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Original Research

Portrait of *Hafnia alvei* HA4597® – the first precision probiotic changing the game and paradigm in probiotic developments

Abstract

This article aims to summarize the development of *Hafnia alvei* HA4597® as the first precision probiotic – from its discovery and selection through preclinical and clinical evidence to commercialization.

Hafnia is a leading probiotic strain in weight management backed by a precise mechanism of action – its effect is mediated through the production of the protein Caseinolytic peptidase B (ClpB), which is a molecular mimetic of the satiety hormone alpha-MSH. As ClpB activates the receptors of alpha-MSH in the gut, it stimulates the anorexigenic pathway, reducing the feeling of hunger and supporting weight loss. This result has been demonstrated in several mice models of obesity (Legrand *et al.*, 2020 and Lucas *et al.*, 2020) and in a double-blind, randomized, placebo-controlled study with overweight adults following a 20% caloric restriction diet for 12 weeks (Déchelotte *et al*., 2021).

Tennoune *et al.* (2014) showed through sequence alignment the homology of sequence between alpha-MSH and ClpB, and by Western Blot the recognition of ClpB by anti-alpha-MSH immunoglobulins. Subsequently, Breton *et al.* (2015) showed by electrophysiology that ClpB activates the hypothalamic proopiomelanocortin neurons, and Dominique *et al.* (2019 also showed ClpB to stimulate PYY release. Legrand *et al.* (2020) Lucas *et al.* (2020) and Déchelotte *et al.* (2021) then confirmed the physiological effects of ClpB-delivering *Hafnia alvei* on food intake and weight loss in obese mice and overweight men and women.

As such, *Hafnia alvei* HA4597® is referred to as a precision probiotic because of its targeted action and well-characterized mechanism of action at the molecular level.

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The discovery of *Hafnia*

Hafnia alvei (*H. alvei*) was named by Danish biologist Vagn Møller who first described it in 1954 [1]. It gets its name from the Latin name of Copenhagen – Hafnia – where it was first discovered and the epithet alvei is chosen because the bacterium has been isolated from the intestinal tract of honeybees [2].

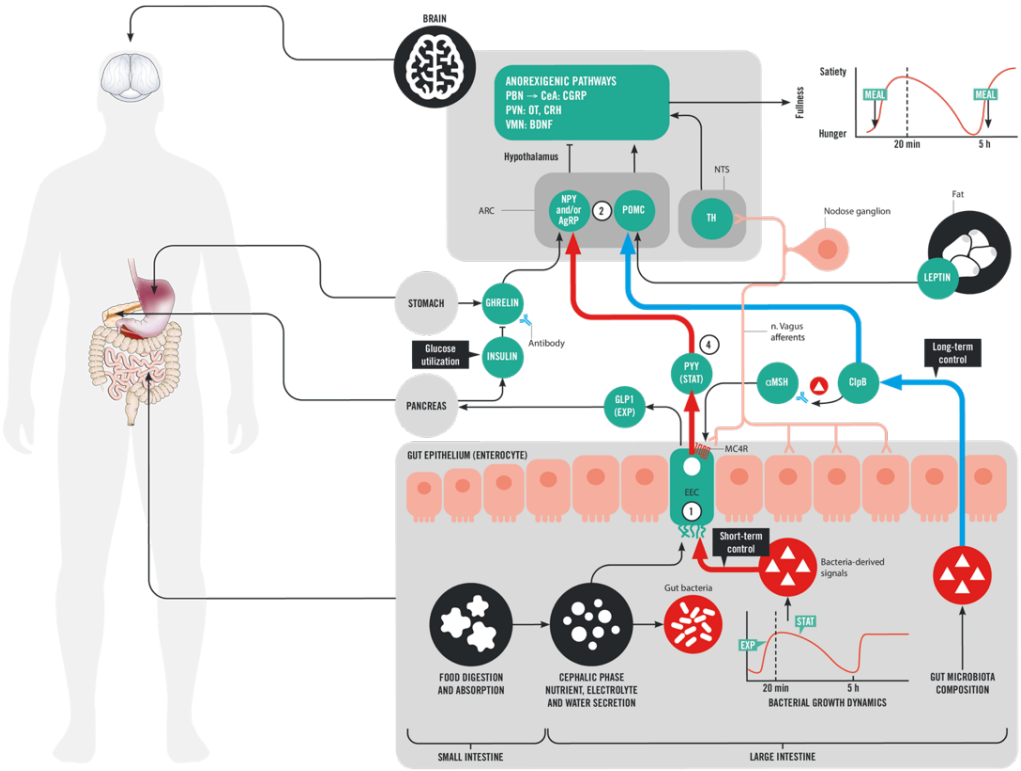
In the 1970s and 1980s, *Hafnia* was identified as the dominant species in camembert cheese [2]. Later, it was found in raw milk, raw sheep milk, vegetables, and other food products, from chorizo to kimchi.

In the 1990s, the French cheese industry started to pasteurize milk for improved safety. *Hafnia* was inoculated back into the milk to maintain the texture and taste properties typical of camembert. It was also added to the production of gouda, cheddar and Livarot cheeses.

The role of Caseinolytic peptidase B in appetite regulation

Prof. Sergueï Fetissov, a neuroendocrinologist and physiology professor, investigated the gut microbiota's roles in host appetite control. As early as 2002, he identified human autoantibodies reacting with the key hormone of satiety alpha-Melanocyte Stimulating Hormone (alpha-MSH). He discovered an anorexigenic peptide produced by the microbiota presenting a homology of sequence with alpha-MSH [3].
In a review published in *Nature Reviews Endocrinology*, he showed that metabolites produced by the microbiota have the ability to regulate food intake. More specifically, pointed to a bacterial protein called Caseinolytic peptidase B (ClpB), a conformational antigen mimetic of alpha-MSH [4, 5] (**Fig. 1**).

Figure 1: Bacteria-host integrative homeostatic model of appetite control. Adapted from Sergueï Fetissov, *Nature Reviews* 2016 [4]



ClpB levels in human faecal or serum samples are negatively associated with Body Mass Index (BMI) [6, 7]. Together with Prof. Pierre Déchelotte, gastroenterologist, professor in human nutrition, and at this time director of the Gut-Brain Axis Laboratory at the French National Institute of Health and Medical Research (Inserm), Prof. Fetissov and his team carried out investigations to demonstrate the cause-and-effect relationship between ClpB and body weight, to establish the proof of their concept on animal models and translate the discovery into a lever of action in the battle against obesity.

In 2014, Tennoune *et al.* showed that ClpB and alpha-MSH amino acid sequences align, and that ClpB is recognized by anti-alpha-MSH Immunoglobulins G [5]. In the following years, Breton *et al.* showed that ClpB produced by E. coli activates hypothalamic proopiomelanocortin neurons (POMC) [8] and Dominique *et al.* demonstrated in primary intestinal mucosa cell cultures that ClpB stimulates PYY release in a dose-response relationship [9].

From mechanism of action to proof of efficacy in animal models

A preclinical study was conducted to validate *H. alvei* and its production of ClpB as a potential probiotic for appetite and body weight management in overweight and obesity. Legrand *et al.* [7] tested *H. alvei* HA4597® on two mice models of obesity: leptin-deficient ob/ob mice and High-Fat Diet-fed (HFD) mice. Their results confirmed that *H. alvei* HA4597® significantly reduces body weight gain and fat mass in both models. In the hyperphagic ob/ob animals, *H. alvei* HA4597® treatment reduced total food intake by 20.8% vs placebo over 18 days ($p<0.001$), the difference becoming significant as early as day 8.

Thus, by triggering anorexigenic and lipolytic effects in hyperphagic mice resulting in decreased body weight gain and fat mass, *H. alvei* HA4597® exhibits the desired probiotic properties of an appetite and body weight management supplement.

A second trial conducted by Lucas *et al.* [10] evaluated the efficacy of *Hafnia* on a combined model of both HFD-fed and genetic ob/ob mice, which may most closely represent hyperphagia and diet-induced obesity in humans. This study also compared the efficacy of the strain to the drug Orlistat, a lipase inhibitor used in humans for the management of obesity.

Mice given a daily dose of 1.4×10^{10} Colony Forming Units (CFU) of *H. alvei* HA4597® showed significantly decreased food intake, body weight gain, and total fat mass while preserving lean mass. In addition, during treatment with *H. alvei*, metabolic parameters were improved, including glycaemia, total cholesterol, and hepatic alanine aminotransferase (ALAT).

Although Orlistat is effective for weight loss, in contrast to *H. alvei* HA4597®, it is accompanied in this study by a hyperphagic effect which may be linked to the increased glycaemia observed for the mice receiving Orlistat [10].

From animal models to supporting weight loss in humans

After *H. alvei* proved successful in the regulation of appetite and weight in mice models, the next step was to evaluate its efficacy in humans. A study by Pierre Déchelotte *et al.* [11] tested the strain in a 12-week multicentric, double-blind, randomized placebo-controlled trial including 236 overweight adults. All subjects were on a

-20% calorie diet and were asked to maintain their usual physical activity. They received either two capsules per day providing 10^{11} cells of *H. alvei* HA4597® (HA) or a placebo (P).

The primary outcome was the percentage of subjects losing 3% or more of their body weight after 12 weeks. Indeed, significantly more subjects (+38%) lost at least 3% of their initial weight during the treatment period when compared to the placebo group (57.7% vs. 41.7%, p=0.028, per protocol results) (**Fig. 2A**). Furthermore, 51% more of the HA group lost in excess of 4% of their body weight compared to the P group (46.2% vs. 30.6%, p=0.024) (**Fig. 2B**).

This enhanced weight loss was accompanied by a significant reduction in hip circumference in the HA group (p<0.001) (**Fig. 2C**) and an improvement in fasting glucose (p<0.05) (**Fig. 2D**). The trial also confirmed an increased feeling of fullness assessed by Visual Analogous Scale (VAS) in the HA group (p=0.009 at 8 weeks vs P) (**Figs. 2E, 2F**) despite the caloric restriction. Importantly, this result confirms the mechanism of action of this precision probiotic on the physiological pathways of satiety and the higher level of satisfaction reported by the HA group compared to the P group (p=0.019).

With clinically relevant efficacy as soon as in the first 12 weeks, excellent tolerability, and no adverse events, the results of the Déchelotte study support the use of *H. alvei* HA4597® in the global management of overweight.

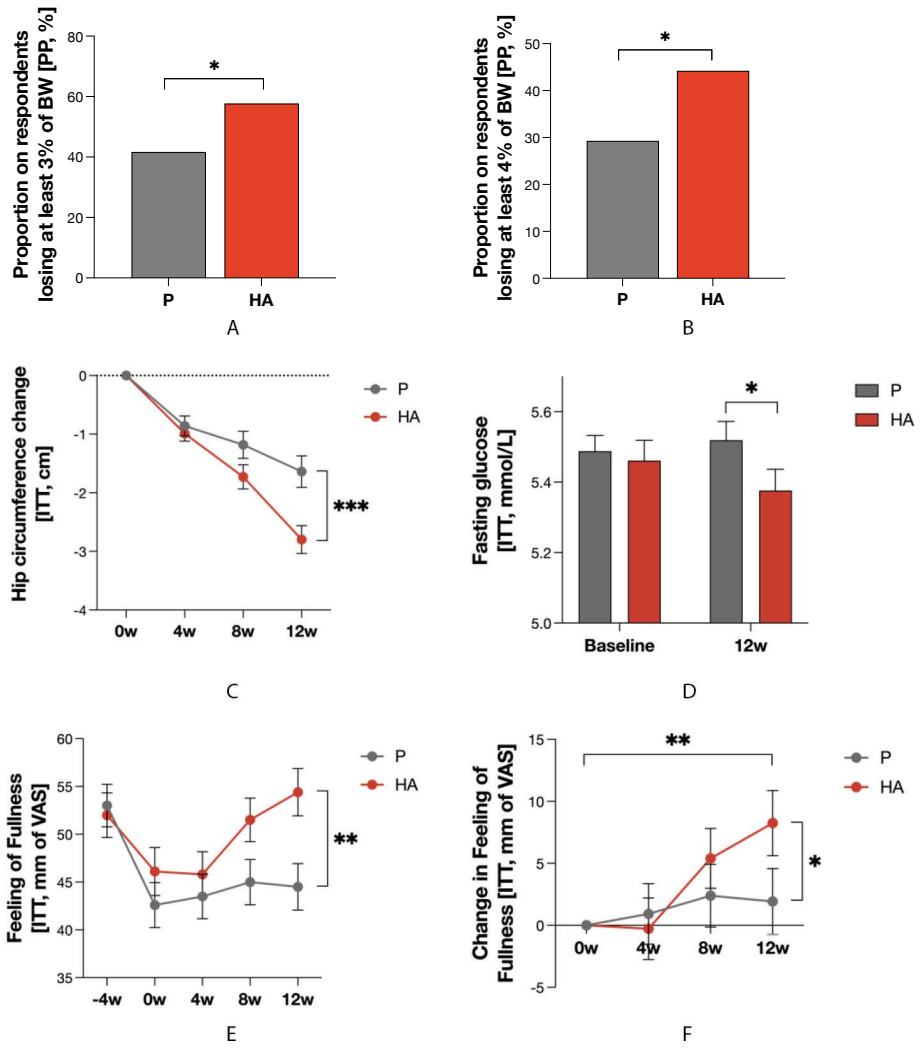


Figure 2: Results of the 12-week clinical study on overweight adults with *H. alvei* HA4597® or placebo, adapted from Déchelotte et al. (2021), except Fig. 2D, previously published in IntechOpen [12].

(A) Proportion of subjects who lost at least 3% of body weight after 12 weeks PP population, Exact Fisher's test P. vs HA. *p≤0.05.
(B) Proportion of subjects who lost at least 4% of body weight after 12 weeks PP population, Exact Fisher's test P. vs HA. *p≤0.05.
(C) Hip circumference change vs T0 ITT population, Mann-Whitney-U test (w12-w0) P. vs (w12-w0) HA. *pU≤0.001.
(D) Serum glucose concentration before and after supplementation ITT population, Mann-Whitney-U test (w12) P. vs. (w12) HA. *pU≤0.05.
(E) Feeling of fullness ITT population, Mann-Whitney-U test (w12) P. vs. (w12) HA. **pU≤0.01.
(F) Change in Feeling of fullness ITT population, Mann-Whitney-U test; (w12-w0) P. vs (w12-w0)HA. *pU≤0.05.
Paired Wilcoxon test; HA(w0) vs HA(w12). **pwi≤0.01.

Commercialisation of *H. alvei*

H. alvei HA4597® was launched for the first time as a probiotic in France in December 2018 and was immediately recognized for its outstanding potential in weight management; it was welcomed as the best ingredient of the year in the weight management category by the internationally recognized NutraIngredients Awards. This success was followed by being elected best probiotic of the year in 2021 [13], just before the clinical study results were published.

It was then marketed in Portugal and Italy in 2021, followed by Croatia, Turkey, Germany and Poland in 2022–2023. It is currently being expanded beyond Europe.

A consumer survey in 2021 confirmed similar levels of efficacy for the food supplement in a real-life setting as in the clinical study. Benefits were observed in a wide scope of BMI and enabled the assessment of long-term use of the product in everyday life. This unpublished data showed that continuing the supplementation beyond the posology of three months allowed participants to keep losing weight in the long-term (an average of 7.16% weight loss after eight months, equivalent to 5.77 kg and 1.98 points of BMI reduction, n=11). Three participants in four had a BMI above 25 kg/m^2, four in five had tried diets before, and 81% reported feeling a positive effect on eating behaviour and/or weight loss (unpublished internal report, TargEDys SA).

Conclusion

The history of the first probiotics was mostly based on trial and error, with strains selected based on the physical constraints of the laboratory – an environment where bacteria were exposed to oxygen, unlike in the physiological conditions of a healthy anaerobic colon.

They were isolated, grown, fermented in reactors, and turned into end products, with or without studies of efficacy. But mostly, it was a mystery how they worked. As recently as 2019, a review on how probiotics exert their benefits [14] stated that 'the mechanism of action of probiotics, which are diverse, heterogeneous, and strain-specific, have received little attention'.

This lack of mechanical studies explains in part the difficulty to convince the medical community of the importance of probiotics as tools for the maintenance of health. The studies using *H. alvei* are inspiring, showing that despite the complexity of working with live cells and the heterogeneity of reactions depending on the recipient's microbiome and lifestyle, it is possible to understand which molecules mediate probiotics' effects, which receptors they activate in the host and for which macroscopic benefits.

Understanding the mechanism of action of a probiotic strain is the only way to optimize product development and the quality control of an active molecule for consistent batch-to-batch efficacy in mass production. Leading brands in the future market of probiotic supplements will naturally turn to precision probiotics for the credibility and inherent satisfaction that when you know how it works, you know that it works.

Conflict of Interests

Nina Vinot and Joséphine Gehring are all employees of TargEDYs, SA; the company commercializing food supplements based on *Hafnia alvei* HA4597®.

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