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HAFNIA ALVEI AS A NEXT-GENERATION PROBIOTIC: MECHANISMS, SAFETY, AND THERAPEUTIC POTENTIAL

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ABSTRACT

Background: Differences in the lifestyle of people that this century brought on us resulted in an increase in obesity and metabolic disorders. This growing prevalence resulted in an intensified interest in next- generation probiotics (NGPs). They are microorganisms that enable a more targeted therapy to various diseases. Hafnia alvei, a commensal bacteria inhabiting the human gut, has gained attention due to it's potential to influence appetite regulation and energy homeostasis.
Aim of the study: This article reviews the information about Hafnia alvei with particular emphasis on it being the next-generation probiotic, as well as the mechanisms, safety and potential therapeutic effect
Scope of review: The goal of this review was to gather knowledge about Hafnia alvei, its effects on human metabolism and the potential of Hafnia alvei becoming a NGP.
Conclusion: Forty eight studies were included in this review. We found that specific strains of Hafnia alvei can produce caseinolytic protease B (ClpB), a protein that mimics α-melanocyte-stimulating hormone (α-MSH) that is a well known regulator of appetite. Through this mimicry, H. alvei may influence food intake and body weight regulation. Experimental and clinical studies suggest that applying H. alvei as a treatment results in a reduced caloric intake, improved metabolic parameters and weight loss. Moreover it has been demonstrated that Hafnia can influence the release of pro- and anti-inflammatory cytokines. Studies suggest that this bacteria can regulate the balance of inflammatory effects, which could be of importance regarding inflammatory diseases, especially obesity where the inflammation can be implicated in it's pathophysiology. Despite promising findings, the available data is very limited and there is a need of conducting larger-scale, well-controlled trials to confirm the efficacy of this treatment.
Further research is crucial to ensure the safety of this therapeutic option and confirm these findings.

KEYWORDS

Hafnia Alvei; Microbiome; Microbiota; Gut; Health

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1. Introduction

1. In recent years the gut microbiota has been assigned a significant role in the health of a gut. A vast population of bacteries have an influence on a wide range of biological processes such as nutrient metabolism, immune system development, and protection against pathogenic microorganisms. Over the past two decades due to the improvement of genomics, molecular biology, bioinformatics and sequencing technology our understanding of it's significance has dramatically improved. Different diseases and dysbiosis can disrupt the composition of gut microbiota. Metabolic diseases are often associated with an inflammatory process started by dysbiosis of the gut microbiome. Obesity, type 2 diabetes as well as other gastrointestinal diseases can stem from the microbial disruptions. These observations have created an interest in modulating the microbiota in order to restore the microbial balance and improve the hosts' life. Among many, the next generation probiotics have emerged as a potential way.

2. Probiotics are generally described as live microorganisms that can positively influence the host's health when applied in an adequate amount [1]. They can be found in certain fermented foods, yoghurts, cheese as well as pills. The first discovery of a probiotic was made in 1905 when microbiologist Stamen Grigorov had encountered Lactobacillus bulgaricus. Since then, many other probiotics have been found. Amongst the most famous probiotics Lactobacillus has made it's mark. It has the ability to ferment food, prevent it's spoilage and improve the nutritional value of meals. Pickled vegetables, kimchi, sauerkraut and sour bread are just a few examples of products that use Lactobacillus in the process. It is important to notice that using probiotics in immunodeficient patients may increase the chance of adverse effects. There have been reports of children with loss of immunity who had severe bacteriemia and even sepsis after consuming probiotics.

Probiotic supplements should be used according to the recommended dose, as its abundance can cause unintended effects [2]. In the last few years the growing interest has caused the creation of a concept of the next-generation probiotics.

3. Next-generation probiotics are microorganisms that have been carefully engineered to offer a targeted approach to various diseases [3]. The difference between probiotics and NGP is that NGP allows a more tailored approach to battling an illness. Advancements in technology have allowed us to identify potential NGP candidates. Amongst them were: Faecalibacterium prausnitzii, Akkermansia muciniphila, Bacteroides fragilis [4] and Hafnia alvei. One of the examples was Lactobacillus that could be engineered to produce a transforming growth factor (TGF- beta) or interleukin 10 (IL-10) that can help with the symptoms of inflammatory bowel disease (IBD) [5]. Akkermansia muciniphila is another example of potential NGP. It could be used as a diabetes treatment, because it can improve glucose metabolism. It is important to note that the use of NGP faces some challenges. The biggest one is the need for more extensive research that could ensure it's safety.

4. Within this concept there has been a growing interest in the potential of Hafnia Alvei being the next-generation probiotic. This gram negative bacteria has been investigated because of it's targeted properties, especially in the appetite and energy balance. Thanks to it's production of ClpB protein that mimics the anorexigenic hormone alfa- MSH, it has a potential to influence the satiety pathways resulting in gut and dietary changes. Despite this ability, there still needs to be more research done in order to establish Hafnia's efficacy and long-term safety.

5. Therefore the aim of this review is to analyze the potential of hafnia alvei as the next generation probiotic using a vast range of research. There has been an emphasis placed on the mechanisms in which the H. alvei can influence the gut balance, safety of the proposed method and the therapeutic potential of using h. Alvei as the next- generation probiotic. By combining the experimental, clinical, microbiological studies, this overview serves a purpose in finding the probiotical opportunities of H. Alvei.

Methods

Our search focused on the potential of Hafnia alvei becoming the next- generation probiotic and it's influence on gut health. Our systematic search was conducted of PubMed and SCOPUS databases on 7 November 2025. Initially, we searched the databases with Hafnia Alvei, microbiome, microbiota, gut, health. Additionally, we manually inspected the reference list to check for any publications of interest. We included only records written in English and related to humans and mice and rats.

The goal of this review was to investigate and analyse the potential benefits of applying Hafnia alvei.

After screening, 320 articles on Pubmed and 286 on scopus were identified. In summary 606 studies have been found, 164 of them were duplicates, most of them were rejected due to the fact that they differed significantly from the main topic of this work. Most of them involved case reports, studies about Hafnia's protein membrane, LPS and quorum censoring without addressing the potential probiotic effect. Additional exclusion involved work about non- representative study populations such as non- mammals. Case reports and studies with a small group of patients were not used due to insufficient clinical significance.

Data Collection Process

Microsoft Excel program was used to carry out the data collection. This process was carried out by one author, while another reviewed it for reliability. The dataset included information about study characteristics, interventions, participant details, and microbiome shift outcomes. All discrepancies were resolved by consensus between the authors.

2. Biological Characteristics of Hafnia alvei

Hafnia Alvei is a facultative anaerobic, gram negative rod that stems from the Hafniaceae family [6]. The genus was first described by Møller in 1954. Hafnia is a flagellate bacterium and thus is motile. It tests positive for catalase, ornithine decarboxylase, lysine decarboxylase but negative for oxidase. Moreover, different strains of Hafnia are known to produce N-acyl homoserines lactones (AHLs) and form biofilm [7]. AHLs are a type of quorum signaling (QS) [8] molecule that enable regulation of behavior depending on the environment [9]. This is an advantageous model as it can increase resistance to environmental factors such as the host immune system or antibiotics. Studies demonstrate that several H. alvei strains share the same core oligosaccharide structure within their lipopolysaccharide (LPS). LPS contained components that are typical for gram negative bacteria: Kdo, heptose residues, phosphate groups, and ethanolamine [10]. In this case LPS is a highly conserved core region, that due to phosphate and phosphoethanolamine substitutions may contribute to the bacteria's immunomodulary properties [11].

It has been discovered that Hafnia is particularly resistant to colistin placing it amongst enterobacterial genera naturally resistant to this antibiotic because of the substructural modifications of LPS, especially within the lipid A moiety [12].

Sazakis' serological classification dictates that hafnia strains have 68 O-antigens and 34 H-antigens [13]. Seven O-subgroups can cross-react with Enterobacter cloaceae, Citrobacter freundii, Escherichia coli and Shigella [13].

Additionally Hafnia possesses similar characteristics as Salmonella, which often times has led to misidentification. False identification can distort data for studies and may result in inappropriate findings.

Hafnia is also known to be an often cause of food spoilage of fish, cream and milk where the population can reach up to 8 × 1010 CFU/g [14]. The reason for that is the production of AHLs and mediating the spoilage factors through QS [7]. Moreover the food decomposition can often happen in vacuum packagings [15]. In the food industry this bacteria is used to ripen certain types of cheeses such as camembert contributing to the flavour development [9, 16]. Other studies suggest that incorporation of Hafnia as a part of a probiotic group in post- harvest wash treatments may reduce Salmonella contamination in eggs. [17]

This bacteria is widely distributed throughout nature and exists in various places. It is commonly found in food, soil, water and mammals where it colonises the gastrointestinal tract. Hafnia can rarely act as an opportunistic pathogen usually in patients that are immunocompromised, with malignancies or infected with HIV virus [18]. Most of the infections are nosocomial [19]. It can cause different symptoms such as diarrhea [19], fever [20], abdominal pain and various diseases: gastroenteritis [8, 21], septicemia [22, 23], meningitidis [24], pneumonia [25, 26], urinary tract infections. Fecal samples from patients have been reported to be bloody or tarry [18]. H. alvei is considered to be a commensal bacterium, which means that it causes no harm to the host. It is being proposed that the bacterium can additionally benefit the place it colonises- the gut microbiome. [27].

A significant aspect of understanding the Hafnia genus is differentiating various strains of this bacterium. As with many other bacteria, the properties of the organism vary depending on the strain. This is a very important aspect when looking for a next-generation probiotic. One of the most broadly studied strains in this case is Hafnia alvei 4597. This commercial strain of hafnia produces a caseinolytic protease B (ClpB). ClpB is a 96kDa heat-shock protein that is involved in cellular stress response and protein disintegration. It has been found that it mimics the α-melanocyte-stimulating hormone (α-MSH) [28] that plays a role in regulation of appetite, satiety, glucose levels and losing weight [29]. There have been conducted few pre-clinical and clinical studies that have shown promising results over this beneficial role of Hafnia, but it still needs further investigation to notice adverse effects and long-term safety. At the same time it is crucial to notice that some of the strains may act as opportunistic pathogens, particularly in patients with underlying diseases. It is imperative to notice this dual nature and be cautious when conducting the studies using different strains.

In another study it was found that the growth of Hafnia lowers the inflammatory response of endothelial cells by regulation of adhesion molecules and decreases in pro-inflammatory cytokines such as IL-6 and IL-8 [30]. Moreover it has been discovered that some of Hafnia strains can induce high levels of Interleukin-10 in dendritic cells [31]. IL-10 is an anti-inflammatory cytokine that has effects on immunoregulation. It lowers Th1 expression and improves B cell survival. This context can be relevant in the context of immune diseases and allergies.

3. Mechanisms of Action

3.1 Modulation of Appetite and Energy Homeostasis

The potential of Hafnia alvei being the next generation probiotic is closely correlated to the mechanism it presents. Unlike the traditional probiotics which mostly have a local impact in the gut, Hafnia may work though more complex mechanisms like other gut microbiota. It may involve microbial signaling, neuroendocrine and immune modulation [32, 33].

One of the ways that it can exert it's effects is through it's commensalism in the gut microbiota. Although Hafnia is the smaller part of the microbiota, it still can influence the balance by competing with other bacteria for nutrients or adherence sites. This can affect the proportional composition of bacteria and their metabolites in the gut.

A distinctive feature of Hafnia is it's ability to produce the ClpB protein, which has been identified as the key to the potential next- generation probiotic effect. ClpB is a heat shock protein that is involved in stress response. Moreover it exhibits a similar structure to alfa-MSH hormone [28]. This mimicry suggests that ClpB could act as alfa-MSH hormone and have a role in appetite suppression, regulating the food intake. The primary site of alfa-MSH regulatory effect is located in the brain, however there are also more peripheral targets such

as the gut, where it can stimulate the PYY secretion by expressing the MC type 4 receptor (MC4R) [34]. Mutation that causes a loss of function of MC4R results in severe obesity with hyperphagia and hyperinsulinemia [35]. MC4R is the second most enriched G protein-coupled receptor (GPCR) in PYY and glucagon-like peptide (GLP-1) expressing L cells which are located in the distal small intestine and colon [34]. Activating the MC4R by alfa-MSH leads to reduced caloric intake and often results in weight loss [34]. PYY acts by slowing the emptying of the stomach and satiety promotion, while GLP-1 promotes insulin secretion and inhibits glucagon release thus suppresses appetite.

There is a proposition that hafnia alvei may influence the gut- brain connection, especially the vagus nerve. It is suggested that the hormone secretion can affect the vagal afferents thus impacting the central appetite control circuits. This could be an additional way that the microbiome can affect the metabolism. Another reason that suggests that Hafnia alvei may influence the gut- brain connection are increased serum IgM and IgA responses to H. alvei's LPS in patients with major depression disorder due to leaky gut [36].

3.2 Immunomodulatory Effects

An additional reason that Hafnia can become a great new generation probiotic is the ability to regulate anti- and proinflammatory cytokines. The gut microbiota plays a crucial role in controlling homeostasis and any disruptions can induce inflammation observed in metabolic disorders [37]. Experimental studies have demonstrated that H. Alvei can induce production of crucial pro-inflammatory cytokines such as TNF-alfa and IL-6 [31]. Additionally, this bacteria has also influenced the production of secretion of an anti- inflammatory cytokine IL-10. Even though studies about Hafnia's immunomodulatory effect are limited, findings from broader microbiome research suggest that commensal bacteria may shift the cytokine balance to the anti-inflammatory state. Modulation of the cytokines may also influence the balance between T helper cells subsets, particularly Th1 and Th2. Th cells are a part of the adaptive immune system that work by releasing cytokines. While Th1 is responsible for cell-mediated response, usually through macrophages and T cytotoxic cells, activation of the Th2 leads to a humoral immune response with the help of B-cells, eosinophil and mast cells. Equilibrium between Th1 and Th2 is crucial to maintain immune homeostasis as disruptions can lead to metabolic and inflammatory diseases. Microbiota can regulate this balance through the impact on the gut-associated lymphotic tissue (GALT) and dendritic cells. Such observations have been reported for other commensal bacteria and it is believed that Hafnia has the same effect.

Another mechanism that Alvei presents is the influence of the intestinal barrier integrity. The intestinal epithelium is a single layer made out of simple columnar epithelium that covers the small and large intestine. It's main function is the absorption of useful substances and secretion of mucins and peptides. The barrier of the intestinal epithelium is made out of 4 types of cell junctions, in which the most important are tight junctions. They provide a selective, narrow paracellular transport that prevents the passage of microorganisms and their toxins [38]. Disruptions of these structures can lead to an increased intestinal permeability otherwise known as a "leaky gut". Leaky gut can contribute to systemic inflammation [39]. Although direct evidence for Hafnia is limited, it has been studied that other commensal and probiotic bacteria can enhance the tight junction stability. In experimental and clinical studies Akkermansia muciniphila has shown to improve the gut barrier and reduce gut permeability [40]. Hafnia may indirectly exert similar abilities thanks to promoting a favorable microbial environment and reducing inflammation in the epithelial structures.

4. Safety Considerations

The safety of Hafnia alvei is a crucial aspect to consider when talking about the possibility of this bacteria becoming a next-generation probiotic. H. Alvei belongs to the order of Enterobacterales which includes several pathogenic species. Despite that, Hafnia is generally considered to have a low pathogenic potential. It can be found in a lot of different niches such as food, water, soil and the gastrointestinal tract suggesting a long history of exposure to the human immunity system. It's frequent detection in familiar places may indicate that Hafnia does not cause widespread adverse effects. The safety of h. Alvei 4597 strain has been investigated in experimental models [experimental hafnia]. The studies reported no acute toxicity, significant adverse effects or pathogenic behavior. Furthermore the preliminary clinical studies also did not find any side effects, suggesting a safe profile [41]. Nevertheless, further studies need to be conducted to ensure the absolute safety of using Hafnia as the next-generation probiotic.

Despite Hafnia being considered a low- pathogenic bacterium, there have been reports of opportunistic infections in hospitalised or immunocompromised patients. These infections include: bacteriemia, respiratory infections and urinary tract infections, however such cases remain a rarity [18]. Often there were cases of

polymicrobial infections where it was difficult to say if Hafnia was the cause of the infections or just a secondary colonizer.

Another key safety concern is the risk of horizontal gene transfer within the gut microbiome and the potential presence of antibiotic resistant genes. Bacteria from the Enteriobacteriaceae family are known to have diverse antibiotic resistance mechanisms such as Beta-lactamase production and multidrug efflux system.These features can raise concerns as to the safety of applying Hafnia alvei as a therapeutic. Comprehensive research is needed to ensure that other members of the gut microbiota won't undergo the horizontal gene transfer which could lead to the spread of antibiotic resistance. Strict regulations and continuous monitoring should be applied to minimize these potential risks.

5. Evidence from Preclinical and Clinical Studies

Preclinical studies can provide an important insight into the possibilities of Hafnia alvei, especially regarding obesity and appetite regulation. Several studies have used a HA4597 strain to conduct their research, amongst them some employed a diet-indused obesity (DIO) model on ob/ob mice to evaluate the physiological effects of H.alvei. This model is used to imitate the modern human high-fat diet (HFD). Other studies included C57BL/6J mice and KK.Cg-a/a and KK.Cg-Ay /a mice that imitate a patient with type 2 diabetes mellitus.

Results of such studies have yielded promising results. Supplementing Hafnia Alvei HA4597 by an intragastric gavage to HFD mice for 38 days has resulted in decreased body mass, reaching a 15.3% difference to the untreated HFD group [42]. Another study reported a 50,1% decrease in body weight gain in ob/ob mice after the course of treatment. Body composition in H.alvei HA4597-treated ob/ob mice changed with 38.3% lower gain of fat and 126.8% decrease in lean mass. The HFD model also resulted in 51.9% decrease in fat mass in mice, while the changes in lean mass were not significant [43]. Studies that used C57BL/6J mice showed no significant changes in weight or body composition after the course of treatment. [44] Administration of Hafnia alvei to KK.Cg-a/a and KK.Cg-Ay /a mice did not significantly change the body weight, adiposity, glucose concentration, or insulin levels compared to the placebo (C57BL/6J) group. [45]

H. alvei has shown similar results to orlistat regarding the improvement of the lean/fat ratio. Interestingly this strain of bacteria has shown potential to reduce the food intake, whereas orlistat did not affect the hyperphagia. The HFD mice group treated with Hafnia resulted in a 26.7g lower cumulative food intake compared to the HFD control group [42]. In hyperphagic ob/ob mice the treatment yielded a 20,8% decrease in food intake, while there was no significant difference in HFD model [43].

Moreover mice treated with h. alvei have shown a significant decrease in the basal glucose level compared to control groups. HFD Hafnia- treated mice had about 1.5-fold of the basal glucose plasma level compared to the HFD controlled and standard diet- groups [42].

Studies have shown that the decrease in glucose was bigger when the Hafnia alvei bacteria was applied, in comparison to H. Alvei protein extract on C57BL/6J mice [44]. However the bacterial protein extract improved glucose tolerance (only in IP-GTT and not IG-GTT). Pretreatment with either Hafnia alvei bacteria or protein extract did not change plasma glucose levels in IG-GTT. These findings suggest that the observed metabolic effects are probably not mediated by alterations in intestinal glucose absorption. The mice receiving the bacterial suspension also showed a visible elevation of the incretin effect. That can suggest that Hafnia can influence gut- derived hormonal signaling pathways. ($F(2, 32) = 4.48$, $p < 0.02$; effect of time $F(6, 192) = 3.22$, $p < 0.005$; probiotic × time $F(12, 192) = 1.02$, $p > 0.42$)).

Interestingly there have not been demonstrated any significant effects on the insulin levels neither when given the hafnia alvei protein extract nor when applied hafnia alvei bacterium. Pretreatment with either one of them did not significantly alter the basal plasma insulin level. (one-way ANOVA: $F(2, 26) = 1.02$, $p > 0.37$; $p> 0.34\sim 0.90$,Turkey post hoc test $p > 0.36$). However there was a tendency of a lower level in mice treated with bacteria (Student's t-test $p = 0.08$ vs control). Probiotic implementation had no significant effect on a response to insulin tolerance test. ($F(2, 27) = 0.32$, $p > 0.72$; effect of time $F(3, 81) = 231.42$, $p < 0.0001$; probiotic × time $F(3, 81) = 1.32$, $p > 0.25$) [44]. These findings suggest that Hafnia alvei metabolic effects are unlikely mediated through classical insulin- dependent pathways. The alternative way may use modulation of appetite regulation, incretin pathways, gut-brain signaling as a more significant role.

Additionally, studies demonstrated that the relative mass of pancreas have decreased by 21% in mice receiving H.alvei protein extract and 13% in mice receiving h. Alvei bacteria (compared to the control group) [44]. The reduction in relative mass of pancreas also occurred in C57BL/6J (B6) control mice while in KK or Ay mice there weren't any changes [45].

No compelling results have been shown on the effects on plasma triglycerides [42], However h. Alvei prevented alanine aminotransferase (ALAT) and total cholesterol increase in the HFD group [44]. Treatment of H. alvei in ob/ob mice resulted in a significant increase in pHSL levels compared to the control group. In the HFD mice model there was a trend of increasing pHSL levels. Administration of H. alvei did not yield substantial improvements in liver morphology or metabolic parameters in KK or Ay mice [45].

Applying Hafnia to ob/ob mice resulted in an increased ClpB DNA content and plasma concentration of ClpB [43]. The HFD obese mice model also resulted in an increase in ClpB DNA content, however the ClpB plasma concentration did not change.

The studies show that there wasn't any change to the expression levels of NPY and POMC mRNA in hypothalamus in ob/ob, HFD and C57BL/6J mice, which suggest that the treatment does not influence the hypothalamic metabolism regulation. [43, 44] However there has been a lower mRNA expression level of orexigenic neuropeptide AgRP in ob/ob mice and HFD obese mice [43]. Agouti-related protein (AgRP) is a neuropeptide that increases appetite and decreases metabolism.

Experimental studies have not shown any adverse effects.

As to Hafnia's probiotic properties, a study about how H. alvei influences survival rates of narrow clawed crayfish (Astacus leptodactylus) has been made. This treatment did not affect growth or survival rates of mentioned crayfish. This bacterium did not show any harmful effects towards the animals, however it showed antagonistic effects towards crayfish's pathogen- Aeromonas hydrophila [46].

Clinical studies have been conducted to further verify the effects of Hafnia alvei 4597 strain (HA). A 12- week placebo-controlled, randomized, double-blind explorative study had been conducted and has shown promising results [41]. The HA group had significantly more patients reach the endpoint than the placebo group. (54.9 vs. 41.4%, p = 0.048). In both Intended to treat (ITT) and per protocol (PP) the amount of patients who lost at least 3% of their weight the was significantly higher in HA group (54.9% and 57.7%) compared to the placebo group 41.4% and 41.7%, for ITT and PP, respectively ((p = 0.048 and 0.028, respectively) [24]. Likewise, there were more patients who lost at least 4% of baseline weight in the HA group both in ITT and PP ((44.2% and 46.2%) compared to the P group (29.3% and 30.6%, for ITT and PP, respectively).

There has been a BMI reduction of about 0.97 kg/m2 in HA groups compared to 0.82 kg/m2 in placebo groups (pU = 0.048). In both the HA and placebo group the weight loss was considered significant (2.89 kg (HA) and 2.49 kg (P)). The difference was more significant in PP (p=0.046) than ITT (p=0.1) Despite that, there wasn't a significant increase in lean mass/fat ratio. Additionally there has been a slightly higher loss in the hip circumference in HA group vs Placebo. Moreover, while there were no significant changes in the ITT group, the PP has noted a trend in a lower waist circumference in HA (−2.95 cm) in comparison to the P group (−2.76 cm.) (pu=0.1)

The change in fullness has drastically improved after the 12 week supplementation, with the HA group having 8.24 mm in Visual Analogue Scale (VAS), while the placebo group had 1.92 mm VAS. The HA group reported a higher reduction in gastrointestinal symptoms than the placebo group (pHA = 0.003 and pP = 0.16, ITT analysis).

In ITT analysis, the Hafnia alvei supplemented group had lower fasting glucose than the p group at 12 weeks ((pU = 0.027)). There has been no findings of any change to haemoglobin, haematocrit, erythrocytes, thrombocytes, leukocytes, ALAT, ASAT AP, gGT, bilirubin, creatinine, urea, uric acid, HbAc1, cholesterol and triglycerides levels [41]. There weren't any significant differences in systolic, diastolic pressure or pulse rates during screening visits. The ITT analysis did not report any differences in quality of life in global IWQOL-LITE score. Tolerability of treatment was assessed as "very good" in 98.2% of cases in both groups.

There have been reported a total of 55 adverse effects in 43 out of 236 patients (25 in HA, 18 in P group). None of them were classified as a serious adverse effect and the causal relationship was assessed as unlikely.

No safety concerns have been reported.

No.	Article	Type of Study	Description of Experiment and Study Groups	Method of Analysis	Conclusion
1	Lucas N 2020 [31]	Preclinical (animal study, mouse model)	Mice with hyperphagic obesity; intervention: Hafnia alvei HA4597 vs control	Glucose tolerance tests, body weight, food intake, lipid profile	H. alvei reduces food intake, body weight gain and improves glucose & lipid metabolism → suggests anorexigenic mechanism (ClpB–α-MSH mimicry)
2	Legrand 2020 [32]	Preclinical (animal study, obese mice)	Obese mice treated with commensal H. alvei strain vs control	Food intake monitoring, fat mass measurement	Reduction in food intake and fat mass → supports role as appetite-regulating probiotic (NGP candidate)
3	Zolotarev VA 2023 [33]	Preclinical (mechanistic, mouse study)	Mice treated with protein extract (ClpB) from H. alvei vs controls	Glucose tolerance tests, metabolic assays	ClpB protein improves glucose tolerance → confirms mechanism via gut-brain axis & insulin sensitivity
4	Déchelotte P 2021 [34]	Clinical trial (RCT, double-blind, placebo-controlled)	Overweight humans on hypocaloric diet; H. alvei HA4597 vs placebo	Body weight, BMI, satiety scores, metabolic parameters	Significant weight loss improvement and better satiety → supports clinical efficacy and safety as NGP

6. Therapeutic Applications

6.1 Obesity and Metabolic Syndrome

Obesity and metabolic diseases that are becoming major global epidemics are defined by adipocity, chronic low-grade inflammation and disrupted energy balance. The genetics, environmental, metabolic and neuroendocrine factors play a major role in the development of these diseases. In recent years the gut microbiota has gained an interest as a potential regulator of appetite control and host metabolism.

Within this context, Hafnia alvei, especially the HA 4597 strain has emerged as a potential treatment for metabolic interventions. Experimental and clinical studies have shown promising results [41, 42, 43, 44]. Pre-clinical studies have shown that implementation of HA 4597 caused a reduced food intake, weight loss and improvement in metabolic parameters. Although more studies are needed, these findings suggest that H. Alvei may present a new approach to treating obesity.

6.2 Type 2 Diabetes

Type 2 diabetes (T2D) is a metabolic disorder that is characterised by resistance to insulin or failure to produce it causing spikes in sugar. Recent studies have suggested that it can be linked to profound dysbiosis [47]. Dysbiosis can be associated with reduced production of beneficial bacterial metabolites such as short-chain fatty acids (SCFA) and elevated levels of endotoxins, which may contribute to insulin resistance and inflammation. Indirectly H. alvei can improve the glucose tolerance. By improving satiety and reducing food intake it can contribute to the loss of weight, which can improve the insulin sensitivity. Although direct studies about the influence Hafnia alvei has on diabetes is limited, findings from other studies can provide indirect support for this potential role. Future clinical studies must be conducted to decide on this treatment's potential.

6.3 Eating Disorders

Eating disorders can be defined as abnormal behaviour around food that can affect a person's mental or physical health. They involve neurobiological, metabolic and psychologic factors. Studies suggest that altered gut microbiota may be observed in individuals with eating disorders [48]. This may imply that microbial factors can influence appetite regulation. H.alvei can affect eating behaviours through the ClpB protein that mimics alfa-MSH and enteroendocrine signaling pathways. Further research is required to determine if H. alvei can be used as an eating disorder treatment.

6.4 Inflammatory Disorder

Inflammatory disorder can be characterised by a persistent activity of the immune system and dysregulation of the inflammatory signaling pathways. Inflammatory responses can be triggered in the gastrointestinal tract by an impaired barrier function. Increased intestinal permeability can allow microorganisms or their toxins to enter the systemic circulation and cause an inflammatory reaction. In this situation Hafnia can turn out to be a helpful treatment. Studies show that H. Alvei can regulate the cytokine levels, produce anti-inflammatory interleukins such as IL-10 and additionally can help stabilize tight junctions. Although this aspect needs further investigation, Hafnia remains a promising treatment option.

7. Limitations and Knowledge

Despite the growing interest in H. alvei becoming the next-generation probiotic, the number of studies is very scarce and several limitations need to be considered when analysing the current studies. Most of the current knowledge about Hafnia comes from a small group of studies, where most of them focus primarily on HA 4597 strain. Moreover there is a prevalence of preclinical studies that may not fully translate to the human model. Although the studies seem comprehensive, the translation of these works to human psychology may not take into account factors such as genetics, lifestyle and environmental factors.

Clinical studies not only are limited in quantity but have generally involved a small sample of people and short intervention periods. Moreover the studies have mainly focused on the weight management potential, leaving other potential probiotic advantages largely unexplored.

Lastly, the mechanism of how Hafnia alvei works still remains incomplete. Despite knowing the role of ClpB protein, other mechanisms that involve microbiome modulation and metabolite production still need further investigation. Addressing these concerns will be crucial to determine whether Hafnia could become the NGP.

8. Future Perspectives

There still need to be conducted further research about the probiotic potential of Hafnia. The studies that are now available have some gaps that need to be addressed. It’s crucial to plan larger and longer-term studies to evaluate safety and efficacy of this treatment. Such studies should be done not only on overweight patients but also on patients with other metabolic disorders such as insulin resistance or type 2 diabetes. In addition to clinical research, there should be a further interest in understanding how H. alvei interacts with gut microbiota. This could identify potential adverse effects or give a better understanding of mechanisms that are or aren’t even discovered yet. Moreover an area that deserves more investigation involves microbiome- based therapies, where Hafnia alvei would be used as a part of microbial consortium rather than a single therapeutic agent. The use of next- generation probiotics is based on the idea of targeted therapies and H. alvei may be considered a valuable component of polibacterial therapy.

9. Conclusions

In recent years there has been a growing interest in the microbiome-based therapies that led to finding potential next-generation probiotics. Amongst them, thanks to experimental and clinical evidence Hafnia alvei has emerged as a strong candidate. One of the most distinctive feature is the production of ClpB protein, which mimics anorexigenic alfa-MSH hormone that signals satiety. Preclinical studies used a strain HA 4597 and demonstrated that its use can reduce food intake and body weight in obese mice [42, 43, 44]. These findings have been partially supported by clinical studies that included overweight patients [41].

Despite the promising results, the amount of studies remains very limited. Most studies have only focused on a single strain and a limited range of metabolic outcomes. Further studies need to be conducted to explore broader therapeutic effects and possible long-term adverse effects. Moreover the mechanisms that happen between the bacterium and the host need more research.

Overall, Hafnia alvei is a promising next-generation probiotic. Continuing interdisciplinary research will prove to be crucial to determine whether hafnia should be used as a treatment.

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REFERENCES

1. Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., . . . Sanders, M. E. (2014). The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology, 11*(8), 506–514.
2. Singhi, S. C., & Kumar, S. (2016). Probiotics in critically ill children. *F1000Research, 5*, F1000 Faculty.
3. Vijayaganapathi, A., & Mohanasrinivasan, V. (2025). A review of next-generation probiotics—as a gateway to biotherapeutics. *Probiotics and Antimicrobial Proteins, 17*(4), 1985–1997.
4. Tiwari, A., Ika Krisnawati, D., Susilowati, E., Mutalik, C., & Kuo, T. R. (2024). Next-generation probiotics and chronic diseases: A review of current research and future directions. *Journal of Agricultural and Food Chemistry, 72*(50), 27679–27700.
5. Ji, J., Jin, W., Liu, S. J., Jiao, Z., & Li, X. (2023). Probiotics, prebiotics, and postbiotics in health and disease. *MedComm, 4*(6), e420.
6. Vallianou, N. G., Kounatidis, D., Tsilingiris, D., Panagopoulos, F., Christodoulatos, G. S., Evangelopoulos, A., . . . Dalamaga, M. (2023). The role of next-generation probiotics in obesity and obesity-associated disorders: Current knowledge and future perspectives. *International Journal of Molecular Sciences, 24*(7), 6755.
7. Souza Viana, E. L., Martino Campos, M. E., Reis Ponce, A., Cuquetto Mantovani, H., & Dantas Vanetti, M. C. (2009). Biofilm formation and acyl homoserine lactone production in *Hafnia alvei* isolated from raw milk. *Biological Research, 42*(4), 427–436.
8. Huys, G., Cnockaert, M., Abbott, S. L., Janda, J. M., & Vandamme, P. (2010). *Hafnia paralvei* sp. nov., formerly known as *Hafnia alvei* hybridization group 2. *International Journal of Systematic and Evolutionary Microbiology, 60*(8), 1725–1728.
9. Ramos-Vivas, J., Tapia, O., Elexpuru-Zabaleta, M., Pifarre, K. T., Armas Diaz, Y., Battino, M., & Giampieri, F. (2022). The molecular weaponry produced by the bacterium *Hafnia alvei* in foods. *Molecules, 27*(17), 5585.
10. Lugowski, C., Niedziela, T., Jachymek, W., Klonowska, A., Czarny, A., Rowiński, S., . . . Kenne, L. (1995). Structural and serological characterization of *Hafnia alvei* lipopolysaccharide core region. *Acta Biochimica Polonica, 42*(1), 51–54.
11. Maes, M., & Leunis, J. C. (2008). Normalization of leaky gut in chronic fatigue syndrome (CFS) is accompanied by a clinical improvement: Effects of age, duration of illness, and the translocation of LPS from gram-negative bacteria. *Neuroendocrinology Letters, 29*(6), 902.
12. Turbett, S. E., Bronson, R. A., Worby, C. J., McGrath, G. E., Hodgkins, E., Becker, M., . . . Pierce, V. M. (2023). Intrinsic resistance to colistin in the genus *Hafnia*. *Journal of Clinical Microbiology, 61*(5), e01326-22.
13. Sakazaki, R. (1984). Serology of *Enterobacter* and *Hafnia*. In *Methods in microbiology* (Vol. 14, pp. 165–186). Academic Press.
14. Kilonzo-Nthenge, A., Rotich, E., & Nahashon, S. N. (2013). Evaluation of drug-resistant Enterobacteriaceae in retail poultry and beef. *Poultry Science, 92*(4), 1098–1107.
15. Padilla, D., Remuzgo-Martínez, S., Acosta, F., & Ramos-Vivas, J. (2012). *Hafnia alvei* and *Hafnia paralvei*: Taxonomy defined but still far from virulence and pathogenicity. *Veterinary Microbiology, 163*(1–2), 200–201.
16. Casado, A., Francés, Á., Fernández, E., Alvarez, M. A., & Ladero, V. (2025). Agmatine-producing *Hafnia* spp. dairy strains: Isolation, technological characterization, and potential in functional food development. *LWT*, 118635.
17. Reddyvari, R., Lu, S., Kosuri, P., & Amalaradjou, M. A. (2025). Incorporation of probiotics in post-harvest wash treatments reduces *Salmonella* contamination and improves egg safety. *Poultry Science, 104*(6), 105146.
18. Janda, J. M., & Abbott, S. L. (2006). The genus *Hafnia*: From soup to nuts. *Clinical Microbiology Reviews, 19*(1), 12–28.
19. Günthard, H., & Pennekamp, A. (1996). Clinical significance of extraintestinal *Hafnia alvei* isolates from 61 patients and review of the literature. *Clinical Infectious Diseases, 22*(6), 1040–1045.
20. Berger, S. A., Edberg, S. C., & Klein, R. S. (1977). *Enterobacter hafniae* infection: Report of two cases and review of the literature. *The American Journal of the Medical Sciences, 273*(1), 101–104.
21. Westblom, T. U., & Milligan, T. W. (1992). Acute bacterial gastroenteritis caused by *Hafnia alvei*. *Clinical Infectious Diseases, 14*(6), 1271–1272.
22. Englund, G. W. (1969). Persistent septicemia due to *Hafnia alvei*: Report of a case.
23. Mobley, D. F. (1971). Hafnia septicemia. *Southern Medical Journal, 64*(4), 505–506. https://doi.org/10.1097/00007611-197104000-00028
24. Mojtabaee, A., & Siadati, A. (1978). *Enterobacter hafnia* meningitis.
25. Washington, J. A., Birk, R. J., & Ritts, R. E., Jr. (1971). Bacteriologic and epidemiologic characteristics of *Enterobacter hafniae* and *Enterobacter liquefaciens*. *Journal of Infectious Diseases, 124*(4), 379–386.

26. Klapholz, A., Lessnau, K. D., Huang, B., Talavera, W., & Boyle, J. F. (1994). *Hafnia alvei*: Respiratory tract isolates in a community hospital over a three-year period and a literature review. *Chest, 105*(4), 1098–1100.
27. Dragacevic, L., Tsibulskaya, D., Kojic, M., Rajic, N., Niksic, A., & Popovic, M. (2025). Identification and characterization of new *Hafnia* strains from common carp (*Cyprinus carpio*), potentially possessing probiotic properties and plastic biodegradation capabilities. *International Journal of Molecular Sciences, 26*(3), 1119.
28. Tennoune, N., Chan, P., Breton, J., Legrand, R., Chabane, Y. N., Akkermann, K., . . . Fetissov, S. O. (2014). Bacterial ClpB heat-shock protein, an antigen-mimetic of the anorexigenic peptide α-MSH, at the origin of eating disorders. *Translational Psychiatry, 4*(10), e458.
29. Anderson, E. J., Çakir, I., Carrington, S. J., Cone, R. D., Ghamari-Langroudi, M., Gillyard, T., . . . Litt, M. J. (2016). 60 years of POMC: Regulation of feeding and energy homeostasis by α-MSH. *Journal of Molecular Endocrinology, 56*(4), T157–T174.
30. Kuntz, S., Kunz, C., Domann, E., Würdemann, N., Unger, F., Römpp, A., & Rudloff, S. (2016). Inhibition of low-grade inflammation by anthocyanins after microbial fermentation in vitro. *Nutrients, 8*(7), 411.
31. Wittmann, A., Lamprinaki, D., Bowles, K. M., Katzenellenbogen, E., Knirel, Y. A., Whitfield, C., . . . Kawasaki, N. (2016). Dectin-2 recognizes mannosylated O-antigens of human opportunistic pathogens and augments lipopolysaccharide activation of myeloid cells. *Journal of Biological Chemistry, 291*(34), 17629–17638.
32. Li, X., Zhang, S., Guo, G., Han, J., & Yu, J. (2022). Gut microbiome in modulating immune checkpoint inhibitors. *EBioMedicine, 82*.
33. Chen, Y., & Tang, D. (2026). From neuro-immune command circuits to microbiota-mediated regulation in the gastrointestinal tumor microenvironment. *Frontiers in Immunology, 17*, 1739357.
34. Panaro, B. L., Tough, I. R., Engelstoft, M. S., Matthews, R. T., Digby, G. J., Møller, C. L., . . . Cone, R. D. (2014). The melanocortin-4 receptor is expressed in enteroendocrine L cells and regulates the release of peptide YY and glucagon-like peptide 1 in vivo. *Cell Metabolism, 20*(6), 1018–1029.
35. Huszar, D., Lynch, C. A., Fairchild-Huntress, V., Dunmore, J. H., Fang, Q., Berkemeier, L. R., . . . Lee, F. (1997). Targeted disruption of the melanocortin-4 receptor results in obesity in mice. *Cell, 88*(1), 131–141.
36. Mass, M., Kubera, M., & Leunis, J. C. (2008). The gut-brain barrier in major depression: Intestinal mucosal dysfunction with an increased translocation of LPS from gram-negative enterobacteria (leaky gut) plays a role in the inflammatory pathophysiology of depression. *Neuroendocrinology Letters, 29*(1), 117–124.
37. Cani, P. D., Bibiloni, R., Knauf, C., Waget, A., Neyrinck, A. M., Delzenne, N. M., & Burcelin, R. (2008). Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet–induced obesity and diabetes in mice. *Diabetes, 57*(6), 1470–1481.
38. Groschwitz, K. R., & Hogan, S. P. (2009). Intestinal barrier function: Molecular regulation and disease pathogenesis. *Journal of Allergy and Clinical Immunology, 124*(1), 3–20.
39. Maes, M., Kubera, M., Leunis, J. C., & Berk, M. (2012). Increased IgA and IgM responses against gut commensals in chronic depression: Further evidence for increased bacterial translocation or leaky gut. *Journal of Affective Disorders, 141*(1), 55–62.
40. Everard, A., Belzer, C., Geurts, L., Ouwerkerk, J. P., Druart, C., Bindels, L. B., . . . Cani, P. D. (2013). Cross-talk between *Akkermansia muciniphila* and intestinal epithelium controls diet-induced obesity. *Proceedings of the National Academy of Sciences, 110*(22), 9066–9071.
41. Déchelotte, P., Breton, J., Trotin-Picolo, C., Grube, B., Erlenbeck, C., Bothe, G., . . . Lambert, G. (2021). The probiotic strain *H. alvei* HA4597® improves weight loss in overweight subjects under moderate hypocaloric diet: A proof-of-concept, multicenter, randomized, double-blind, placebo-controlled study. *Nutrients, 13*(6), 1902.
42. Lucas, N., Legrand, R., Deroissart, C., Dominique, M., Azhar, S., Le Solliec, M. A., . . . Lambert, G. (2019). *Hafnia alvei* HA4597 strain reduces food intake and body weight gain and improves body composition, glucose, and lipid metabolism in a mouse model of hyperphagic obesity. *Microorganisms, 8*(1), 35.
43. Legrand, R., Lucas, N., Dominique, M., Azhar, S., Deroissart, C., Le Solliec, M. A., . . . Fetissov, S. O. (2020). Commensal *Hafnia alvei* strain reduces food intake and fat mass in obese mice—A new potential probiotic for appetite and body weight management. *International Journal of Obesity, 44*(5), 1041–1051.
44. Zolotarev, V. A., Murovets, V. O., Sepp, A. L., Sozontov, E. A., Lukina, E. A., Khropycheva, R. P., . . . Fetissov, S. O. (2023). Protein extract of a probiotic strain of *Hafnia alvei* and bacterial ClpB protein improve glucose tolerance in mice. *International Journal of Molecular Sciences, 24*(13), 10590.
45. Zolotarev, V. A., Murovets, V. O., Novikova, N. S., Ermolenko, E. I., Sepp, A. L., & Khropycheva, R. P. (2024). Effect of *Hafnia alvei* on morphophysiologic parameters and gut microbiota of mice with inherited type 2 diabetes mellitus. *Bulletin of Experimental Biology and Medicine, 177*(3), 313–317.
46. Didinen, B. I., Bahadir Koca, S., Metin, S. E. Ç. İ. L., Diler, O., Erol, K. G., Dulluc, A., . . . Kucukkara, R. (2016). Effect of lactic acid bacteria and the potential probiotic *Hafnia alvei* on growth and survival rates of narrow-clawed crayfish (*Astacus leptodactylus* Esch., 1823) stage II juveniles. *Iranian Journal of Fisheries Sciences, 15*(4), 1307–1317.
47. Tilg, H., & Moschen, A. R. (2014). Microbiota and diabetes: An evolving relationship. *Gut, 63*(9), 1513–1521.
48. Cryan, J. F., & Dinan, T. G. (2012). Mind-altering microorganisms: The impact of the gut microbiota on brain and behaviour. *Nature Reviews Neuroscience, 13*(10), 701–712.