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*Published in:*  
Food Microbiology

*Link to article, DOI:*  
[10.1016/j.fm.2023.104372](https://doi.org/10.1016/j.fm.2023.104372)

*Publication date:*  
2024

*Document Version*  
Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

*Citation (APA):*  
Kothe, C. I., Rasmussen, J. A., Mak, S. S. T., Gilbert, M. T. P., & Evans, J. (2024). Exploring the microbial diversity of novel misos with metagenomics. *Food Microbiology*, 117, Article 104372. <https://doi.org/10.1016/j.fm.2023.104372>

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## Exploring the microbial diversity of novel misos with metagenomics

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### ARTICLE INFO

#### Keywords:

Miso  
Plant-based  
Fermentation  
Metagenomics  
MAG

### ABSTRACT

Interest in fermented foods, especially plant-based ones, has increased considerably in the last decade. Miso—a Japanese paste traditionally fermented with soybeans, salt, and koji (*Aspergillus oryzae* grown on grains or beans)—has gained attention among chefs for its rich flavour and versatility. Some chefs have even been experimenting with making novel misos with untraditional substrates to create new flavours. Such novel fermented foods also offer new scientific opportunities. To explore the microbial diversity of these new traditional foods, we sampled six misos made by the team at a leading restaurant called Noma in Copenhagen (Denmark), using yellow peas (including a nixtamalised treatment), lupin seeds, Swedish Vreta peas, grey peas, and Gotland lentils as substrates. All misos were made with the same recipe and fermented for 3 months at 28 °C. Samples were collected at the end of fermentation for subsequent shotgun metagenomic sequencing and a genome-resolved metagenomic analysis. The taxonomic profile of the samples revealed the presence of koji mould (*A. oryzae*) and *Bacillus amyloliquefaciens* in all misos. Various species of the genera *Lactilactobacillus*, *Lactiplantibacillus*, *Pediococcus* and *Staphylococcus* were also detected. The Metagenome-Assembled Genomes (MAGs) revealed genomic sequences belonging to 12 different species and functional analyses of these MAGs were performed. Notably, we detected the presence of *Exiguobacterium*—the first reported instance of the genus in miso—and Average Nucleotide Identity (ANI) analyses suggest a potentially new species. We hope these results will improve the scientific literature on misos and contribute to developing novel fermented plant-based foods.

### 1. Introduction

Our planet is currently home to around 8 billion people, with UN projections suggesting that, by 2050, this number will reach 10 billion (United Nations, 2022). The consequential challenge now and in the future is to provide quality food while simultaneously safeguarding and even enriching the environment. One approach to tackling this challenge involves plant-based protein-rich food products, which can help meet the demand for protein-rich foods while avoiding the exponentially higher environmental cost of animal products and, in the case of legumes, increasing soil fertility by fixing nitrogen (Willett et al., 2019).

Fermentation, as a process that breaks down food substrates and makes them more flavourful and nutritionally bioavailable, can aid the development of satisfying and nourishing plant-based foods (Graham and Ledesma-Amaro, 2023; Jahn et al., 2023). It is an ancient and ubiquitous process that has been used as a preservation technique in

cuisines around the world since the Neolithic era (as early as 10,000 B.C. E.), producing a variety of foods and beverages such as breads, yogurts, cheeses, vinegars, beers, and wines (Craig, 2021; McGovern et al., 2004). In addition to its food preservation capabilities and nutrient availability enhancement, fermentation also imparts unique sensory characteristics to foods. These qualities have contributed to a resurgence in popularity of fermented foods, potentially also attributed to its recognized advantages for gut, mental, and overall health (Pasolli et al., 2020; Wastyk et al., 2021).

Fermented foods feature prominently in many Asian cuisines. Their rich and savoury flavours may be why certain traditional fermentation techniques—especially from Japan, China, and Korea—have excited many fermenters and consumers worldwide (Redzepe et al., 2018). These cuisines often use plant-based ingredients such as rice, wheat, barley, millet, soybeans, and vegetables in their fermented products (Steinkraus, 1997). One example is miso—a Japanese fermented paste

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<https://doi.org/10.1016/j.fm.2023.104372>

Received 17 March 2023; Received in revised form 17 August 2023; Accepted 25 August 2023

Available online 26 August 2023

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traditionally made with soybeans, salt, and *kōji* (*Aspergillus oryzae* grown on rice, barley, wheat, or soybeans), used as a flavour enhancer in soups, sauces, marinades, and even sweets (Shurtleff and Aoyagi, 2018). Beyond the traditional soy-based miso, chefs, cooks, and fermenters are exploring alternative versions made with other legumes, nuts and seeds (Katz, 2021; Redzepi et al., 2018).

Alongside these products' growing culinary popularity, scientific studies of these products are also emerging. Metagenomic studies investigating *kōji*, a key ingredient in miso production, have revealed that the dominant genera in this starter product may include *Aspergillus*, *Weissella*, *Kurthia*, and/or *Staphylococcus* (Tan et al., 2022; Wang et al., 2017). However, the number of publications describing the microbial communities in miso is limited, and those available have relied on culture-dependent and PCR-DGGE techniques (Kim et al., 2010; Onda et al., 2003a, 2003b). The existing studies on miso mainly focus on production process, health implications, chemistry, flavour and food safety (Allwood et al., 2021, 2023; Kusumoto et al., 2021; Saeed et al., 2022; Wang et al., 2021). While there is a lack of culture-independent studies of miso, recent reports have emerged of other Asian fermented soybean products, predominantly from China and Korea (Tamang et al., 2022; Xie et al., 2019, 2020). These studies have identified a wide diversity of species, including *Bacillus*, *Enterococcus* and *Staphylococcus* genera, and various lactic acid bacteria. This diversity suggests that the composition of these fermented products can vary significantly based on the quality of raw materials, production location, processing techniques employed and handlers.

The objective of this research is to begin to address the lack of culture-independent studies of microbial communities in miso, both traditional and novel, focusing on how the fermentation process and culinary experimentation could shape its microbial composition. By investigating these questions, we aim to contribute to the broader field of microbial ecology and evolution, providing insights into the assembly and dynamics of microbial communities when introduced to new environments. Our approach involves sampling misos made with different proteinous substrates and performing shotgun metagenomic analysis to reveal the taxonomy and potential functions of the species present. These data can provide valuable information for producers and consumers and contribute to developing and producing novel fermented plant-based foods. They might also interest scientists working on microbial ecology in the fermented food environment.

## 2. Material and methods

### 2.1. Preparation of the misos

#### 2.1.1. *Kōji*

Pearl barley (Lantmännen Cerelia A/S, Vejle, Denmark) was soaked in water overnight (2 parts water: 1 part dry grain) and steamed at 100 °C for 40 min in a Combi oven (Rational iCombi Pro, Germany). The grains were then cooled to room temperature until they reached 37 °C, and inoculated with 0.2% pure commercial albino white rice *kōji* spores (from Bio'c, Japan). The inoculated grains were mixed with gloved hands, transferred to damp wrung linen cloths in perforated stainless steel trays, and incubated at 32 °C and 70% relative humidity for 18h. Afterwards, the grains were mixed to redistribute the growing mycelia and then were returned to the trays, covered with freshly wrung linen cloths, and placed back in the incubator at 28 °C for 24h, allowing the *kōji* to form.

#### 2.1.2. Selected substrates

To develop the novel misos, the team at the Noma Fermentation Lab selected regional Nordic proteinous substrates, including yellow peas (Unifood A/S, Denmark), lupin seeds, Swedish Vreta peas, Swedish grey peas, and Gotland lentils (Nordisk Råvara AB, Sweden). The legumes were soaked in water overnight (2 parts water : 1 part dried legume), then drained and boiled in fresh water. Additionally, they developed one

miso using the nixtamalisation process. For this miso, they chose yellow peas as the substrate, which were cooked in a 0.1% aqueous calcium hydroxide (CaOH) solution. Once cooked, the peas were left soaking in the solution at room temperature for 12h. Afterwards, the peas were drained, rinsed under cold water, and drained again before being ground (Redzepi et al., 2018).

#### 2.1.3. Miso fermentation and sampling

Using Noma's standard recipe, the Fermentation Lab team developed six misos with the above substrates, each around 3 kg (Redzepi et al., 2018). Ground pearl barley *kōji* and ground protein substrates were combined in a 2:3 part ratio, 4% salt of the combined weight was added, the ingredients were mixed thoroughly with gloved hands, and the mixture was packed tightly into sterilized 5-L glass jars (Utopia Biscotti jars, d = 19 cm, h = 24 cm). The packed misos were covered on the surface with cling film, weighed with ceramic weights, and fermented for 3 months at 28 °C.

At the end of the fermentation, the top surface was removed and the remaining miso was sampled. Samples were put into 150 ml plastic containers (Condi ApS) and placed into the freezer at -20 °C.

### 2.2. DNA extraction and shotgun sequencing

The DNA of the six misos (0.25g) was extracted using the Qiagen DNeasy PowerSoil Kit according to the manufacturer's instructions and quantified using a Qubit 1.0 fluorometer with a dsDNA High Sensitivity Assay kit (ThermoFisher Scientific). The samples were constructed into libraries using the Blunt End Multi Tubes (BEMT) protocol (Sirén et al., 2019), and sent to BGI Group (Hong Kong, China) for circularization and sequencing using the BGISEQ-500 platform with PE150 chemistry. Quality control and pre-processing of the fastq files were performed with fastp 0.20.1 (Chen et al., 2018, Table S1, 'metadata' sheet).

### 2.3. Bioinformatic analyses

#### 2.3.1. Taxonomic composition by metagenomics

From the generated fastq files, we first estimated microbial composition by mapping the sample reads against the representative catalogue contained in the MetaPhlAn tool, v.3.0.4 (Truong et al., 2015), using default parameters, -read\_min\_len 70, and -add\_viruses. Additionally, we performed taxonomic profiling using an assembly-based marker gene analysis, which allows the non-supervised binning of metagenomes. To do this, *de novo* assembly was performed using megahit 1.2.9 with -min-contig-len 500 -k-list 21,33,55,77 options (Li et al., 2015). Genes were then predicted using prodigal (v.2.6.3) using default settings and meta procedure, and marker genes were extracted using fetchMG, v.1.2 (Ciccarelli et al., 2006; Sunagawa et al., 2013). Then, to perform the taxonomic assignment, we used the *leuS* marker gene (COG0495), whose closest homologue was assigned by a blastp search on all the available sequences from the NCBI protein database. Summary species composition plots were created in R (v.3.6.1) using the ggplot2 package, v.3.3.2.

#### 2.3.2. Enterotoxin gene analyses

Since we found some *Staphylococcus* species in the samples, we found it relevant to investigate whether they possessed genes encoding enterotoxins. Using BLAST, we looked for the presence of pyrogenic superantigenic toxins (PTSAgs) (*sea-see*, *seg-sevu*, *selv*, *selx*, *sey*, *selz*, *sel26*, *sel27* and *TSST1*) and exfoliative toxins (*eta*, *etb*, *etd*) in the assembled metagenomes.

#### 2.3.3. Metagenome-assembled genomes (MAGs)

Genome binning and refinement were performed using metawrap 1.3.2. Bins were grouped into metagenomic bins using the metaWRAP binning module (-maxbin2-concoct-metabat2 options), and the resulting bins were refined with the bin\_refinement module (-c 70 -x 10 options). The quality of the resulting prokaryotic bins was assessed with

CheckM (Parks et al., 2015). Annotations of each MAG were performed using Rapid Annotation using Subsystem Technology, RAST, server (Aziz et al., 2008). Then, we concatenated the functions and heatmap was generated using the R package ComplexHeatmap.

The genome-type strains closely related to each MAG were downloaded from the RefSeq NCBI database, and Average Nucleotide Identity (ANI) was calculated using JSpecies (Richter et al., 2016). The whole genome sequences of all *Exiguobacterium* species were collected from the RefSeq NCBI database, as well as the outgroup *Bacillus amyloliquefaciens* DSM7. The sequences of these genomes were aligned with the *Exiguobacterium* MAG obtained in this study using the BPGA pipeline (Bacterial Pan Genome Analysis tool) to calculate a core phylogenomic tree (Chaudhari et al., 2016).

#### 2.4. Data availability

The raw sequences of shotgun metagenomic reads were deposited on the European Nucleotide Archive (ENA) under the BioProject ID PRJNA943417. The MAGs are available in [Mendeley Data](#).

### 3. Results & discussion

The description of the misos and their respective substrates, as well as the results of the quality control of the sequences, are presented in [Table S1](#) ('metadata' sheet). The samples presented a high read depth, with more than 25 million 150-nucleotide paired-end reads per sequencing run. Here we present our main findings: 1) the similarities and differences among the microbiota of the novel misos; 2) the safety of the Staphylococci in the misos, and a general discussion of the safety of Staphylococci in fermented foods; 3) the functional features of the MAGs recovered; and 4) the first report of an *Exiguobacterium* in miso, and a potentially new species.

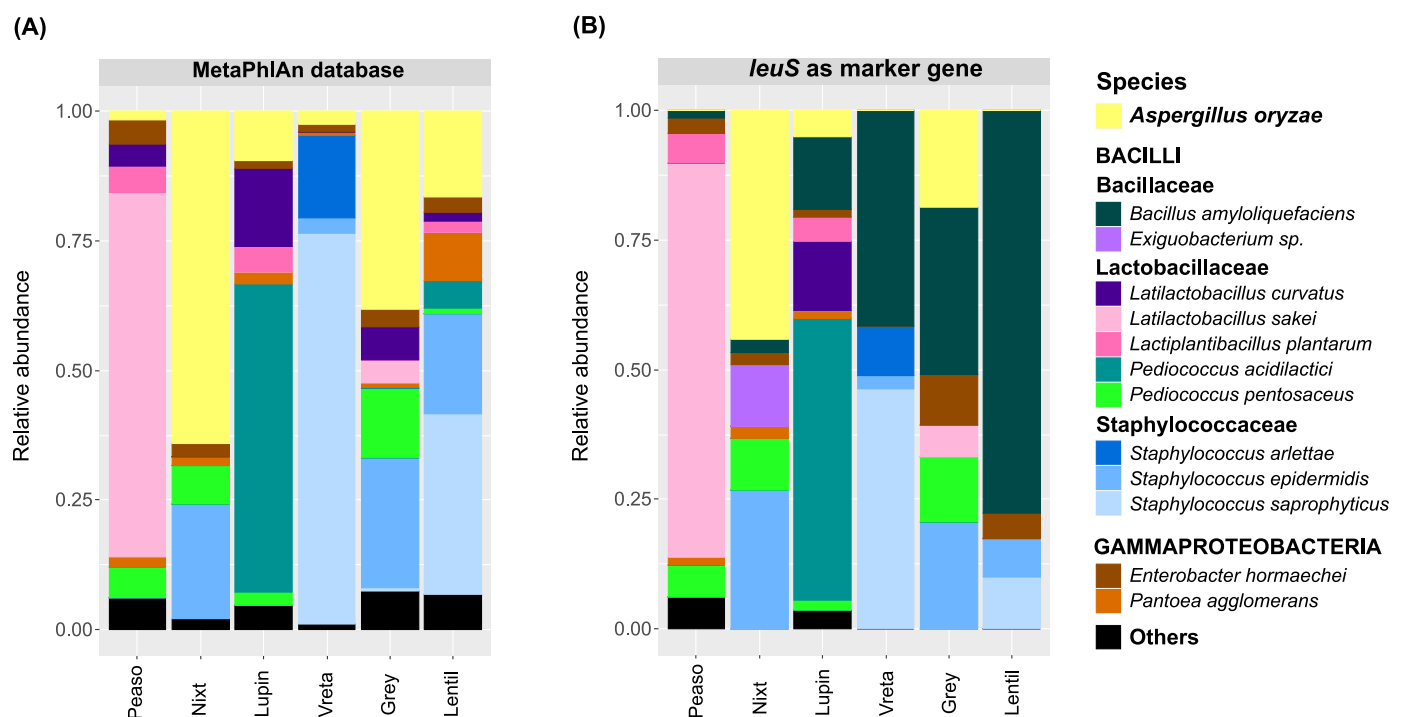
#### 3.1. Microbiota of the novel misos

The shotgun metagenomic sequence analysis conducted on the miso

samples revealed the presence of bacteria and eukaryotes; no archaea or viruses were detected. This absence in the data does not necessarily mean there were no bacteriophages in our samples. Virus extraction is delicate and may need a specific protocol (Dugat-Bony et al., 2020); and according to Shaffer et al. (2022), the extraction kit used in this study (PowerSoil) does not extract RNA. It is also important to highlight that this study only had access to samples collected from the final product at the end of the 3-month fermentation period. Questions of how the microbial communities changed over the course of the fermentation are thus not possible to address here. Additionally, it should be acknowledged that the absence of replicate biological fermentations limited our ability to capture possible variability across misos made using the same substrate. Despite these limitations, this exploratory study suggests some similarities and differences in microbial communities among the finished misos.

##### 3.1.1. Similarities among the misos

As expected from miso, using the MetaPhlAn tool, we detected *Aspergillus oryzae* in all samples. However, in the yellow peas (or 'Peaso', as the Noma team call their original yellow pea miso) and Vreta samples, the koji mould had a minority presence (<2.5%). Characterising the novel miso microbiota independently of fixed references by applying a marker gene analysis from the assembled metagenomes, we detected similar results with the previous analysis using read profiling ([Table S1](#)). However, some differences merit being highlighted, such as the presence of *Bacillus amyloliquefaciens* in all samples, a species likely absent from the MetaPhlAn database ([Fig. 1B](#)). This species is especially dominant in the misos made with Vreta peas, grey peas, and Gotland lentils. Using culture-dependent approaches, [Onda et al. \(2003a\)](#) also identified this species in soy misos. *B. amyloliquefaciens* has been widely used as a starter culture in the fermentation of different cereals, vegetables, beverages, meat and dairy products, and even in the production of koji ([Woldemariam et al., 2020](#)). It is a beneficial bacterium in fermentation because some strains have high proteolytic activity, producing complex aromas and improving the sensory quality of foods ([Zhang et al., 2019, 2020](#)). *B. amyloliquefaciens* strains have also shown the potential to



**Fig. 1.** The relative abundance of the microbial composition of the six novel misos. Values were calculated from the MetaPhlAn database (A) and the coverage of the *leuS* marker gene assembled in the metagenomes (B).

synthesise bioactive peptides (Yang et al., 2019) and antimicrobial compounds (Sim and Kim, 2016), as well as having been used as prebiotics and probiotics (Jers et al., 2017).

### 3.1.2. Differences between the misos

Overall, we observed that each substrate may shape the microbial composition of the miso ecology differently. For example, in the miso made with yellow peas (Peaso), we identified a dominance of *Lactobacillus sakei*. In contrast, in the lupin seed miso, the most abundant species was *Pediococcus acidilactici* (Fig. 1A). Both species are lactic acid bacteria commonly used as starter cultures, mainly in fermented meat products (Laranjo et al., 2019). While *L. sakei* has never been reported in miso, *P. acidilactici* has been isolated from miso and some strains have been tested for their antimutagenic and anticarcinogenic potential (Asahara et al., 1992; Onda et al., 2003b).

The nixtamalised yellow pea (Nixt) and the grey pea misos displayed some similarities concerning the dominance of *A. oryzae*, *Staphylococcus epidermidis* and *P. pentosaceus* species. *P. pentosaceus* has been detected in previous microbiological studies in miso (Kim et al., 2010; Onda et al., 2003). This species is found in many fermented foods and could improve flavour and nutrition, and it may have active roles in food quality and safety due to its antimicrobial capabilities (Hu et al., 2021; Jiang et al., 2021). Meanwhile, the Vreta pea and Gotland lentil misos are distinguished by the presence of *Staphylococcus saprophyticus* (Fig. 1A). This variability may be also due to the small sampling (0.25g), rather than the different pulses used. Further experiments should be performed to confirm this.

### 3.2. The safety of *Staphylococci*, in these misos and in fermented foods in general

Although the dominant *Staphylococcus* species in miso are coagulase-negative and part of the normal human flora, they can carry enterotoxin genes (Becker et al., 2014). A search for pyrogenic toxin superantigens and exfoliating toxins in the metagenomes showed no homology with these genes, suggesting they are safe for consumption. While none of these genes were detected in the final samples, in principle there is a small possibility that such genes may have been present in *Staphylococcus* species earlier in the fermentation. Toxigenic gene-carrying *Staphylococcus* spp. could have produced toxins then died, their cells losing integrity and their DNA degraded. In this case, their DNA would not show up in the final analysis, but the toxins could remain. To clarify this possibility, future studies could sample the fermentation at multiple timepoints and/or also analyse the final product for toxins directly.

The likely safety of these food-related *Staphylococcus* strains is supported by other studies of *Staphylococcus* in fermented foods. Coton et al. (2010) investigated *S. epidermidis* strains from cheese, dry fermented sausages, and clinical sources, and showed by pulsed-field gel electrophoresis (PFGE) that the food strains did not share any common pattern with the clinical strains. Other *Staphylococci* are also often safely present in diverse fermented foods. *S. saprophyticus*, for example, has been frequently isolated and used as a starter culture for the fermentation of salami (Fonseca et al., 2013; Montel et al., 1993) and fermented soybeans (Jeong et al., 2016). Many *S. saprophyticus* strains exhibit proteolytic and lipolytic activities and have been associated with important flavour compounds in cheeses and during meat fermentation (Bertuzzi et al., 2017; Ravyts et al., 2010), showing that they are not only safe but necessary for the proper development of certain fermentations. Previous studies have not identified these species in misos but reported the presence of other coagulase-negative *Staphylococci*, such as *S. gallinarum* and *S. kloosii* (Kim et al., 2010; Onda et al., 2003a).

While the role of *S. epidermidis* in food is still unknown and can be treated as a possible contamination from handling, it is possible that, as with *S. saprophyticus*, *S. gallinarum*, and *S. kloosii*, *S. epidermidis* may be not a contaminant, but a typical and even beneficial part of the ecosystem of certain fermented foods. It is common for certain strains to

circulate innocuously between human bodies and fermented food ecologies (Dunn et al., 2021; Reese et al., 2020), and growing research around the emerging concept of the holobiont (Faure et al., 2018; Simon et al., 2019)—reconceptualising individual organisms as ecologies formed of the ‘host’ and their microbiota—provides a promising framework to further investigate and normalise these microbial circulations from bodies to foods and back again. When it comes to this particular species, more studies are needed to understand its behaviour and potential in food and to establish the conditions of its safety.

### 3.3. Functional features of MAGs

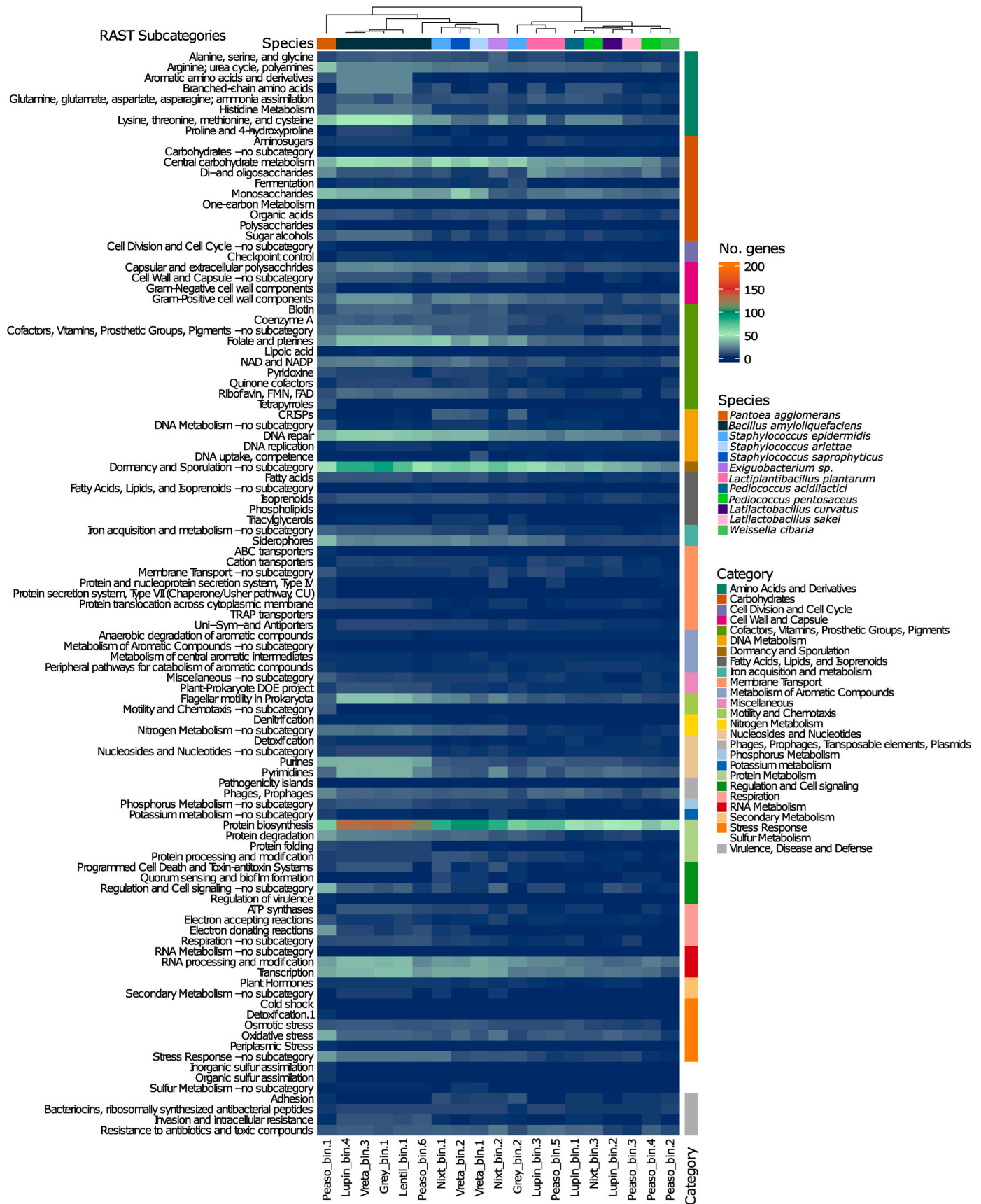
To increase the functional knowledge of the MAGs recovered from the misos, we annotated the functions using RAST. Our analyses revealed that species present in the misos analysed in this study have a wide range of functionalities (Fig. 2, Table S1, ‘RAST\_functions’ sheet). We observed that the functional clusters are aligned with the meta-taxonomic analyses, whose MAGs were divided into three main groups: the first containing *Pantoea agglomerans*, the second mainly *B. amyloliquefaciens*, *Staphylococcus* and *Exiguobacterium* species, and the third containing lactic acid bacteria, such as species belonging to the family *Lactobacillaceae*. Here we point out that the miso strains, mainly the *B. amyloliquefaciens* group, have many potential genes associated with amino acids, co-factors, dormancy and sporulation, as well as protein metabolism.

Previous studies have emphasised the key role of cofactors and vitamins in fermentation processes (Liu et al., 2021; Sun et al., 2023). In this study, we found potential genes associated with folate, a cofactor derived from vitamin B, as well as pterins, which play an indispensable role in facilitating enzymatic reactions and metabolic pathways during fermentation. Additionally, umami taste, which is highly desirable in miso, is predominantly attributed to the presence of amino acids, especially glutamate (Lee et al., 2023). Here we detected several potential genes associated with the aromatic amino acids, glutamate, lysine, threonine, methionine, and cysteine, as well as protein biosynthesis, that could contribute to the sensory properties of miso. Furthermore, a study focusing on metabolomic profiles during rice kōji fermentation revealed that using *B. amyloliquefaciens* increases aromatic and branched-chain amino acids (Lee et al., 2016). Consequently, it is not surprising that *Bacillus* species have been widely used as a starter culture within fermented food production (Kimura and Yokoyama, 2019).

In this study, we investigated the potential functions present in the miso strains. A deeper investigation into specific metabolic activities and gene functions could be facilitated by integrating metagenomic data with metabolomics. Such an integrated approach would provide a more detailed and holistic perspective on the relationship between gene expression and metabolic pathways within the miso ecosystem. Future research could explore these connections to illuminate the mechanisms of the fermentation process and resulting sensory attributes, offering new insights for miso production craft.

### 3.4. The first report of *Exiguobacterium* in miso, and a potentially new species

A final point to highlight is the presence of *Exiguobacterium* sp. in the nixtamalised miso (Fig. 1B). *Exiguobacterium* strains have been isolated from various environments, such as soils, sediments, seawater, and permafrost (Vishnivetskaya et al., 2009). The genus was first described from a strain found in an alkaline potato processing plant (Collins et al., 1983), and has since also been found in a processing plant for fish (Yumoto et al., 2004). *Exiguobacterium* has been found only once before in a fermented food, the Mexican fermented maize beverage, *pozol* (Omar and Ampe, 2000), made by fermenting nixtamalised corn dough (*masa*), diluting it with water or milk, and sometimes adding sweeteners like sugar or honey and seasonings like cocoa powder or dried chili



**Fig. 2.** Heatmap showing the functions (RAST subtypes) belonging to each MAG recovered in this study. The color bar represents the number of genes, with red representing high number and blue representing low.

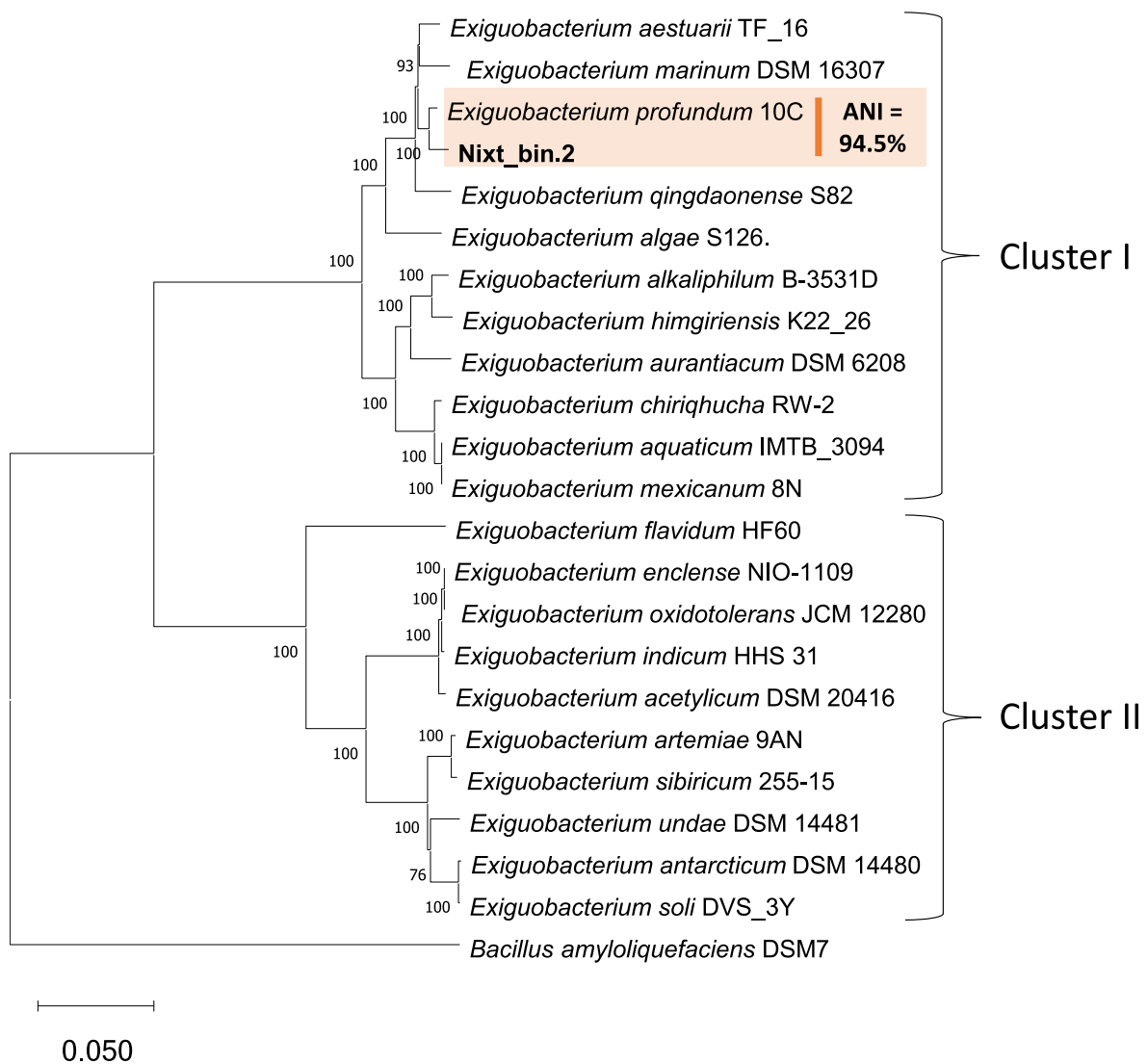
flakes. These previous occurrences of the *Exiguobacterium* in the alkaline environments of the potato processing plant and the *pozol* suggest that it could grow in the miso because of the alkalinity of the nixtamalised peas. To our knowledge, this is the first report of the genus having been detected in miso. Its presence may even have contributed desired characteristics. Further studies are needed to explore this possibility. Furthermore, due to many *Exiguobacterium* species' halotolerance and ability to survive at varying temperatures (Crapart et al., 2007), they may have unexplored potential for fermentation in general.

To know more about this *Exiguobacterium* sp. as well as the other strains from the misos, MAGs were reconstituted (Table S1, 'MAGs' sheet). All MAGs were assigned to a species and, sometimes, subspecies, except the one belonging to the *Exiguobacterium* genus. This MAG represents a potentially novel species since it presents ANI values lower than 95% with the most closely related reference genome, *E. profundum* 10C (threshold defined by Goris et al., 2007). To support this finding, we downloaded the whole genomes of all *Exiguobacterium* species present in the RefSeq NCBI database, and analysed the ANI (Table S1, 'Exiguo' and 'ANIB' sheets). As found in previous studies (Crapart et al., 2007; White et al., 2019), our analysis also revealed the presence of two large distinct clusters within the *Exiguobacterium* genus (Fig. 3) and confirmed that the species closest to the MAG obtained in this study is *E. profundum*. Since

the *Exiguobacterium* MAG showed more than 99% completeness and 0% contaminations in the quality assessment, as well as 86.3% alignment with the reference, it is likely this strain belongs to a new species. Isolation of the strain, together with genomic, phenotypic and chemotaxonomic characterization, is necessary to confirm and describe this potentially new species.

#### 4. Conclusion & perspectives

The findings presented in this study reveal the diverse microbiota present in misos produced with different novel legumes, suggesting the possibility that each substrate shapes its own particular microbial diversity. Further studies with experimental replicates and statistical analysis are needed to confirm this possibility. Notably, all misos contain not only *A. oryzae*, the fungus added with the *kōji*, but also *B. amyloliquefaciens*, a known bacterial species of technological interest in food production. Other species with technological applications in food were detected in the misos, belonging mainly to the lactic acid bacteria group, such as *L. sakei*, *L. plantarum*, *L. curvatus*, *P. acidilacti* and *P. pentosaceus*. We also identified coagulase-negative *Staphylococcus* species commonly found on human skin, such as *S. epidermidis*, *S. saprophyticus* and *S. arlettae*, where genes encoding toxins were not detected



**Fig. 3.** Pan-genome phylogenetic tree of *Exiguobacterium* species with the concatenated core-genome content using a neighbor-joining method at 1000 bootstrap. *Bacillus amyloliquefaciens* DSM7 was used as the outgroup. The *Exiguobacterium* MAG extracted from nixtamalised miso is highlighted in bold, and the ANI value with the closest species is indicated.

in the metagenomes, indicating their likely safety for consumption. To confirm this, the microbial dynamics during fermentation must also be investigated. Functional analysis of MAGs recovered from the misos reveal a range of functionalities, highlighting genes related to amino acids, cofactors, and protein metabolism, which are essential for fermentation processes and contribute to the sensory properties of miso. The recovery of MAGs also suggests a potentially new species of *Exiguobacterium*, most closely related to *E. profundum*. This notable finding suggests that experimental chefs and fermenters around the world, perhaps unknowingly, are creating new niches for novel microbial biodiversity, which could enrich local ‘microbial terroirs’ (Felder et al., 2012; Wolfe et al., 2014) and open up new possibilities for flavour, nutrition, food safety, and diverse culinary and cultural expression.

While it is important to note that this study is exploratory and based on a limited number of samples without replicates, to our knowledge this is the first study using shotgun metagenomic analyses to investigate the microbiota of misos, traditional or novel. This preliminary investigation indicates that there is still much to explore about miso, not only in microbiology but also in flavour chemistry, nutrition, sensory analysis, and consumer acceptance. Further metagenomic studies with additional substrates, batch replicates, temporal parameters, treatments, and fermentation sites could also offer more robust insights about both traditional and novel misos, with potential applications for other plant-based foods.

#### Authors’ contributions

JE conceived the study and its experimental design and gathered samples of the misos. SM and JAR extracted DNA from the samples and submitted the DNA for sequencing. JAR performed QC and preliminary bioinformatic analysis. MTPG provided computational and laboratory resources. CIK performed the metagenomic, genomic and safety analyses and data visualizations used in the paper. CIK wrote the first draft. JE and CIK developed and revised the manuscript. All authors read and approved the final manuscript.

#### Declaration of competing interest

The authors Caroline Isabel Kothe, Jacob Agerbo Rasmussen, Sarah S. T. Mak, M. Thomas P. Gilbert and Joshua Evans have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers’ bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements) in the subject matter or materials discussed in this manuscript.

#### Data availability

The raw sequences of shotgun metagenomic reads were deposited on the European Nucleotide Archive (ENA) under the BioProject ID PRJNA943417. The MAGs are available in Mendeley Data. Supplementary data can be found in Appendix A.

#### Acknowledgements

First and foremost, we would like to acknowledge the countless generations of miso makers, domestic and professional, within and outside of Japan, without whose knowledge and craft this study would not be possible and on which it attempts to build. We would also like to thank David Zilber and Lars Williams at the Noma Fermentation Lab for creating and providing samples of the misos and René Redzepi for giving access to his restaurant. We would also like to thank our two reviewers whose valuable comments helped improve the manuscript. The sequencing and preliminary analyses were funded by The Danish National Research Foundation award to MTPG (DNRF143); the rest of the

project was funded by The Novo Nordisk Foundation, NNF Grant number: NNF20CC0035580.

#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fm.2023.104372>.

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