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# Pre-Incubation of Probiotics Before Intake: A Reasonable Recommendation?

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**Małgorzata Bernatek**[1]
https://orcid.org/0000-0001-8687-9383

**Jacek Piątek**[1]
https://orcid.org/0000-0003-2152-0876

**Katarzyna Wojtyła-Blicharska**[2]

**Henning Sommermeyer**[1]
https://orcid.org/0000-0003-1991-9327

**Address for correspondence**

[1] Faculty of Health Sciences, Calisia University, Kalisz, Poland
[2] Pleszewskie Centrum Medyczne Sp. z o.o., Pleszew, Poland

Jacek Piątek
Department of Health Sciences
Calisia University
4 Nowy Swiat St.
62-800 Kalisz, Poland

## *Abstract*

***Background:*** *Protecting the bacteria contained in probiotics against the effects of low pH is one of the key issues for the development and production of such products. In earlier research we demonstrated that the inactivation of bacteria in probiotics by low pH can be prevented by an effective enteric coating formulation of the product.*

***Objective:*** *The present study aimed to evaluate whether the producer's recommended pre-incubation step for its probiotic product A prior to intake is having an effect on the survival rate of the product's bacteria in low pH solutions.*

***Method:*** *For the investigated product A (and an enteric coated reference product B) amounts of colony forming units (CFU) before and after pre-incubations for 0, 1 and 30 min followed by exposure to 0.1 N HCl (pH 1.2) at 37°C for 90 min were determined. The loss of living bacteria was calculated using the $CFU_1/CFU_0$-ratio, with $CFU_1$ and $CFU_0$ being the amount of CFU at the end and at the beginning of the experiment, respectively.*

***Results:*** *The exposure of the product A to a low pH (1.2) resulted in a strong reduction of the amount of CFU (about -99.9%). Pre-incubation of product A neither for one nor for 30 min had a significant effect on the strong loss of viability of the probiotic bacteria contained in product A when compared to the loss without the pre-incubation process. In contrast, exposing the enteric coated product B to low pH resulted only in a minor loss (about -7%) of living bacteria.*

***Conclusions:*** *The pre-incubation before intake recommended by the producer of product A is not having a significant influence on a major reduction of CFU caused by the exposure to low pH. However, the observed considerable loss of CFU in a solution that mimics the condition in the human stomach triggers doubts about product A. We strongly suggest basing probiotic product related statements on scientific data.*


***Key words:*** *activation of probiotics, enteric coating, hydrochloric acid, low pH, pre-incubation of probiotics, probiotics, stomach, bacterial survivability*

## Introduction

The World Health Organization (WHO) working committee on probiotics has defined probiotics as "living microorganisms which when administered in adequate amounts confer health benefits on the host" [1]. The health benefits attributed to the administration of probiotics originate from the action of the living probiotic microorganisms in the intestine of the host [2, 3]. One of the big challenges for orally taken probiotic bacteria is to reach the gut alive [4]. The key challenge of this journey is to travel through the stomach without being inactivated by the stomach acid. Among other roles, the low pH of the stomach serves to reduce the bacterial load of food [5]. In this role, the stomach cannot differentiate between potentially harmful bacteria and potentially beneficial probiotic bacteria. Protecting the bacteria contained in probiotics against the effects of low pH is therefore one of the key issues for the development and production of such products [6]. In previous research we demonstrated that the inactivation of bacteria in probiotics by low pH can be prevented by an effective enteric coating formulation of the product [7].

We were puzzled when we learned about the broadly communicated recommendation of a certain producer of a probiotic product (product A) who required the user to perform what he called an 'activation step' before taking the product. The 'activation step' was either a one-minute or 30-minute incubation in water before intake [8]. According to the rationale for this step provided, it would 'prepare' the bacteria for proliferation in the intestine. Based on our knowledge, we rather considered the 'activation step' to potentially increase the risk that the probiotic bacteria in the product could become more sensitive to inactivation by low pH. From a scientific perspective, there is no evidence that this step might result in an 'activation' of the probiotic bacteria; consequently, we will refer to it in the remaining part of our article as a 'pre-incubation' step.

The present study aimed to evaluate the hypothesis that the pre-incubation of product A prior to intake might increase the risk that the product's probiotic bacteria become more vulnerable to inactivation by the stomach acid. Experimentally, we used an in-vitro model of survivability of bacteria in

a low pH solution. The results of the present study will allow us to determine whether the pre-incubation of product A prior to intake is reasonable or not.

## Materials and methods

The tested product, referred to as product A, (Omni-Biotic®10, Institut AllergoSan Deutschalnd (privat) GmbH, Grünwald, Germany) is a probiotic containing 10 different probiotic bacterial strains. Product A is formulated as a powder with a total of 1 x $10^9$ colony forming units (CFU) per 1 g of powder. No information is provided about the individual CFU-values per individual bacterial strains or about any measures used to protect the bacteria against inactivation by low pH (e.g., enteric coating). The product leaflet contains the recommendation to 'activate' the product prior to intake by either a one minute or a thirty minute long incubation in water.

In the present study product A was either 'not activated' or 'activated' for one or thirty minutes by incubation in water, before exposing it to a low pH solution. As an additional control, we examined product B (Vivatlac® Synbiotikum, Vivatrex GmbH, Rees, Germany). Product B contains 9 different probiotic bacterial strains in defined individual quantities, which add-up to a total CFU amount of 4.5 x $10^9$ per capsule. Probiotic bacteria in product B are protected against inactivation by low pH by an enteric coating of the capsules provided during production.

Survival rates of bacteria were evaluated by exposing the product to 'in-vitro' conditions mimicking the acid conditions existing in the stomach lumen of adults. The artificial stomach solution used was a 0.1 N hydrochloric acid solution of pH 1.2. The products were exposed to this solution at 37°C under gentle stirring for 90 min [7]. The amounts of colony forming units (CFU) before ($CFU_0$) and after ($CFU_1$) the test were determined. The survival rate was calculated using the following formula:

$$\text{Survival rate [\%]} = (CFU_1)/(CFU_0)\ \%$$

The results shown are means ± SD of three independent experiments calculated using Excel (Excel, Microsoft, Redmont, WA, USA). P-values were determined

by independent T-test statistical analyses of datasets using GraphPad Prism software version 8.2 (GraphPad Software, San Diego, CA, USA).

## Results

The effect of different pre-incubation regimes followed by 90 min exposure to a solution of pH 1.2 at 37°C on the amount of CFU is presented in Table 1. As the data revealed, exposing product A to low pH resulted in an extensive reduction of CFU. The effect of the three investigated pre-incubation regimes administered to product A did not result in significant differences in the losses of CFU, while there might have been a trend for a slightly higher loss of CFU when product A was pre-incubated for 30 min (Figure 1). In contrast, the reduction in the CFU amounts after exposure to low pH observed for product B was by far less pronounced.

**Table 1.** Amount of colony forming units (CFU) at the beginning ($CFU_0$) of the experiment and after the 90 min incubation in 0.1 N HCl, pH 1.2 solution at 37°C ($CFU_1$). The number of surviving CFU is expressed as the $CFU_1/CFU_0$ ratio. The data shown are means ± SD from three independent experiments. Product A is the key product evaluated in the study, while product B is a reference product in which the probiotic bacteria are protected by enteric coating

| Product<br>Pre-incubation | $CFU_0$ [$10^6$]<br>Mean ± SD | $CFU_1$ [$10^6$]<br>Mean ± SD | $CFU_1/CFU_0$ [%] |
|---|---|---|---|
| Product A<br>No pre-incubation | 8,000.0 ± 0.0 | 8.5 ± 0.4 | 0.11 |
| Product A<br>1 min pre-incubation | 8,000.0 ± 0.0 | 8.8 ± 0.6 | 0.11 |
| Product A<br>30 min pre-incubation | 8,500.0 ± 0.0 | 7.8 ± 0.2 | 0.09 |
| *Product B*<br>*No pre-incubation* | *820.0 ± 0.0* | *760.0 ± 21.6* | *92.7* |

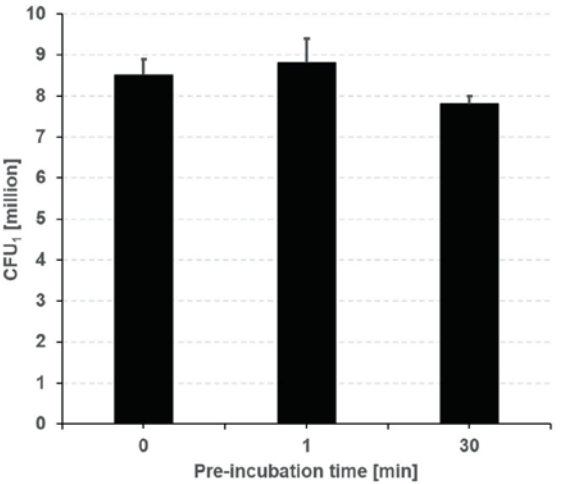


**Figure 1**. Influence of the length of pre-incubation of product A on the survival of its probiotic bacteria after the exposure to a 0.1 N HCl pH-1.2 solution for 90 min at 37°C. The data shown are means ± SD from three independent experiments. Statistical analysis using the independent T-test of the data showed that there is no significant difference between the number of $CFU_1$ without pre-incubation and its number after 1 min (p-value = 0.323) or 30 min (p-value = 0.280) long pre-incubation

## Discussion

The results of the present study showed that the probiotic bacteria of the evaluated product A were inactivated to a large extent (about 99.9%) by the exposure to a low pH solution mimicking the conditions the bacteria would encounter during their passage through the stomach of an adult human being. The two pre-incubation periods before intake recommended by the producer of product A [8] had no significant influence on the excessive loss of viability of product A's probiotic bacteria. One obvious explanation is that the effect of the pre-incubation period in comparison to that of the low pH exposure is so minor that it was difficult to detect experimentally. As the aim of probiotic administration is to deliver living bacteria to the gut, the observed low survival of product A's probiotic bacteria is hardly making product A an ideal member of the product category. While we found

that our study hypothesis ("pre-incubation of a probiotic prior to intake has a negative effect on its survival in low pH-solution") turned out to be false, our study identified a major issue with product A, namely the high vulnerability of its probiotic bacteria that become inactivated when being exposed to low pH.

As shown in the present study for product B, and in previous studies from our research group [7], protecting the bacteria by an effective enteric coating against inactivation by low pH solutions is an appropriate measure in order to avoid the 'loss of life' of probiotic bacteria travelling through the upper part of the human digestive tract.

The present study has certain limitations. One of them is the fact that in the study an artificial in-vitro experimental setting was used that mimics the harsh environment encountered by probiotic bacteria in the human stomach. A further limitation is that our in-vitro results might have little predictive value in regard to the clinical effects that might be achievable with product A. Despite the large number of probiotic bacteria killed at low pH, the few survivors might be enough to form colonies in the host's intestine, which may result in meaningful clinical effects. However, if this could be the case would have to be demonstrated in clinical trials, which we were unable to identify.

Product claims made by producers of probiotics should be not only of relevance for healthcare providers recommending these products (physicians and pharmacists) and users of these products (patient and consumers in general), but they should be also backed by at least some scientific evidence. Unsupported marketing claims can do significant harm to this product category, which has increased its medical relevance in recent years thanks to the growing evidence for its health benefits originating from the increasing number of scientific reports published in peer-reviewed scientific journals.

## Conclusion

The data from the current study revealed that the pre-incubation step for product A recommended by the producer prior to intake is not making a difference, since there is a huge loss of CFU occurring when the product is

exposed to a low pH solution. It is this loss in CFU which should be of major concern, as our current working hypothesis is that taking a probiotic should result in the delivery of the product's probiotic bacteria alive to the intestine. At least for the majority of probiotic bacteria contained in product A, this is not the case.

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