Safety of pasteurised Akkermansia muciniphila as a novel food pursuant to Regulation (EU) 2015/2283.

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Safety of pasteurised *Akkermansia muciniphila* as a novel food pursuant to Regulation (EU) 2015/2283

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Abstract

Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on pasteurised *Akkermansia muciniphila* as a novel food (NF) pursuant to Regulation (EU) 2015/2283. *A. muciniphila* is a well-characterised non-toxin producing, avirulent microorganism that has been reported as part of normal gut microbiota. The NF, pasteurised *A. muciniphila*, is proposed by the applicant to be used as a food supplement at max. 5×10^{10} cells/day by adults excluding pregnant and lactating women, and in foods for special medical purposes. The Panel considers that the production process of the NF is sufficiently described and that the information provided on the composition of the NF is sufficient for its characterisation. Taking into account the composition of the NF and the proposed conditions of use, the consumption of the NF is not nutritionally disadvantageous. Based on literature data, and by applying an uncertainty factor of 200 to the no observed adverse effect level (NOAEL) of a 90-day repeated dose oral toxicity study in rats, the Panel concludes that the consumption of 3.4×10^{10} cells/day is safe for the target population under the provision that the number of viable cells in the NF is < 10 colony forming units (CFU)/g (i.e. limit of detection).

Keywords: *Akkermansia muciniphila*, novel food, food supplement, gut microbiota, safety

Requestor: European Commission

Question number: EFSA-Q-2019-00767

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

On 24 October 2019, the company A-Mansia Biotech S.A., submitted a request to the Commission in accordance with Article 10 of Regulation (EU) No 2015/2283 to place on the EU market pasteurised *Akkermansia muciniphila* as a novel food.

Pasteurised *Akkermansia muciniphila* is intended to be used in food supplements as defined in Directive 2002/46/EC, and in foods for special medical purposes as defined in Regulation (EU) No 609/2013.

The applicant has requested data protection according to the provisions of Article 26 of Regulation (EU) 2015/2283.

In accordance with Article 10(3) of Regulation (EU) 2015/2283, the European Commission asks the European Food Safety Authority to provide a scientific opinion on pasteurised *Akkermansia muciniphila* as a novel food.

2. Data and methodologies

2.1. Data

The safety assessment of this NF is based on data supplied in the application, information submitted by the applicant following two EFSA requests for supplementary information and information provided by the EFSA BIOHAZ Panel in the context of the QPS assessment for *Akkermansia muciniphila*.

Administrative and scientific requirements for NF applications referred to in Article 10 of Regulation (EU) 2015/2283 are listed in the Commission Implementing Regulation (EU) 2017/2469[1].

A common and structured format on the presentation of NF applications is described in the EFSA guidance on the preparation and presentation of an NF application (EFSA NDA Panel, 2016). As indicated in this guidance, it is the duty of the applicant to provide all of the available (proprietary, confidential and published) scientific data (including both data in favour and not in favour) that are pertinent to the safety of the NF.

This NF application includes a request for protection of proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283. The data requested by the applicant to be protected comprise: bacterial reverse mutation test (Brient, 2019a, unpublished), *in vitro* micronucleus assay (Brient, 2019b, unpublished), 14-day dose range-finding toxicity study (Bracken, 2019a, unpublished), 90-day toxicity study (Bracken, 2019b, unpublished), published toxicity-data (Druart et al., 2020), flow cytometry validation (Jensen, 2019, unpublished), antimicrobial resistance study (Gueimonde, 2019, unpublished).

2.2. Methodologies

The assessment follows the methodology set out in the EFSA guidance on NF applications (EFSA NDA Panel, 2016) and the principles described in the relevant existing guidance documents from the EFSA Scientific Committee. The legal provisions for the assessment are laid down in Article 11 of Regulation (EU) 2015/2283 and in Article 7 of the Commission Implementing Regulation (EU) 2017/2469.

This assessment concerns only the risks that might be associated with consumption of the NF under the proposed conditions of use, and is not an assessment of the efficacy of the NF with regard to any (claimed) benefit.

3. Assessment

3.1. Introduction

The NF which is the subject of the application is pasteurised *Akkermansia muciniphila*. The NF is produced by anaerobic growth of the bacterium followed by pasteurisation and freeze-drying. The NF is proposed by the applicant to be used in food supplements and in foods for special medical purposes (as defined in Regulation (EU) No 609/2013). The proposed target population is the general adult population excluding pregnant and lactating women.

The NF falls under Article 3(2)(a)(ii) foods consisting of, isolated from or produced from microorganisms, fungi or algae, as defined in Regulation (EU) 2015/2283.

[1] Commission Implementing Regulation (EU) 2017/2469 of 20 December 2017 laying down administrative and scientific requirements for applications referred to in Article 10 of Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. OJ L 351, 30.12.2017, pp. 64–71.

3.2. Identity of the NF

The NF is pasteurised *Akkermansia muciniphila* Muc^T which is a Gram-negative, strictly anaerobic, non-motile and non-spore-forming human gut bacterium.

The species *A. muciniphila* has been shown to be a mucin-degrader, i.e. it uses intestinal mucin as source for carbon and nitrogen (Derrien et al., 2004). The mucins are high-molecular mass glycoproteins (produced by intestinal goblet cells) which form a viscous gel, i.e. the mucus, that lines and protects (as a barrier defence) the gastrointestinal tract.

The full taxonomic classification of the employed strain is the following; Empire: Prokaryota; Kingdom: Bacteria; Phylum: Verrucomicrobia; Class: Verrucomicrobiae; Order: Verrucomicrobiales; Family: Verrucomicrobiaceae; Genus: *Akkermansia*; Species: *Akkermansia muciniphila*; Strain: *Akkermansia muciniphila* Muc^T (ATCC BAA-835^T=CIP 107961^T).

The strain is deposited at two culture collections, i.e. at the American Type Culture Collection (ATCC) under accession number ATCC BAA-835^T and at the Collection de l'Institut Pasteur (CIP) under accession number CIP 107961^T, for which a certificate of deposition was provided by the applicant.

The isolation of the strain Muc^T from human faeces and its morphological, physiological and phylogenetic features were first described by Derrien et al. (2004). The whole genome was sequenced by PacBio and Illumina shotgun genome sequence analysis (National Center for Biotechnology Information (NCBI) Ref. Sequence: NC-010655.1).

The taxonomic identification of the strain Muc^T as *A. muciniphila* was additionally established by several phylogenetic analyses using the whole genome sequence (WGS). A dendrogram using genome-aligning software MUMmer and based on average nucleotide identity (ANI), an SNP tree based on the core genome phylogeny and the associated SNP distance matrix showed that the Muc^T strain clustered with the NCBI genome of the reference species.

3.3. Production process

According to the information provided, the NF is produced in line with good manufacturing practice (GMP) and hazard analysis critical control points (HACCP) principles.

The process flow diagram together with a detailed description of the methods involved at each step and a complete list of the culture media and processing aids plus the respective certificates of analysis were provided (confidential information).

In short, an inoculum of *A. muciniphila* is made by successive preculture steps in liquid media in strictly anaerobic conditions at increasing volumes in order to prepare for the inoculation of the main fermenter. The main fermentation is conducted under strictly anaerobic conditions at a certain temperature and pH (confidential information), while stirring. The growth of *A. muciniphila* is monitored via spectrophotometry at regular time points.

The production process for pasteurised *A. muciniphila* consists of an anaerobic fermentation followed by pasteurisation and concentration of the bacterial cells. The cells are then mixed with cryoprotectants and freeze-dried in order to produce a powder. Total bacterial cell counting is performed and, if required, the powder is mixed with stabilising agents. The applicant informed that the addition of stabilising agents is an optional step in the manufacturing process, in order to dilute the powder at a given cell counting (i.e. the proportion and the quantity added depends on the production yield and the quantification of the cell count in the 'raw' powder). At the current production practice, the NF is standardised to 1×10^{11} cells (measured as total fluorescent units (TFU))/g of powder.

The bulk powder is packaged into water- and airproof multi-layer pouches, heat sealed and stored at $\leq -18°C$ until the powder is packed in final products which are to be stored (e.g. by wholesalers, retailers, consumers) at 15–25°C, protected from light and moisture. The proposed shelf-life is 1 year.

The Panel considers that the production process is sufficiently described.

3.4. Compositional data

The NF, pasteurised *A. muciniphila*, consists of carbohydrates (~ 45%), proteins (~ 30%), ash (~ 18%), moisture (~ 6%) and minor amounts of fat.

In order to confirm that the manufacturing process is reproducible and adequate to produce on a commercial scale a product with certain characteristics, the applicant provided analytical information for five independent batches of the NF (before the addition of stabilising agents) (Table 1). The

appearance of all batches was that of an off-white to beige homogenous powder, as confirmed by visual inspection.

Table 1: Batch to batch analysis of the NF

Parameter (unit)	Batch number					Method of analysis
	#1	#2	#3	#4	#5	
Species identification (*A. muciniphila*)	Conforms	Conforms	Conforms	Conforms	Conforms	16S rRNA gene sequencing (Sanger Method)
A. muciniphila – total cell count (cells (as TFU)/g)	2.23×10^{11}	1.78×10^{11}	1.13×10^{11}	9.34×10^{10}	9.94×10^{10}	Flow cytometry (LoQ = 1×10^5 TFU/g)
A. muciniphila – viable cell count (CFU/g)	< 10	< 40	400	30	20	Plating (LoQ = 10 CFU/g)
Protein (%)	31.6	26.5	30.2	29.8	32.2	Kjeldhal or Dumas method
Fat (%)	1.2	0.6	0.5	0.5	0.2	Extraction after hydrolysis/ gravimetry
Moisture (%)	5.5	7.4	8.5	3.9	4.9	Vacuum drying method
Ash (%)	18.4	19.7	19.3	19.5	13.2	Dry ashing
Carbohydrates (%)	43.3	45.8	41.5	46.3	49.5	By calculation(1)
Water activity	0.17	0.28	0.35	0.32	0.29	NF ISO 21807:2004
Heavy metals						
Arsenic (mg/kg)	0.25	0.22	0.23	0.18	0.17	ICP-MS
Cadmium (mg/kg)	0.005	0.009	0.006	0.006	0.015	ICP-MS
Mercury (mg/kg)	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	ICP-MS
Lead (mg/kg)	0.04	0.043	0.038	0.04	< 0.02	ICP-MS
Microbial						
Aerobic mesophilic total count (CFU/g)	< 10	< 10	< 40	< 10	< 10	NF EN ISO 4833-1
Sulfite-reducing anaerobes (CFU/g)	< 10	< 10	< 10	40	< 10	ISO 15213
Coagulase + Staphylococci (CFU/g)	< 10	< 10	< 10	< 10	< 10	NF EN ISO 6888-2
Enterobacteriaceae (CFU/g)	< 10	< 10	< 10	< 10	< 10	ISO 21528-2
Bacillus cereus (CFU/g)	< 10	< 10	< 10	< 10	< 10	NF EN ISO 7932
Listeria spp. (in 25 g)	Not detected	Not detected	Not detected	Not detected	Not detected	ISO 11290-1:2017
Salmonella spp. (in 25 g)	Not detected	Not detected	Not detected	Not detected	Not detected	ISO 6579-1:2017
Escherichia coli (in 1 g)	Not detected	Not detected	Not detected	Not detected	Not detected	NF ISO 16649-1
Yeasts (CFU/g)	< 10	< 10	< 10	< 10	< 10	ISO 21527-2:2008
Moulds (CFU/g)	< 10	< 10	< 10	< 10	< 10	ISO 21527-2:2008

CFU: colony forming units, ICP-MS: inductively coupled plasma mass spectrometry, ISO: International Organization for Standardization, LoQ: limit of quantification, NF: norme française (French Standard), rRNA: ribosomal ribonucleic acid, TFU: total fluorescent units.
(1): Carbohydrates% = 100% – protein% – fat% – moisture% – ash%).

The total cell count of pasteurised *Akkermansia muciniphila* in the batch testing was measured (as TFU) by flow cytometry according to the 'A-Mansia method' (denominated as VC01_V1.0 in the respective certificate of analysis), for which the validation report was provided.

Analyses of mycotoxins and polycyclic aromatic hydrocarbons in the NF were not performed. However, the applicant provided certificates of analysis for the raw materials, which state the absence of these contaminants in the raw materials used for the fermentation. These certificates of analysis confirmed also the absence of pesticide residues in the raw materials. Two batches of the NF were nonetheless analysed for the presence of pesticides and related compounds. No residues of these pesticides or related compounds were detected above their respective limits of quantification/detection.

The applicant also provided a particle size analysis (by laser diffraction), where 50% of the cumulative distribution ranged between 50 and 100 μm. The smallest particle sizes detected were between 1 and 5 μm.

Information was provided on the accreditation of the laboratories that conducted the analyses presented in the application.

The Panel considers that the information provided on the composition of the NF is sufficient for characterising the NF.

3.4.1. Stability

The applicant performed stability tests with three independently produced batches of the NF. The tests were carried out at ambient (at 25°C and 60% relative humidity (RH), Table 2) and at accelerated conditions (at 40°C and 75% RH, Table 3) for up to 12 months. The batches were analysed for total and viable *A. muciniphila* cell counts and additional microbiological parameters (not shown) as per the specifications for the NF. The analysed parameters remained within the limits as set in the specifications as originally proposed by the applicant. A shelf-life of one year was proposed by the applicant.

Table 2: Total and viable *A. muciniphila* cell counts in the NF during the stability testing under ambient conditions (25°C and 60% RH)

Parameter	Time	Batch number		
	(months)	#1	#2	#3
Total *A. muciniphila* cell count (TFU (cells)/g)	0	2.23×10^{11}	1.78×10^{11}	1.13×10^{11}
	3	1.89×10^{11}	1.80×10^{11}	1.23×10^{11}
	6	1.84×10^{11}	1.54×10^{11}	1.32×10^{11}
	12	2.86×10^{11}	1.41×10^{11}	1.05×10^{11}
Viable *A. muciniphila* cell count (CFU/g)	0	< 10	10 ne	400
	3	< 10	240	390
	6	< 10	< 10	< 10
	12	30 ne	20 ne	< 10

CFU: colony forming units; ne: estimated number; TFU: total fluorescent units.

Table 3: Total and viable *A. muciniphila* cell counts in the NF during the stability testing under accelerated conditions (40°C and 75% RH)

Parameter	Time	Batch number		
	(months)	#1	#2	#3
Total *A. muciniphila* cell count (TFU (cells)/g)	0	2.23×10^{11}	1.78×10^{11}	1.13×10^{11}
	1	1.54×10^{11}	8.11×10^{10}	1.16×10^{11}
	3	1.19×10^{11}	9.11×10^{10}	9.23×10^{10}
	6	9.84×10^{10}	1.06×10^{11}	8.40×10^{10}
	12	2.34×10^{11}	1.66×10^{11}	9.47×10^{10}
Viable *A. muciniphila* cell count (CFU/g)	0	< 10	10 ne	400
	1	< 10,000[1)]	< 10,000[(1)]	< 10,000[(1)]
	3	< 10	< 10	36
	6	< 10	< 10	< 10
	12	< 10	30 ne	< 10

CFU: colony forming units; ne: estimated number; TFU: total fluorescent units.
(1): A different less sensitive detection method was used at that time point.

The Panel considers that the data provided sufficient information with respect to the stability of the NF.

3.5. Specifications

The specifications of the NF are indicated in Table 4.

Table 4: Specifications of the NF

Description: pasteurised *Akkermansia muciniphila* (strain ATCC BAA-835) produced by anaerobic fermentation, followed by pasteurisation and freeze-drying	
Parameter	**Specification**
Total *A. muciniphila* cell count (cells/g)	2.5×10^{10}–2.5×10^{12}
Viable *A. muciniphila* cell count (CFU/g)	< 10 (LoD)(1)
Water activity	≤ 0.43
Moisture (%)	≤ 10(2)
Protein (%)	25–35(2)
Fat (%)	0–2(2)
Crude ash (%)	17–21(2)
Carbohydrates (%)	36–48(2)
Microbiological	
Aerobic mesophilic total count (CFU/g)	≤ 500
Sulfite-reducing anaerobes (CFU/g)	≤ 50
Coagulase + Staphylococci (CFU/g)	≤ 10
Enterobacteriaceae (CFU/g)	≤ 10
Yeasts (CFU/g)	≤ 10
Moulds (CFU/g)	≤ 10
Bacillus cereus (CFU/g)	≤ 100
Listeria spp.	Not detected in 25 g
Salmonella spp.	Not detected in 25 g
Escherichia coli	Not detected in 1 g

CFU: colony forming units; LoD: limit of detection.
(1): For cultivation conditions, see Van der Ark et al. (2018).
(2): The numbers given refer to the undiluted NF. The ranges for these parameters are expected to change according to the amount of stabilising agents added.

With respect to the parameter of viable *A. muciniphila* cell count, the applicant originally specified up to 0.0001% viable cells of the overall cell count. Following a request from EFSA to lower this parameter to < 10 CFU/g (i.e. limit of detection), the applicant proposed a specification limit of < 500 CFU/g. The Panel notes that at the proposed conditions of use (5×10^{10} cells/day) and considering < 500 CFU/g, the intake could be up to 1,000 viable *A. muciniphila* cells per day.

The Panel considers that taking into account the presence of *A. muciniphila* in the human gut and the instance that infants can be exposed to *A. muciniphila* via the breast milk, the risk following consumption of up to 1,000 live *A. muciniphila* cells per day via the NF is likely to be low. However, at this exposure level, a possible adverse impact on the gut barrier integrity and balance of the microbiota in susceptible people cannot be ruled out based on the available information (see section 3.10.1). Therefore, the Panel retains that the parameter for viable *A. muciniphila* should be set to < 10 CFU/g (i.e. limit of detection).

The Panel considers that the information provided on the specifications of the NF is sufficient and does not raise safety concerns.

3.6. History of use of the NF and/or of its source

The NF does not have a history of use as or in food.

The applicant informed that the source, i.e. *A. muciniphila*, has been detected in human milk by quantitative polymerase chain reaction (PCR) (Collado et al., 2012). *A. muciniphila* has also been detected by quantitative PCR in colostrum by Aakko et al. (2017).

3.7. Proposed uses and use levels

3.7.1. Target population

The target population proposed by the applicant is the general adult population excluding pregnant and lactating women.

3.7.2. Proposed uses and use levels

The NF is proposed by the applicant to be used in food supplements at up to 5×10^{10} TFU (i.e. cells)/day.

At the proposed specifications for the NF (i.e. from 2.5×10^{10} to 2.5×10^{12} cells/g), the maximum proposed dose of 5×10^{10} cells/day corresponds to 0.02–2 g of the NF.

The NF is also proposed by the applicant to be used in foods for special medical purposes as defined by Regulation (EU) No 609/2013, with the use level to be determined on a case-by-case basis (in any case not more than 5×10^{10} TFU per day).

3.8. Absorption, distribution, metabolism and excretion (ADME)

The applicant did not provide any ADME data for the NF. Instead, the applicant pointed out that *A. muciniphila* is a common member of the human intestinal tract (Collado et al., 2007; Derrien et al., 2008).

The Panel considers that given the nature of the NF, no ADME testing is required for the safety assessment of the NF.

3.9. Nutritional information

The applicant provided proximate analyses for three batches of the NF, which showed that the NF is mainly composed of carbohydrates (41–49%), protein (26–32%), ash (13–20%) and moisture (5–9%).

For two batches of the NF, additional analyses were provided on the concentrations of a number of minerals including trace elements.

The Panel considers that taking into account the composition of the NF and the proposed conditions of use, consumption of the NF is not nutritionally disadvantageous.

3.10. Toxicological information

3.10.1. Microbiological information

Prevalence of *A. muciniphila* in human gut

In a study comprising 249 subjects across various age groups, *A. muciniphila* was detected in faecal samples from 8 of 50 1-month-old infants (16%), 36 of 50 infants aged 6 months (72%), 45 of 50 children at 12 months of age (90%), 54 out of 54 in adults (100%) and 43 out of 45 in elderly (96%). In adults, a median of about 1×10^8 *A. muciniphila* cells per gram faeces (as determined by quantitative PCR) was reported (Collado et al., 2007).

According to a study by Derrien et al. (2008) performed with faecal samples, *A. muciniphila* accounts for about 1–3% of naturally occurring human intestinal microbes.

In a combined analysis of large global data sets (N combined = 3,948 subjects) of faecal samples, the genus *Akkermansia* was present in 77.7% of the total assessed cohorts globally and in 81.8% of two cohorts of Western populations (Falony et al., 2016).

Qualified presumption of safety (QPS) of *A. muciniphila*

In 2020, the species *A. muciniphila* was assessed by the EFSA Panel on Biological Hazards (BIOHAZ) for its suitability to be added to the list of qualified presumption of safety (QPS)-recommended biological agents intentionally added to food or feed (EFSA BIOHAZ Panel, 2020). For this purpose, the BIOHAZ Panel considered the identity, the body of knowledge, safety concerns and antimicrobial resistance aspects of this taxonomic unit.

The BIOHAZ Panel acknowledged that *A. muciniphila* is abundant in the colon of humans and animals and has also been detected in human milk samples (Collado et al., 2012). The Panel also

stated that the prevalence of *A. muciniphila* appears to be decreased in gut microbiota of people suffering from the 'metabolic syndrome' (including obesity, diabetes, cardiometabolic disease and low-grade inflammation). However, the BIOHAZ Panel pointed out that the prevalence of *A. muciniphila* was found to be increased in gut microbiota of people suffering from Parkinson's disease, multiple sclerosis, Alzheimer's disease and autism spectrum disorders, although for autism depletion of the microorganism was reported as well. The BIOHAZ Panel concluded that *A. muciniphila* cannot be recommended for the QPS list due to safety concerns (EFSA BIOHAZ Panel, 2020).

A. muciniphila in disease

There are a number of studies available in the literature on the relationship of viable *A. muciniphila* with disease. The Panel reviewed those that were identified by the EFSA BIOHAZ Panel (2020) and also reviewed further studies which were identified by the experts of the Panel or the Working Group on NF or were submitted by the applicant subsequently to EFSA's requests for additional information. The studies comprised human observational studies and animal studies (including murine models of (neurological) diseases and experiments in gnotobiotic/germ-free mice).

The reviewed studies were concerned with Alzheimer's disease (Vogt et al., 2017; Zhuang et al., 2018; Ou et al., 2020), arthritis (Stoll et al., 2014, 2019; Asquith et al., 2016), autism (Finegold et al., 2010; Wang et al., 2011; De Angelis et al., 2013; Xu et al., 2019; Zou et al., 2020), colitis/inflammatory bowel disease (Png et al., 2010; Ganesh et al., 2013; Desai et al., 2016; Seregin et al., 2017; Bian et al., 2019; Ring et al., 2019; Zhai et al., 2019), colorectal cancer (Weir et al., 2013; Zackular et al., 2013; Baxter et al., 2014; Wang et al., 2020), multiple sclerosis (Jangi et al., 2016; Berer et al., 2017; Cekanaviciute et al., 2017; Liu et al., 2019) and Parkinson's disease (Keshavarzian et al., 2015; Hill-Burns et al., 2017; Heintz-Buschart et al., 2018).

A number of the human observational studies, which were a mix of quite different approaches, reported a higher abundance of some bacteria including *A. muciniphila* in diseased population groups, but the reverse was also observed. In addition, these studies lacked a longitudinal design and, therefore, may be subject to reverse causation and other sources of bias.

Transfer of microbiota from diseased population groups or transfer of *A. muciniphila* to gnotobiotic/germ-free/transgenic mice (Ganesh et al., 2013; Zackular et al., 2013; Baxter et al., 2014; Asquith et al., 2016; Desai et al., 2016; Berer et al., 2017; Cekanaviciute et al., 2017; Seregin et al., 2017; Bian et al., 2019; Liu et al., 2019; Ring et al., 2019; Stoll et al., 2019; Zhai et al., 2019; Ou et al., 2020; Wang et al., 2020) gave some results, albeit also in different directions, and the relevance of such studies for humans is questionable.

The Panel notes that associations of *A. muciniphila* with diseases (neurological, autoimmunity and other) are inconsistent and that causal relationships have not been established.

Information on antimicrobial resistance (AMR) and potential mobile elements in *A. muciniphila* BAA-835

The applicant provided an AMR phenotypic study (Gueimonde, 2019, unpublished) evaluating the presence of AMR factors in *A. muciniphila* BAA-835, according to the provisions of the EFSA Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA FEEDAP Panel, 2018). *A. muciniphila* BAA-835 presented high resistance levels to aminoglycosides, vancomycin and ciprofloxacin. Minimum inhibitory concentrations (MICs) obtained for all antimicrobials tested were similar among the five *Akkermansia muciniphila* strains tested by the applicant, showing a similar level of phenotypic resistance within the species. Other studies show that the species *A. muciniphila* is intrinsically resistant to vancomycin (MIC > 64 μg/mL), metronidazole (MIC > 64 μg/mL) and penicillin G (MIC 2.8 μg/mL) (Derrien et al., 2008; Dubourg et al., 2013; Gómez-Gallego et al., 2016).

Additionally, the applicant carried out an *in silico* interrogation regarding the presence of AMR genes by comparing the genome sequence to AMR genes included in CARD and NDARO databases. According to the applicant, the findings suggest that the strain does not harbour any AMR genes of concern. A previous *in silico* genome sequencing analysis (Van Passel et al., 2011) identified some putative antibiotic resistance genes (two beta-lactamases and one metronidazole resistance gene) in *A. muciniphila* BAA-835.

The presence of mobile genetic elements in *A. muciniphila* BAA-835 was investigated using the MobileElementFinder database. One insertion sequence, ISAmu1 (Accession: NC_010655), was identified showing 100% coverage and 100% identity, but is unlikely to contribute to the exchange of AMR genes. According to Gómez-Gallego et al. (2016), some of the AMR genes present in *A. muciniphila* have been acquired through evolution from other commensal bacteria species i.e.

Bacteroides and *Bifidobacterium* by horizontal transfer. Guo et al. (2017) described the presence of three antibiotic resistance genes in *A. muciniphila* strain GP36, which originated from plasmid pRSF1010 (8,684 bp) of *Salmonella enterica*. This indicates that some strains of *A. muciniphila* might acquire antibiotic resistance genes through lateral gene transfer (Geerlings et al., 2018).

3.10.2. Toxicological studies

The applicant provided four toxicological studies with the NF (Table 5), three of which were conducted in compliance with OECD principles of good laboratory practice (GLP) (OECD, 1998) and in accordance with the respective OECD test guidelines (TG).

Table 5: List of toxicological studies with the NF

Reference	Type of study	Test system	Dose
Brient (2019a, unpublished)	Bacterial reverse mutation test (GLP, OECD TG 471)	*S.* Typhimurium TA98, TA100, TA102, TA1535 and TA1537	Up to 5,000 μg/plate (in absence and presence of S9 mix) except for TA98
Brient (2019b, unpublished)	*In vitro* mammalian cell micronucleus test (GLP, OECD TG 487)	Human lymphocytes	Up to 750 μg/mL for 3 h (in the absence and presence of S9 mix); up to 375 μg/mL for 24 h
Bracken (2019a, unpublished)	Preliminary 14-day dose range-finding study	Wistar rats (Crl:WI (Han))	Up to 1,500 mg/kg bw per day (9.6×10^{10} cells/kg bw per day)
Bracken (2019b, unpublished)	90-day repeated dose oral toxicity (GLP, OECD TG 408)	Wistar rats Crl:WI(Han)	Up to 1,500 mg/kg bw per day (9.6×10^{10} cells/kg bw per day)

Genotoxicity

The applicant provided a bacterial reverse mutation test (Brient, 2019a, unpublished; Druart et al., 2020) and an *in vitro* mammalian cell micronucleus test (Brient, 2019b, unpublished; Druart et al., 2020) that were performed with the NF as a suspension in water.

The Panel notes that the NF did not show any mutagenic activity in the bacterial reverse mutation test and it was not clastogenic or aneugenic in the *in vitro* micronucleus test.

The Panel notes that genotoxicity testing should have been performed with suitable extracts and not with whole bacteria. Especially the bacterial reverse mutation test is not a suitable test for whole bacteria. However, taking into consideration that *A. muciniphila* lacks toxicity traits and that the production process does not raise safety concerns, the Panel considers that further genotoxicity testing is not required.

Subacute toxicity

A 14-day dose range-finding study in Wistar rats (Crl:WI(Han) strain) was provided (Bracken, 2019a, unpublished). The study was not carried out according to any specific guideline.

In the preliminary phase of the study, three males and three females were administered by gavage 1,500 mg/kg body weight (bw) per day of the NF (9.6×10^{10} TFU/kg bw per day) once daily for 3 days. That phase was immediately followed by a dose range-finding phase, where groups of five males and five females received 0, 1,125 mg (7.2×10^{10} TFU/kg bw per day) or 1,500 mg (9.6×10^{10} TFU/kg bw per day) per kg bw per day of the NF once daily for 14 days.

There were no deaths and no test item-related clinical signs. Animals given pasteurised *A. muciniphila* showed similar weight gains and ate similar amounts of food as controls. No test item-related macroscopic or microscopic changes were observed at necropsy. A dose-related decrease in absolute and relative prostate gland weight (for males) and a dose-related higher absolute and relative pituitary gland weight for females was observed for rats that received the test item as compared to controls. In the absence of any histopathological findings, these differences were considered non-adverse by the study authors. Thus, the highest dose tested (i.e. 9.6×10^{10} TFU/kg bw weight per day) was considered a suitable high dose for the 90-day study.

Subchronic toxicity

The applicant provided a 90-day repeated dose oral toxicity study with the NF in Wistar rats (Crl:WI (Han) strain) (Bracken, 2019b, unpublished study report; Druart et al., 2020). The study was

conducted in compliance with the OECD principles of GLP and according to OECD TG 408 (OECD, 2018).

Groups of 10 male and 10 female rats received by gavage 0, 75, 375 or 1,500 mg/kg bw per day of the NF for at least 90 days. The dosages corresponded to 0, 4.8×10^9, 2.4×10^{10} and 9.6×10^{10} cells (measured as TFU)/kg bw per day. The viable *A. muciniphila* cell count of the batch of the NF as tested was < 50 CFU/g of the test item.

One animal (female in the high-dose group) was euthanised on day 24, following a dosing error (perforated oesophagus). There were no further deaths nor were there any clinical signs observed. There were no differences across study groups in the neurophysiological and behavioural tests.

Animals that were administered the NF had similar weight increase, feed consumption and water consumption as control animals.

No statistically significant or biologically relevant differences were observed in blood chemistry or coagulation parameters between controls and the groups that received the NF.

Dose-related increases in absolute neutrophil count and total white blood cell (WBC) count were observed in males and were statistically significant in the high-dose group. The increase in total WBC count was due to the increase in neutrophils and to a dose related, but not statistically significant, increase in lymphocytes. The Panel notes that the increase in WBC counts was small (i.e. 7.8×10^9/L vs. 6.1×10^9/L) and that the difference to the control group, even though statistically significant, might have been a consequence of a rather low WBC count in the control group (6.1×10^9/L) when compared to historical controls (mean: 9.3×10^9/L; range 5.0–13.7×10^9/L). The Panel therefore considers that the findings reflect normal biological variation rather than an adverse effect.

Dose-related statistically significant reductions in relative (but not absolute) eosinophils were seen in males in the low- and mid-dose groups but not in the high-dose group. Statistically significant increases were observed for triiodothyronine (T3) and thyroxine (T4) in males in the low-dose group and for T4 in males in addition in the high-dose group, as compared to controls. Thyroid-stimulating hormone (TSH) was slightly (not statistically significantly) increased in the low-dose group. The Panel considers that these findings are unrelated to the NF as there was no clear dose response relationship.

There were no differences in organ weights between control groups and groups that received the NF. There were also no test item-related macroscopic or microscopic (histopathological) findings.

The Panel considers that the no observed adverse effect level (NOAEL) of this study is the highest dose tested, i.e. 1,500 mg NF/kg bw per day, corresponding to 9.6×10^{10} cells/kg bw per day.

Other animal studies

The applicant provided three short-term (4–5 weeks) studies on the effect of oral administration of live or pasteurised *A. muciniphila* or a purified membrane protein from the bacteria on metabolic parameters in obese and diabetic C57BL/6J mice (all three studies published by Plovier et al., 2017).

In the three studies, mice (8–10/group) were administered daily for 4–5 weeks live or pasteurised *A. muciniphila* grown on mucus-based medium or synthetic medium. The mice were kept on high-fat diet (60% fat, 20% carbohydrates) or normal diet. In one study, one additional group of mice was given orally 3 μg/day of the membrane protein Amuc_1100 (estimated to be equivalent to 1.5×10^8 CFU of *A. muciniphila*).

The study authors reported on a number of findings of *A. muciniphila*, e.g. body weight, weight gain, fat mass, adipocytes, insulin resistance, dyslipidaemia, goblet cell density, in obese mice. The authors found that pasteurisation of *A. muciniphila* seemed to enhance its effects compared to live bacteria, presented as beneficial by the authors. Furthermore, the study authors isolated a specific protein, i.e. Amuc_1100, from the outer membrane of *A. muciniphila*. According to the publication, this protein interacts with Toll-like receptor 2, is stable at temperatures used for pasteurisation (analysis indicated a melting temperature of 70°C) and partly showed similar effects as the bacterium in the studies presented (Plovier et al., 2017).

3.10.3. Human studies

The applicant provided a randomised, double-blind, 3-arm placebo-controlled exploratory pilot study with the NF in overweight/obese insulin-resistant subjects (Depommier et al., 2019). According to the study authors, the main objectives of this study were (1) to evaluate the feasibility, safety and tolerance of *A. muciniphila* supplementation, and (2) to explore potential metabolic effects of *A. muciniphila* supplementation in humans.

A total of 40 participants were randomised to receive once daily for 12 weeks either a placebo or 1×10^{10} cells/day of live *A. muciniphila* or 1×10^{10} cells/day of pasteurised *A. muciniphila* (i.e. the NF). The Panel notes that this amount is five times less than what the applicant proposed as conditions of use for the NF.

Eight subjects dropped out of the study (two in the placebo group, one in the pasteurised bacteria (NF) group, five in the live bacteria group), resulting in 32 subjects who completed the trial.

This study, even though designed as an efficacy study, included a number of safety-related endpoints, e.g. systolic and diastolic blood pressure, glycated haemoglobin (HbA1c), blood biochemical and haematological parameters (including WBC count), C-reactive protein, prothrombin time, plasma lipopolysaccharides (LPS), glomerular filtration rate, etc. No adverse effects were observed for the analysed endpoints.

No difference in the frequency of other adverse events (i.e. nausea, flatulence, bloating, cramps, borborygmi, gastro-oesophageal reflux) between the groups was observed during the study.

The Panel considers that this study does not raise safety concerns. However, the Panel notes the limitations of the study, i.e. scope (efficacy study), low number of subjects, low dose of the NF, study population (overweight or obese insulin-resistant subjects), short study duration, limited number of safety-related endpoints. The Panel thus considers that this exploratory pilot study is of limited value for the safety assessment of the NF.

3.11. Allergenicity

The Panel notes the protein content of about 30% in the NF and, therefore, the potential of the NF to elicit allergic reactions.

However, the Panel also notes that *A. muciniphila* is part of a balanced gut microbiota. No allergies are expected to be elicited from its protein composition.

The Panel considers that the risk of allergic reactions to the NF for the general population is expected to be low.

4. Discussion

The NF which is the subject of the application is pasteurised *A. muciniphila*. It is proposed by the applicant to be used as food supplement at max. 5×10^{10} cells/day for adults excluding pregnant and lactating women, and in foods for special medical purposes.

A. muciniphila is a well-characterised non-toxin producing, avirulent microorganism that has been reported as part of normal gut microbiota. The number of *A. muciniphila* in the gut is unknown but it has been reported to be in the range of 1–3% of total number (estimated to be about 3.8×10^{13} (Sender et al., 2016)) of bacteria. *A. muciniphila* has also been detected in human milk.

In 2020, the BIOHAZ Panel expressed safety concerns and did not recommend *A. muciniphila* for inclusion into the QPS list, since the prevalence of *A. muciniphila* in gut microbiota was found to be increased in a number of publications reporting on subjects with various neurological diseases. The Panel reviewed these and further publications which contained information on a potential relationship of viable *A. muciniphila* with disease conditions (neurological, autoimmunity and other). The Panel notes that reported associations of *A. muciniphila* with disease conditions are inconsistent and that causal relationships have not been established.

Nevertheless, in order to prevent possible adverse effects on gut barrier integrity and balance of the microbiota in susceptible people, the Panel considers that the specification parameter for viable *A. muciniphila* should be set to < 10 CFU/g (i.e. limit of detection).

A 90-day repeated dose oral toxicity study with the NF was provided. The Panel considers that the no observed adverse effect level (NOAEL) is the highest dose of total number of cells (viable and non-viable) tested in this study, i.e. 9.6×10^{10} cells/kg bw per day. By considering this NOAEL and by applying an uncertainty factor of 200 (10 (interspecies variability) $\times$ 10 (intraspecies variability) $\times$ 2 (subchronic to chronic study duration)), the Panel derives a safe level of 4.8×10^{8} cells/kg bw per day. For the target population (adults excluding pregnant and lactating women) with a default body weight of 70 kg (EFSA Scientific Committee, 2012), this corresponds to a total number of 3.4×10^{10} cells/day.

5. Conclusions

The Panel concludes that the NF, pasteurised *A. muciniphila*, is safe for the target population at 3.4×10^{10} cells/day, provided that the number of viable *A. muciniphila* is below 10 cells/g NF.

5.1. Request for protection of proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283

The Panel could not have reached the conclusion on the safety of the NF under the proposed conditions of use without the data claimed as proprietary by the applicant, i.e. bacterial reverse mutation test (Brient, 2019a, unpublished), *in vitro* micronucleus assay (Brient, 2019b, unpublished), 14-day dose range-finding toxicity study (Bracken, 2019a, unpublished), 90-day toxicity study (Bracken, 2019b, unpublished), published toxicity data (Druart et al., 2020), flow cytometry validation (Jensen, 2019, unpublished), antimicrobial resistance study (Gueimonde, 2019, unpublished).

6. Steps taken by EFSA

1) On 19 May 2020, EFSA received a letter from the European Commission with the request for a scientific opinion on the safety of pasteurised *Akkermansia muciniphila* Ref. Ares(2020) 2618529.
2) On 19 May 2020, a valid application on pasteurised *Akkermansia muciniphila*, which was submitted by A-Mansia Biotech S.A., was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2019/1366) and the scientific evaluation was initiated.
3) On 23 September 2020, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4) On 20 November 2020, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
5) On 15 December 2020, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
6) On 15 January 2021, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
7) On 15 February 2021, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
8) On 30 April 2021, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
9) During its meeting on 7 July 2021, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of pasteurised *Akkermansia muciniphila* as a NF pursuant to Regulation (EU) 2015/2283.

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Abbreviations

A	Akkermansia
ADME	absorption, distribution, metabolism and excretion
AMR	antimicrobial resistance
ANI	average nucleotide identity
ATCC	American Type Culture Collection
BIOHAZ	EFSA Panel on Biological Hazards
bw	body weight
CARD	comprehensive antibiotic resistance database
CFU	colony forming units
CIP	Collection de l'Institut Pasteur
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis Critical Control Points
HbA1c	glycated haemoglobin
ICP-MS	inductively coupled plasma mass spectrometry
ISO	International Organization for Standardization
LoD	limit of detection
LoQ	limit of quantification
LPS	lipopolysaccharides
MIC	minimum inhibitory concentration
NCBI	National Center for Biotechnology Information
NDA	EFSA Panel on Nutrition, Novel Foods and Food Allergens
NDARO	national database of antibiotic resistance organisms
ne	estimated number
NF	novel food/norme française
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
PCR	polymerase chain reaction
QPS	qualified presumption of safety
RH	relative humidity
rRNA	ribosomal ribonucleic acid
SNP	single nucleotide polymorphism
T3	triiodothyronine

T4	thyroxine
TFU	total fluorescent units
TG	test guideline
TSH	thyroid stimulating hormone
WBC	white blood cells
WGS	whole genome sequence