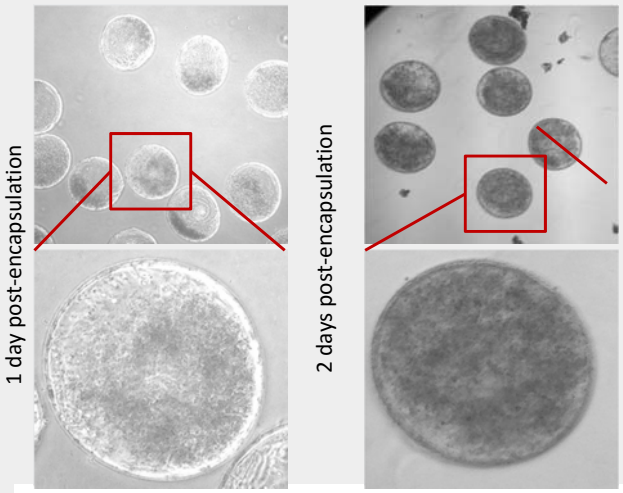
Bac-in-a-Box®: Applications for Probiotics

Probiotics are receiving increasing interest both as potential therapeutics for various human conditions such as inflammatory bowel diseases[1] and colon cancer[2], or as food additives to improve the general health of the gut[3]. In addition there is great interest from the agricultural community in using probiotics as a supplement in animal feed to improve weight gain, intestinal health and performance[4].

Bac-in-a-Box® technology allows:

- Encapsulation of probiotic bacteria and growth within the capsules
- Freeze-drying and recovery of encapsulated bacteria
- Improved product shelf life and ease of transport
- Survival of exposure to simulated gastric conditions
- Biocompatibility in all *in vivo* tests performed to date

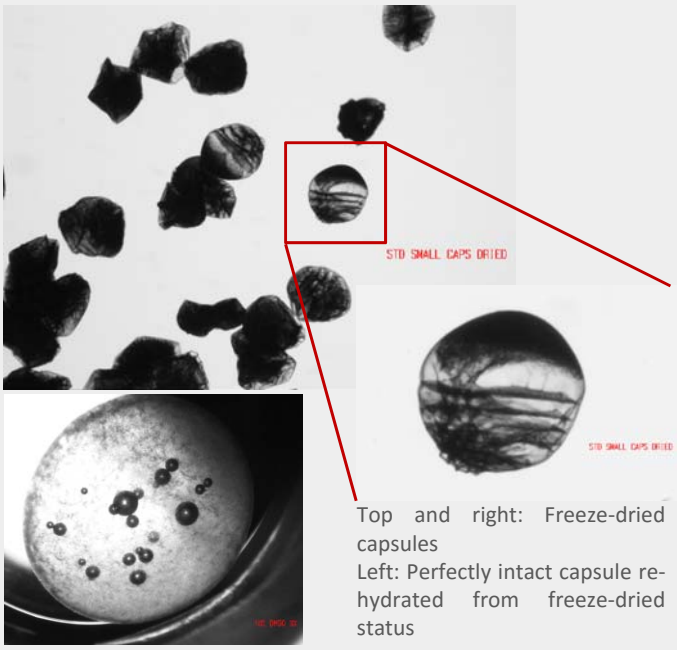
Bac-in-a-Box® Allows Encapsulation of Live Bacteria



- Live probiotic bacteria can be encapsulated and grow in the capsule
- Up to 10 million bacteria in a single capsule
- Protects the bacteria from harsh environmental conditions, e.g. low pH

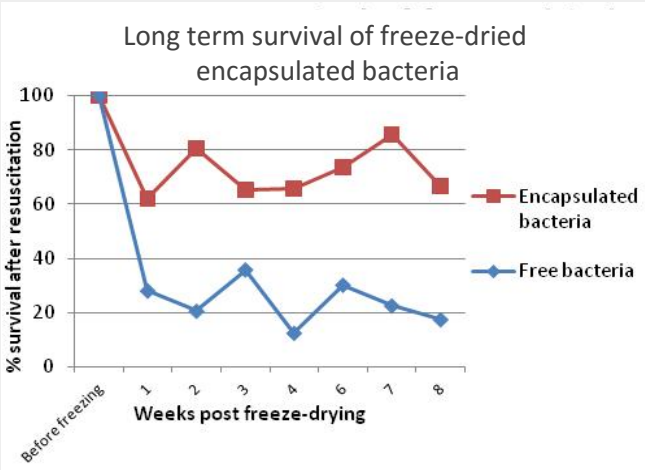
1. Whelan *et al*, 2013, Curr Opin Gastroenterol
2. Behnsen *et al*, 2013, CSH Perspect Med 3.
3. Song *et al*, 2012, InTECH
4. Krehbiel *et al*, 2003, J Anim Sci

Bac-in-a-Box® Supports Freeze-drying of Encapsulated Bacteria



Top and right: Freeze-dried capsules
Left: Perfectly intact capsule re-hydrated from freeze-dried status

- Allows freeze-drying of encapsulated bacteria for ease of storage and transport
- Capsules regain original shape and stability on rehydration, and bacterial growth resumes



- Freeze-dried bacterial capsules remain viable for extended periods of time in ambient storage conditions
- Improves shelf life of product

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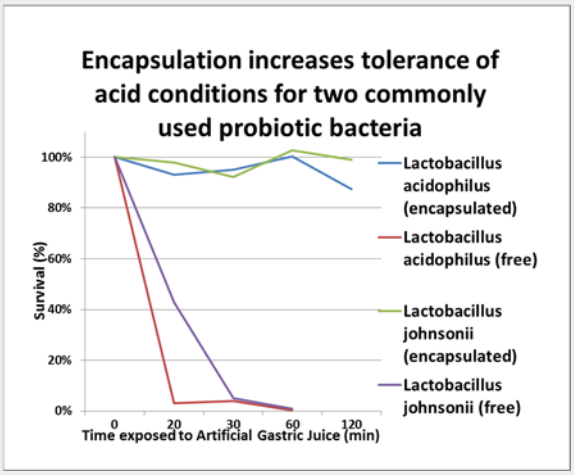
AUSTRIANOVA Encapsulation Technologies **FACTSHEET 7**

Bac-in-a-Box® Allows Encapsulated Bacteria to Survive Acidic Conditions



Encapsulated bacteria survive exposure to Artificial Gastric Juice, unlike free bacteria

- Encapsulation provides protection for probiotics from acidic environments
- Suggests better survival compared to free bacteria during gastric transit
- Increased levels of viable probiotic bacteria reach the gut, enhancing beneficial effects

Bac-in-a-box® Capsules are Digested by Cellulase Activity

There is good evidence for cellulase activity in the human gut. Therefore after surviving gastric transit, capsules may be digested, releasing probiotic bacteria to the optimal site of action.

Capsules exposed to cellulase

Untreated capsules

Bac-in-a-Box® Capsule Material is Biocompatible

There is a large volume of observational and published data supporting the use of the Bac-in-a-Box® encapsulation technology as non-immunogenic, non-toxic and with excellent biocompatibility e.g.:

- intra-arterial administration of capsules into human pancreas
- intra-arterial administration of in pigs
- intrasplenic administration in rats
- intramuscular application in mice
- intratumoral application in dogs
- intraperitoneal application of 300x human dose ground up in rabbits

Summary of Benefits for Bac-in-a-Box® for Probiotic Cells

The use of probiotics products for both human and agricultural applications shows huge potential. However, there are still limitations to their use which Bac-in-a-Box® offers solutions for:

- Biocompatible encapsulation material allows ingestion of encapsulated bacteria
- Freeze-drying of encapsulated bacteria offers improved product stability and shelf-life
- Capsule form provides for ease of packaging and transport
- Provides physical protection from e.g. acidic gastric conditions, improving delivery of viable probiotics to the gut

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