Meeting

# AU+ (Heat Killed Cells of Mycobacterium Aurum) Discussion Paper

### Committee Paper for Discussion - ACNFP/157/09

### Advisory Committee for Novel Foods and Processes

### Application for AU+ (Heat Killed Cells of Mycobacterium Aurum) as a Novel Food from Aurum Switzerland AG

### Application number RP1046

## Issue

An application has been received under the novel food authorisation process (regulation 2015/2283 as repatriated) for heat killed cells of *Mycobacterium aurum*. The Committee is asked to advise on whether the available data provides an adequate basis for a risk assessment, and whether the novel food is safe and not nutritionally disadvantageous under the proposed use and use levels.

## Introduction

1. An application was submitted to the Food Standards Agency in April 2021 by Aurum Switzerland AG, for the authorisation of Au+ (heat killed, whole cells of the species *Mycobacterium aurum* Aogashima) as a food ingredient under the novel foods regulation (EU) 2015/2283.

2. A previous application for *M. aurum* Aogashima (herein referred to as *M. aurum* ) as a food ingredient, from the same applicant, was considered by the Committee in January 2016; where a number of data gaps were identified.

*3. Mycobacterium* is a large bacterial genus with more than 150 species. Although the genus contains pathogenic species, notably *M. leprae, M. tuberculosis* and *M. avium* the applicant points out that these are the very few mycobacterial pathogens causing transmissible disease in immunocompetent people and/or animals. The applicant draws attention to the fact that *M. aurum* is an environmental species that lives harmlessly in the environment and enters the intestinal tract through potable water. The species is considered to be “pseudo-commensal”.

4. The *M. aurum* species intended to be marketed by the applicant is a nonmotile, non-spore-forming, non-pathogenic, non-genetically modified bacterium.

5. The applicant’s intention is to market Au+ as a concentrate of heat-killed *Mycobacterium aurum* Aogashima in suspension in purified water, to be taken as a food supplement. The applicant has declared they do not intend to make an application for assessment under the EU Nutrition and Health Claims Regulation.

6. The European Food Safety Authority (EFSA) has established a framework known as the Qualified Presumption of Safety (QPS) concept which provides a generic assessment system that can be applied to all requests received for the safety assessments of microorganisms deliberately introduced into the food chain. Microorganisms not considered suitable for QPS remain subject to a full safety assessment. *Mycobacterium aurum* has been evaluated and has not been recommended for the QPS list due to a lack of body of knowledge and uncertainty concerning its pathogenicity potential.

# Identity

7. The applicant has provided a range of data which characterises the identity of *M. aurum*. This includes information on the origin of the isolate, the culture bank used to store the strain, as well as studies and data which confirm the identity of the *M. aurum* strain used to produce Au+. Additionally, 16s rRNA gene sequencing information has been provided which includes phenotypic and genomic characterisation of the *M. aurum* strain used to produce Au+. The studies can be found in full within Annex A and a discussion of the results is contained within section 2.10.7.1 of the attached full technical dossier (Annex N – Full Technical Dossier).

8. The applicant relies on commissioned genomic studies performed by The University Of Newcastle, UK, to support the identity and characterisation of the microorganism which comprises Au+. The 16s rRNA sequence obtained from the

*M. aurum* Aogashima strain used in the manufacturing process for Au+ is 100% homologous with the type strain of *M. aurum* retained by the National Collection of Type Cultures (NCTC 10437$^{T}$). The applicant concludes that the information contained within the study report adequately evidences the identity of the microbiological component of Au+.

9. The applicant provides an argument - based on phylogenomic and comparative genomic analyses - for the reclassification of *Mycobacterium aurum* into a new genus '*Mycolicibacterium*', which is distinct from the pathogen-containing genus '*Mycobacterium*'. Please note that the applicant refers to the organism used in this novel food by the name '*Mycolicibacterium aurum*' in some parts of the dossier; whereas for consistency here it will remain to be called '*Mycobacterium aurum* or *M. aurum*'.

## Virulence and Antibiotic Resistance of the Source Microorganism

10. The species *Mycobacterium aurum* can be obtained from the department of Public Health in England (NCTC 10437) and is classified as Hazard group 1 by the Health and Safety Executive. Full details regarding the organisms characterisation and identity can be found within Annex A.

11. The applicant has conducted phenotypic testing for antimicrobial resistance on the Au+ production strain, which highlighted phenotypic resistance to ampicillin only. This was investigated further through screening for genes conferring resistance to ampicillin. There was no association between any genes and resistance to ampicillin, thus the effect was deemed to be solely phenotypic. This further supports the safety of Au+, as the microorganism used is unlikely to transfer any antimicrobial resistance genes throughout the environment, as resistance is phenotypic and the cells are heat-killed during the production of Au+. The full study report can be found attached as Annex H.

12. The *M. aurum* production culture genome was screened for antimicrobial resistance (AMR) conferring genes using the bioinformatic tools ResFinder and CARD webservers. Three hits were reported, suggesting possible antimicrobial resistance to rifampicin, penam, and Fosfomycin. However, the report states that all hits were below 97% homology and further investigation highlighted that the three genes are related to essential cell function and growth. The applicant concludes based on this study that there are no AMR genes associated with resistance to the antimicrobial compounds selected by EFSA for testing. Similarly

to the phenotype testing results, this would mean that the microorganism used to prepare Au+ poses very little risk of transferring antimicrobial resistance genes to the environment. The full AMR study report is attached as Annex I.

13. A study using the disk diffusion method for antimicrobial testing has been performed to investigate the antimicrobial activity of *Mycobacterium aurum.* The applicant states the study was carried out in accordance to the Clinical Laboratory Standards Institute methodology. In conclusion, the organism was not found to produce any antibiotics nor exhibit any antimicrobial activity against any of the test strains used within the study. Therefore, the microorganism used to produce Au+ is not expected to pose any health risks through antibiotic or antimicrobial activity. The full study report can be found attached as Annex J.

14. The *M. aurum* draft genome was screened for pathogenicity islands, and genes coding for toxins using various bioinformatic approaches. The applicant concluded that no pathogenicity islands were found during the study. And as no genes of concern were identified, the applicant suggests that the organism is nonpathogenic and safe. The full study report is attached as Annex K.

# Production Process

15. The product intended for consumption (Au+) is produced through propagation of an isolate of *M. aurum* Aogashima recovered from a GMP-licensed Development Cell Bank. The identity of the starting culture is confirmed through 16s rRNA sequencing and comparison with the National Collection of Type Cultures reference strain for *Mycobacterium aurum*.

16. The propagation is step-wise, starting with a volume of 1L and subsequently scaling-up to either 5L or 20L volumes within a bioreactor. Once propagation is complete, spent media is removed prior to resuspension in water and heat inactivation of living cells at 121°C for ≥ 20 minutes. The resulting product is then diluted in water before being packaged within ampules and shipped to the consumer. Down-stream microbial analyses are used to confirm that the production culture remains axenic. The applicant states that the growth media used within the bioreactor stage is proprietary, the composition of which can be found within Annex B, section 1.i.

17. The applicant demonstrates management of the process through a number of quality tests which ensure the identity, purity, viability and consistency of the culture during production. The identity of the culture is managed through comparison of 16s rRNA gene homology to the National Collection of Type Culture

reference strain; purity is confirmed through testing for other contaminating microbes. Viability assessments are made during the propagation stage and after heatinactivation of the cells – which ensures no viable organisms remain in the final product.

18. Full details of the production process can be found within Annex B. This includes production flow charts, internal specifications, and certificates of analyses relating to the production process.

## Compositional Data and Specifications

19. The final consumer product which the applicant intends to market consists of heatinactivated *M. aurum* suspended in purified water at a concentration of 0.03mg/mL.

20. Please note that both the compositional and analytical specification data for Au+/*M.* aurum has been combined into one section. The analytical data relates to the bulk product prior to dilution in purified water. The applicant states the reason for this is to increase analytical sensitivity. Therefore, any contaminants present in the bulk product are diluted by a factor of 1000 in the consumer product.

21. A specification table, which also contains compositional data for five independent batches of bulk product, has been provided by the applicant and is attached as Annex C. This includes a range of parameters including visual appearance, identity (16s rRNA sequence homology), heavy metal contaminants, and microbial contaminants. Also included are total aerobic microbial counts and viability assessments in order to ensure and evidence effective heat inactivation of living cells.

22. The applicant has provided methodology information as well as limits of detection where appropriate. The certificates of analysis for the compositional analyses are provided and can be found within Annex D. The applicant does not claim to hold any accreditations for GLP/GMP, or analytical techniques. However, they do hold the quality management accreditation ISO 9001:2015. A request for further information on accreditations was sent to the applicant, which revealed the facility responsible for production of their product was previously a licenced GMP facility, but ceased to renew their license in 2021. The applicant’s response to the RFI is attached as Annex M.

## Stability

23. The applicant has provided a range of analytical data which characterises and describes the stability of Au+ over a 24 month period. The data includes five batch, real-time monitoring of bulk samples stored at 5°C (+/-3°C), and a 9-month accelerated study at 40°C (+/-3°C) on one batch of bulk product.

24. The parameters chosen for measurement and monitoring of stability by the applicant were solely physiochemical. The parameters cover appearance, microscopic appearance (scanning electron microscopy), and particle size distribution.

25. The information summary pertaining to the stability of Au+ can be found in section 2.4.2 of the attached full technical dossier (Annex N), and full information can be found within Annex B, from page 11.

## History of Use and History of the Source

26. The applicant conducted a literature search for information on the history of *M. aurum* and its historic use as a food. The search methodology has been clearly described and inclusion and exclusion criteria have been stated.

27. There is a considerable lack of information surrounding the historic consumption of *M. aurum*. However, the applicant states that the microorganism has never been deliberately consumed within the EU, yet environmental exposure to the source organism is common. Environmental exposure is stated to occur through drinking water; a number of reference articles are provided as evidence. Information on the history of use provided by the applicant is found in section 2.6.2 of the full technical dossier (Annex N).

28. The applicant also draws upon the widespread consumption of other microorganism species, both viable and non-viable (heat-inactivated), in products such as yoghurts. This information is being used to support the case for purposeful consumption of microorganisms as food products. However, this does not necessarily support the safe consumption of *M. aurum* as a food supplement, as its use here is different to the use of microorganisms for the production of yoghurt and in its consumption.

## Proposed Uses, Use Levels, and Anticipated Intake

29. The target population for Au+ is stated to be the general population, excluding infants and young children – defined by the applicant as below the age of three years old. The applicant states there are no documented records indicating that the consumption of *M. aurum* by infants or young children is unsafe and that the exclusion is simply precautionary.

30. The proposed use of Au+ is as a food supplement, with a recommended daily intake of 0.33 mL of the product mixed into a beverage or taken directly. This dosing corresponds to an intake of 0.01 mg of *M. aurum* ($10^7$ cfu/mL) and is also the maximum recommended daily dosage. Au+ is not intended to replace any other food or provide any significant nutritional value.

31. The applicant has provided a consideration of combined exposure of *M. aurum* form other sources, in this case - drinking water only. The combined anticipated intake of the novel food is suggested to be 0.01 mg (the amount consumed in one dose of Au+) as there is no significant intake expected from any other food or source, including drinking water – from where the organism is found and consumed naturally.

32. The applicant does not expect there to be any consumer exposure to undesirable substances through consumption of Au+, based on the compositional analyses performed and management of the production process.

## Nutritional Information

33. Au+ has no nutritional value based on its nature and intended use levels. The applicant also states the novel food is not expected to create nutritional imbalance and no antinutritional activity was observed within the toxicological studies performed (see section below). This suggests that the novel food product is able to form part of the diet with minimal risk of altering nutritional intake in consumers.

## Toxicological Information

34. A range of toxicological studies and data have been provided by the applicant in support of the safety of Au+. The applicant states that all studies carried out are GLP-compliant and follow OECD guidelines. Information on GLP compliance and methodologies is included within each respective study report noted below.

## Genotoxicity

35. Both an *in vitro* reverse mutation assay and an *in vitro* micronucleus tests were performed on heat-killed *M. aurum* bulk product, following OECD 471 guidelines. Both studies indicate that the novel food has no mutagenic, clastogenic or aneugenic effects. The full genotoxicity reports are attached as Annex E (Ames Test) and Annex F (Micronucleus Test).

## Sub-chronic Toxicity

36. The applicant performed a 90-day sub-chronic oral toxicity study in rats, using a total of 80 animals (40 male and 40 female), according to OECD 408. Four different dosages were used in the study, ranging from 0 to 2,000 µg/kg/day. The animals used were examined daily and blood samples were taken for clinical pathology at week 13 of the study. No deaths were observed and no adverse effects related to the test item were reported by the applicant throughout the course of the study. The conclusion drawn from the study is that the test item (Au+) was well-tolerated by the test subjects when administered daily, at all dosage levels included.

37. The NOEL has been suggested to be 2,000 µg/kg/day by the applicant, which is the highest dosage used within the study. This value for the NOEL is higher than the proposed maximum dosage set by the applicant, which once adjusted corresponds to just 10 µg per day. This supports the claimed low toxicity of the novel food product, Au+, when consumed under the proposed conditions of use. The full 90-day study report can be found attached as Annex G.

## Allergenicity

38. The applicant provides a consideration of the allergenic potential of *M. aurum* /Au+ within their dossier. A bioinformatic approach was used and includes a 3Dmodelling-based analysis to demonstrate the lack of allergenic potential of Au+.

39. The study compares linear gene sequences which have been highlighted as potential protein/allergen coding regions within the *M. aurum* genome, against a database of known allergens. Sequence matches which have a homology of above thirty-five percent (>35%) are considered to be allergens. Initially the analysis showed fifteen potential allergenic proteins present, although none displayed similarity above the >35% threshold to be of concern. The applicant therefore states that the analysis did not suggest *M. aurum* would be allergenic or cause hypersensitivity reactions in humans, further supporting the safety of Au+

under the proposed conditions of use. The full study report can be found attached as Annex L.

40. The applicant also states that the lack of reports of allergenicity of *M. aurum* within the literature also supports the lack of allergenic potential of Au+. However, this has not been demonstrated with evidence by the applicant.

## Committee Action Required

- The committee is asked whether the available data provide a satisfactory basis for evaluating the safety of this novel food.
- If so, The Committee is asked whether it is content to recommend approval of Au+ as a food supplement.
- If not, The Committee is asked to indicate what additional data would be required.

Secretariat

January 2023

## Annexes:

Annex A – Confidential Identity

Annex B – Confidential Manufacturing

Annex C – Product Specification Table

Annex D – Composition Certificates of Analysis

Annex E – AMES Test Report

Annex F – Micronucleus Test Report

Annex G – Sub-chronic Oral Toxicity Study

Annex H – Phenotyping Study

Annex I – AMR Study

Annex J – Antimicrobial Activity

Annex K - Pathogenicity

Annex L – Allergenicity Study Annex M – Accreditations RFI

Annex N – Full Technical Dossier