cytokines; a large and varied class of proteins that regulate intercellular communication within the immune system.

Probonix contains beneficial bacteria whose cell wall has been lysed via a proprietary system. This process releases vital substances and nutrients from the cell wall and cytoplasm, called ***Probiotic Bacterial Lysates (PBL), into the solution***, making them readily available upon ingestion of Probonix. This is important, because every immune system cell is equipped to synthesize and release a variety of small molecules that travel to other cells and stimulate those cells to become either more (up-regulate) or less (down-regulate) active.

Probonix lysates activate receptors in the cells and result in cytokine production. Cytokines are the immune modulators. They are essential in the maintenance of a balanced immune system and are instrumental in the regulation and orchestration of the immune response.

Several research projects have proved that some molecules contained in cell walls of microorganisms effectively stimulate specific and particularly non-specific immune response. Preparations containing microbial lysates are a useful supplement to support the immune system in a natural way that exploits basic innate defense mechanisms. These immune system modulators exist inside the cell of healthy bacteria and are not readily available in most probiotic formulas. On the basis of the concept that microbial immunogenic components are capable of improving the host defense against pathogens, *PBL* application has been recommended for the prevention of respiratory and gut infections and subsequent inflammatory

⁵ Cazzola M, Rogliani P, Curradi G. Bacterial extracts for the prevention of acute exacerbations in chronic obstructive pulmonary disease: a point of view. *Respir Med* 2008;102:321.e7.

conditions.⁶

Apart from the application of viable bacteria in Probonix, PBLs seem to not only act through direct modification of colonization patterns. Clinical studies, as well as animal experiments, have indicated that increases in (secretory) IgA levels seem to be the most important immunomodulating activity of PBLs.^{7,8,9} According to the age-dependent development of the IgA repertoire,¹⁰ improved IgA release observed after PBL application is attributed to a more unspecific T cell-driven enhancement of IgA maturation in early life and an antigen-defined induction of IgA in patients of preschool age and older (Fig 2).¹¹

Additionally, research indicates that PBL increases the numbers of regulatory T cells (CD4(+)/FoxP3(+)) in mesenteric lymph nodes (MLN), decreases the production of pro-inflammatory cytokines TNF- α and IFN- γ , and anti-inflammatory IL-10 in Peyer's patches and the large intestine, and changes the gut microbiota composition. Moreover, PBL treatment prevents lipopolysaccharide-induced TNF- α expression by down-regulating the NF- κ B signaling pathway.¹²

The efficacy of Humarian Research Lab's probiotic line, Probonix, is indubitable. Saturating the probiotics in the proprietary poly-matrix protection and immuration system while keeping it in a temporary lag phase, allowing the bacteria to adapt and hence survive in the acidic conditions of the stomach, and ultimately multiply in the intestines; using a fermentation process that enables bacteria to eliminate toxins; and utilizing a process of breaking down cells to result in regulating the immune system - these proprietary processes, and innovative technology, that goes into the creation the each individual component in Probonix; Lag Phase Bacteria (LPB), Acid Resilient

⁶ Roz_y A, Chorostowska-Wynimko J. Bacterial immunostimulants—mechanism of action and clinical application in respiratory diseases. *Pneumonol Alergol Pol* 2008;76:353-9.

⁷ Puigdollers JM, Serna GR, Hernandez del Rey I, Barruffet MT, Torroella JJ. Im- munoglobulin production in man stimulated by an orally administered bacterial lysate. *Respiration* 1980;40:142-9.

⁸ Rossi GA, Peri C, Raynal ME, Defilippi AC, Risso FM, Schenone G, et al. Nat- urally occurring immune response against bacteria commonly involved in upper respiratory tract infections: analysis of the antigen-specific salivary IgA levels. *Immunol Lett* 2003;86:85-91.

⁹ Huber M, Mossmann H, Bessler WG. Th1-orientated immunological properties of the bacterial extract OM-85-BV. *Eur J Med Res* 2005;10:209-17.

¹⁰ Rogosch T, Kerzel S, Hoß K, Hoersch G, Zemlin C, Heckmann M, et al. IgA re- sponse in preterm neonates shows little evidence of antigen-driven selection. *J Immunol* 2012;189:5449-56.

¹¹ Cazzola M, Capuano A, Rogliani P, Matera MG. Bacterial lysates as a potentially effective approach in preventing acute exacerbation of COPD. *Curr Opin Pharma- col* 2012;12:300-8.

¹² (Zakostelska Z, Kverka M, Klimesova K, Rossmann P, Mrazek J, Kopecny J, et al. (2011) Lysate of Probiotic *Lactobacillus casei* DN-114 001 Ameliorates Colitis by Strengthening the Gut Barrier Function and Changing the Gut Microenvironment. *PLoS ONE* 6(11): e27961. doi:10.1371/journal.pone.0027961)

Strains (ARS), Toxin-Scavenging Lactobacillus (TSL), and Probiotic Bacterial Lysates (PBL); is what sets it apart from the rest.

Probonix does not just focus on providing the host (user) with probiotics to ingest, but ensures that the bacteria are resilient enough to live and multiply in the gut, thrive in a toxin-challenged environment, as well as enhance the body's immune response.

Among the many health benefits, Probonix supports the natural growth of beneficial bacteria to the gut, enhances the natural detoxification process, inhibits the growth of unhealthy bacteria, improves digestion and supports the regeneration of cells in the gut lining. Furthermore, it is allergen-free and does not contain wheat, gluten, soybeans, eggs, fish/shellfish or peanuts/tree nuts.

A handwritten signature in black ink, appearing to read 'Ryan Bentley', with a long horizontal flourish extending to the right.

Ryan E. Bentley, MD, DC, PhD
Chief Medical Officer