

Safety Assessment of Microbial Based Cleaning Products for Use on Food Contact Surfaces – A Case Study

Sponsored by The American Cleaning Institute
(ACI) and

The International Association for Soaps, Detergents and
Maintenance Products (A.I.S.E.)



Contents

INTRODUCTION	3
CASE STUDY	4
Assessment Framework	4
Available body of knowledge	8
Absence of Toxin	10
VERO Cytotoxicity testing	10
Antimicrobial resistance (AMR) genes	11
Rationalisation of the identified AMR genes using MIC	13
Mobile Genetic Elements	15
Production Process	16
CONCLUSION	17
REFERENCES	18

INTRODUCTION

Microorganisms have been exploited by humans for their many benefits for centuries, for example in food production where they are used for fermentation. However, they are a relatively new innovation for the detergents industry that exploits their ability to metabolise soils and clean in hard-to-reach places. Microorganisms typically used in detergents are spore forming *Bacillus* species (non-pathogenic/ WHO risk group 1/ ACDP hazard group 1) that can be stably formulated into detergent products in their spore form and germinate in the presence of moisture and nutrients once applied to a surface to carry out their cleaning function.

There is no existing published risk assessment framework for assessing the safety of Microbial Based Cleaning Products. Guidance (Kim *et al.*, 2025) has been developed by the detergents industry (A.I.S.E./ACI) and is currently in the process of being peer reviewed for publication. However, that guidance does not specifically address how to carry out a risk assessment for use of a microorganism in a detergent product intended for use on food contact surfaces. Risk assessment frameworks do exist for use of microorganisms under parallel regulations however and these can be used as a starting point to assess the safety of detergent products for use on food contact surfaces (e.g. kitchen counters).

The European Food Safety Authority (EFSA) have published extensively on safety assessment of microorganisms for use in food and feed production. Their guidance on the characterization of microorganisms used as feed additives or as production organisms (EFSA 2018) sets out how a microorganism should be characterized and how this information can contribute to a risk assessment. As part of their process, EFSA have established the Qualified Presumption of Safety (QPS) list (Update to QPS, 2024). This is a list of specified microorganisms that have been pre-assessed for human and environmental safety. Microorganisms on the list are a taxonomic unit (TU) usually at species level for bacteria and, whilst this tells us much about the hazard to human health that a particular microorganism might pose, additional considerations at the strain level (including identified QPS qualifications) are also necessary including considerations about intended use (product type, exposure) and robustness of production methods. EFSA QPS list and associated guidance can be a useful starting point for an assessment for detergent products, provided consideration is given to the consumer exposure arising from use of the product.

Other key groups, who are publishing in this area have broadly taken a similar approach to this case study. These include the International scientific association for probiotics and prebiotics, ISAPP (Merenstein *et al.*, 2023) as well as a United States Pharmacopeia (USP) perspective (Roe *et al.*, 2022) and guidance from the scientific committee advising the Food Safety Authority of Ireland (FSAI, 2024) and these organisations also extensively reference the work of EFSA.

This document describes in detail a case study of a detergent product such as a general-purpose cleaning spray for use on food contact surfaces (e.g. kitchen counters), using a

specific example of *Bacillus amyloliquefaciens* BaXXX to illustrate the pathway through risk assessment.

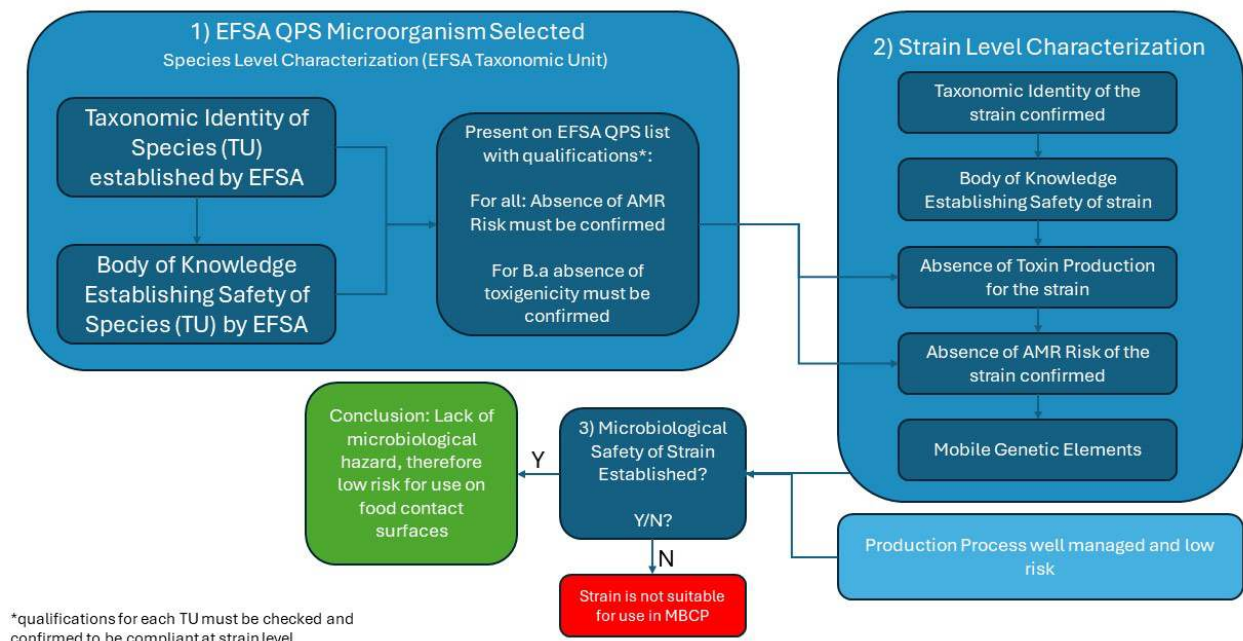
A product risk assessment should cover all relevant health effects arising from the intended and reasonably foreseeable use of a product. For a cleaning product this may include routes of exposure other than oral, e.g. skin, eye (accidental) or inhalation, depending on the product type and its intended use. However, for clarity, this case study will only focus on the hazard characterisation related to exposure via ingestion.

CASE STUDY

Assessment Framework

The below diagram and case study describes the approach taken to assess *Bacillus amyloliquefaciens* BaXXX in this case. It is not intended to be a guideline or definitive approach to safety assessment of microorganisms for use in MBCPs.

Overview of Framework for Risk Assessment of *Bacillus amyloliquefaciens* Intended for use in Products for Food Contact Surfaces



^ this case study is about an actual strain but coded for confidentiality

A. Taxonomic Identity

The taxonomic identity of the microorganism should be unambiguously established at species level (e.g. by whole genome sequencing), since this is crucial to ascertain the general safety of the strain based on the scientific body of knowledge. Additionally, high quality whole genome sequence data is required to perform specific strain level hazard assessment of the microorganism as described in the following steps of the framework (toxigenicity, antimicrobial resistance, and mobile genetic elements).

B. Body of Knowledge

As part of the assessment for pathogenicity, a thorough investigation is conducted of published literature to identify any reports of disease or illness related to the micro-organism under review.

C. Toxigenicity

The genome should be searched for presence of genes encoding toxins. If genes with similarity to known toxin genes are identified, then it should be established whether those genes are expressed (i.e. does the strain actually produce the toxin) in an *in vitro* assay. Only strains that do not produce toxins are considered safe for use in MBCP products, including those used on food contact surfaces.

D. Antimicrobial Resistance Assessment

Deliberately added microorganisms should not add to the pool of bacteria able to cause antibiotic resistant infections, or otherwise increase the spread of AMR genes. The genome should be searched for the presence of genes conferring antimicrobial resistance. Additionally, phenotypic testing against antimicrobials of clinical relevance to the specific organism should be carried out. If genes conferring resistance are found, then it should be established whether they are intrinsic to the species. Together these elements allow a risk assessment for antimicrobial resistance to be conducted. Only strains of low risk for AMR should be considered safe for use in MBCPs.

E. Mobile Genetic Elements

Evaluating the mobile genetic elements is focused on the analysis of WGS for elements that seem to have originated from other prokaryotes or eukaryotes and to understand the agents that effect DNA movement: plasmids, bacteriophages and transposons, which may transfer and potentially activate toxin and AMR genes in other microorganisms.

F. Production Process

A well-managed production process ensures that the strain remains genetically stable and pure over time, so that the hazard characterisation is appropriate at any stage of production. The final material specification remains constant and includes an absence of microbial contamination, including secondary

metabolites. It should be established that the production process is rigorously managed and periodically reviewed to ensure this is the case.

The use of whole genome sequence (WGS) data of the microorganism is a valuable source of information for risk assessment because bioinformatic analysis of the WGS can highlight the genes of interest with regard to antimicrobial resistance, toxin production and mobile elements. WGS is now regarded as the preferred technical method for bacterial characterisation for most purposes (FSAI, 2024).

In addition, a significant proportion of the safety considerations is based on historical data deriving from the body of knowledge available in the published scientific literature; therefore, the correct taxonomical designation is fundamental to attribute the correct information to the strains of interest.

Results

WGS was obtained for *Bacillus amyloliquefaciens* BaXXX, as this provides the most detailed information on the strain identity and offers additional information useful to assess the strain safety, such as the presence of pathogenic traits (e.g. toxin encoding genes) or the potential for antimicrobial resistance.

Available Body of Knowledge

The review of the body of knowledge for *Bacillus amyloliquefaciens* BaXXX was carried out by an independent research facility who were briefed in the following way:

Literature review/Hazard Identification for: *Bacillus amyloliquefaciens*, *Bacillus amyloliquefaciens* BaXXX

Information required:

- **In scope:** Case reports/studies indicating potential for human infection by the *Bacillus* species.
- **Out of scope:** *In vitro/in silico* studies indicating potential for cytotoxicity and/or AMR as these are separately covered below

Examples of information searched for
Is there any report of human infection by the strain? If yes, describe where possible: Severity of the disease (type of symptoms, fatalities; localised or generalised infection) Population (general population or only a subset such as immunocompromised, children etc) Exposure (what type of exposure led to infection: dermal, ocular, inhalation, ingestion?) Antibiotic treatment (was it necessary, was it available, was it effective?) Acquired antibiotic resistance (i.e. is there any report of failed Antibiotic treatment?)
Is there any report of production of toxins affecting human health? If yes, describe where possible: What is the involved toxin? Was the toxin produced under a particular environmental condition? Severity of symptoms (self-limiting – need for treatment/hospitalisation) Exposure route to the toxin causing the disease (inhalation, ingestion etc.)
With the above in mind, a literature search was carried out: Scientific literature search was carried out against <i>Bacillus</i> species in general and also to encompass the particular species of interest: - <i>Bacillus amyloliquefaciens</i> - The primary limit applied was to human health / clinical effects. - Language was limited to English The following were also used to refine the search output: Additional search terms “poisoning OR “food poisoning” OR bacteremia OR infection OR ingest* OR inhal* OR Respir* OR skin OR dermal OR dermis OR eye OR ocular” Search terms were chosen utilising the thesauri (where available) of the selected databases as identified below. Thesaurus terms help to increase the relevance of the search, although free-text terms were also used as applicable i.e. either when there were no thesaurus terms available or to ensure a broad coverage. Search terms, limits and section codes were built into the search strategy using Boolean logic (AND, OR, NOT).

Details of the databases searched are given below:
Main databases: - Biosis Previews (1926-) This database provides comprehensive worldwide coverage of life sciences and biomedical research from journals, meetings, books - Embase (1947-) A key resource for biomedical evidence, from published, peer-reviewed literature, in-press publications and conference abstracts. - Global Health (1910-) This database provides deep subject coverage of information relating to human health and communicable diseases - PubMed (1946-) Covers biomedical literature from MEDLINE and life sciences journals including the fields of biomedicine and health
Secondary databases: - Web of Science: Core Collection (1980-2020) contains over 21,100 peer-reviewed, high-quality scholarly journals published worldwide (including Open Access journals) in over 250 sciences, social sciences, and arts & humanities disciplines. Conference proceedings and book data are also available. - FSTA (1969-2020) covers literature on basic sciences relevant to food (biochemistry, microbiology, toxicology, etc.) as well as biotechnology, food safety, food processing, food products, patents, economics and legislation.
Websites of Governmental, non-governmental and other relevant organisation in individual countries: - US CDC (Centers for Disease Control and Prevention) - WHO (World Health Organization) - ECDC (European Centre for Disease Prevention and Control)

Results

No references were found at all relating *Bacillus amyloliquefaciens* and *Bacillus amyloliquefaciens* BaXXX to disease or human health issues.

For context, summary results of the wider study of *Bacillus* species

1. *Bacillus* species are widely found in the environment. In a food safety setting, the species of prime importance is *B. cereus*. In a clinical setting, *B. anthracis* is of importance. Neither of these species are in the scope of this investigation.
2. A number of *Bacillus* species are reported to be able to produce toxins.
3. There are reports of many *Bacillus* species being associated with food safety and clinical issues. However, the exception to this is *B. amyloliquefaciens*. No references were found to this species having given rise to human health issues.
4. Interestingly, although health issues are associated with some species of *Bacillus* [for example, *Bacillus subtilis*] in contrast in some parts of the world the same species is considered safe to use for production of fermented foods.
5. There are reports in the literature of beta-lactam antibiotic resistance by a number of species of *Bacillus*. However, a number of antibiotics e.g. clindamycin and vancomycin have been shown to be effective against these bacteria.

In summary, despite an extensive study of the available literature, there are no reports of *Bacillus amyloliquefaciens* being associated with any human health issue, and therefore the risk of pathogenicity is low.

Absence of Toxin

Method

Toxin genes were identified using the VFanalyser tool provided by the Virulence Factors Database (Liu et al. 2019). All hits were run using BLAST against the Interpro database (Apweiler et al. 2001) to further understand sequence identity. Historic haemolysis data were retrieved to support understanding of any haemolysis genes present.

Results

From the BLAST results there were three hits however only one of the hits is actually toxin related, Haemolysin III/D. The other 2 hits with *Clostridium* and *Pseudomonas* related sequences were more closely matched to genes for methyltransferase and adenylyl-sulfate kinase in *Bacillus*, respectively. These results are corroborated by the matches to the Interpro database. No plasmid-based toxin hits were found.

Therefore, the results do show that *Bacillus amyloliquefaciens* BaXXX possesses the toxin gene: **Haemolysin III / D**

Next step: Laboratory testing – VERO Cytotoxicity testing to investigate if the Haemolysin III/D gene identified is active against VERO cells

VERO Cytotoxicity testing

VERO Cytotoxicity testing is *in-vitro* testing used to assess the expression of toxin genes. In this case Haemolysin III / D was detected during bioinformatics screening of *Bacillus amyloliquefaciens* (above) so now the phenotypic behaviour needs to be assessed. This is generally done with an *in vitro* cytotoxicity assay on VERO cells.

Method

ASTM F895-11 (Standard Test Method for Agar Diffusion Cell Culture Screening for Cytotoxicity) study using African Green Monkey kidney cells (VERO ATCC CCL-81). The provided material was suspended in sterile Phosphate Buffered Saline (PBS). Cellulosic 13 mm filter discs were inoculated with 40µl of the test solution following placement onto the agar overlaid cell monolayers (Vero; ATCC CCL-81). PBS inoculated discs served as negative controls and wells overlaid with rubber latex sections served as positive controls.

Results

The suspension tested did not exhibit cytotoxicity on the VERO cell line as per the agar diffusion method.

In summary, a negative outcome indicates that the gene is not functional, therefore the potential for toxigenicity upon ingestion is low.

Antimicrobial resistance (AMR) genes

At the same time as being identified for AMR, potential resistance genes will be evaluated for “intrinsic or species-specific resistance”; this is an organism's ability to thrive in the presence of an antimicrobial agent, where this ability is not readily horizontally transferable and is typical of the strains of that given species (Mathur and Singh, 2005). Alternatively, “acquired resistance” can be defined as resistance that is not commonly associated with the given species and this resistance has been acquired either via mutation of indigenous genes or the acquisition from an external vector (EFSA 2012).

The aim of this exercise is to identify all genes on WGS for *Bacillus amyloliquefaciens* BaXXX that confer “acquired resistance” and screen out those that are considered “intrinsic”.

Method of genome analysis for AMR genes

Analysis was done via The Comprehensive Antibiotic Resistance Database (CARD) (Alcock et al. 2020) to highlight all resistance gene determinants and the RESfinder tool to identify potential acquired determinants (Zankari et al. 2012). Each database was interrogated with thresholds fixed to 70% homology and 60% coverage. These thresholds are more stringent than those recommended by EFSA (80% homology and 70% coverage) to increase the sensitivity of the analysis in this case. Plasmid sequences derived from PLASMIDSPAdes were run against CARD and RESfinder.

Genome was interrogated for:

- antimicrobial resistance genes
 - o antimicrobial defined as those relevant by EFSA plus those highly important or critically important antimicrobial as determined by the World Health Organization (WHO) (WHO, 2017).
- Potentially acquired AMR determinants
- Plasmid sequences (identified by PLASMIDSPAdes)

In addition to this, searched in the genome annotation for the keywords: “antibiotic”, “drug”, “resist”, “mycin”, “cillin” “phenicol” and “cycline”.

Results

CARD results at 70% homology/60% coverage thresholds identified **Resistance Gene Identifier (RGI)**

Strict hits:	Loose hits:
Tetracycline and Linezolid	covered a range of potential antibiotic compounds: of which Elfamycin and Tunicamycin were excluded due to their absence from the important/critical WHO lists (WHO. 2017).

RESfinder results 70% homology and 60% coverage threshold:

- Tet (L) likely confer Tetracycline resistance
- Cfr (B) likely to confer linezolid resistance

Direct searching **found** Lincomycin and Tetracycline which needed to be further investigated.

A study of the published material indicated that: the presence of Tetracycline resistance gene is intrinsic to *Bacillus sp.*, that Penicillin has a long history of intrinsic resistance within *Bacillus* (hence the exclusion of ampicillin from the FEEDAP panel), and Bleomycin is a cancer chemotherapy drug that does not appear on the WHO list and nor does Tunicamycin (WHO. 2017), so they could all be excluded from further studies.

So based on the above investigations and including those on the advised EFSA FEEDAP panel, the following antimicrobials are to be sent for MIC testing (below):

EFSA FEEDAP panel	Additional agents identified for MIC
Vancomycin, gentamicin, kanamycin, streptomycin, erythromycin, clindamycin, tetracycline, chloramphenicol.	Ceftriaxone or similar, cephalosporin, Daptomycin, Levofloxacin, Lincomycin, Linezolid, Neomycin, Rifampin, Rifamycin, Streptogramin A, Teicoplanin, Tobramycin, Virginiamycin B, Virginiamycin M.

Next step: Laboratory testing - MIC testing against identified antimicrobials to assess the nature of the resistance genes

Rationalisation of the identified AMR genes using MIC

The aim of the work is to determine the nature of the resistance to any of the antimicrobial compounds screened (previous) and, where resistance cannot be deemed as intrinsic, to determine the risk of that resistance gene.

Minimum inhibitory concentration (MIC) testing was carried out with broth microdilution methods, based on Clinical Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) methodologies where breakpoints were defined from various sources.

Breakpoint determination

In order to determine if an MIC to an antibiotic compound constitutes it as resistant, it needs to be compared to a breakpoint. There is a library of well-established breakpoints for clinically relevant strains for the vast majority of antibiotic compounds.

However, for many strains of *Bacillus*, these data were lacking (at the time this case study was done*) due to low pathogenicity within the genus. Therefore, multiple sources from both clinical institutions and scientific literature were drawn upon to determine breakpoints for compounds lacking breakpoint data for example appropriate epidemiological cutoff values were set using literature data for daptomycin and tobramycin.

Where no specific genus relevant data could be found for a compound, breakpoints for related organisms were taken as an indicator of resistance as per the methods employed in scientific literature (Massilamany et al. 2016).

* More recently *Bacillus* spp breakpoints have been published by EUCAST (2024) for relevant antibiotics which can be considered in future studies.

Analysis of AMR genes of relevance

For all genes that gave MIC results above the breakpoints, either BLASTP or BLASTX was used to find homologous sequences within the Refseq_Protein database against the Bacteria and Archaea genetic code (Atschul et al. 1997). Search results were filtered based on minimum cut-off values of 80% for sequence identity and 75% for sequence coverage and taxonomy was inferred for all gene hits using the taxonomy tool provided with the BLAST web application. Results that yielded significant relation to the *Bacillus* genus and the species in question were considered as evidence of intrinsic resistance as per the methodologies of Glenwright et al. (2017). Literature sources were also mined for each AMR gene of relevance to find evidence of either intrinsic or acquired resistance.

Results

Screening of the *Bacillus amyloliquefaciens* BaXXX strain MIC results revealed 3 MICs above the defined breakpoint parameters: Lincomycin, Streptogramin A and Virginiamycin M.

Lincomycin

The presence of lincomycin resistance gene **LmrB** was found previously via CARD searches and direct grep mining of the genome annotation.

Using BLASTP, 100 instances of the gene were found related to 19 different members of the *Bacillus* genus, 4 in the *Bacillus amyloliquefaciens* group. The other hits were related to a range of members across the *Bacillus* genus indicating that this gene is intrinsic to the *Bacillus* genus (Glenwright et al. 2017).

Following the REScon framework (Martinez et al. 2014) further supported that this gene is intrinsic.

Streptogramin A and Virginiamycin M

The **vmIR** gene was identified via CARD searches.

Using BLASTP 100 instances of the gene were found related to 14 different members of the *Bacillus* genus, 4 in the *Bacillus amyloliquefaciens* group. The other hits were related to a range of members across the *Bacillus* genus indicating that this gene is intrinsic to the *Bacillus* genus (Glenwright et al. 2017).

In summary, screening using WGS for *Bacillus amyloliquefaciens* BaXXX identified 3 potential AMR genes of concern, however further testing and screening confirmed these genes as being intrinsic. Since the strain does not harbour any 'acquired' antimicrobial resistance genes, the potential for AMR development to therapeutic antimicrobials is considered low, as verified by phenotypic MIC testing.

Mobile Genetic Elements

Method

Aim to identify bacterial mobile genetic elements (MGEs), such as conjugative plasmids and integrative and conjugative elements (ICEs), as these have been highlighted as important vehicles for the dissemination of pathogenesis and antimicrobial-resistance genes.

Screen using the OriTfinder tool to look for MGEs and screen the PHASTER tool to identify phage sequences (Arndt et al. 2016).

This approach was taken to identify the most probable types of horizontal gene transfer; however, it is recognised that there are many other factors beyond this including factors such as transposons that may need further consideration (Partridge et al. 2018).

Results

The initial screen via the OriTfinder tool failed to show any complete modules for transfer of AMR genes via conjugation, but screening from the PHASTER tool did reveal intact phage sequences (and a further study was carried out – see below).

Next step: Mobility determination of intact phage sequences

Method

Intact phage sequences were screened for AMR and virulence genes using CARD (Alcock et al. 2020), RESfinder (Zankari et al. 2012) and the virulence factors database (VFDB) (Liu et al. 2019a). ICEberg 2.0 (Liu et al. 2019b) was used to detect ICE (negative and Conjugative Elements) or IME (Integrative and Mobile Elements with any positive sequences then screening for AMR genes and toxin genes as per the aforementioned methodology. Plasmid sequences were considered mobile elements for the purposes of this study.

Results

Screening of intact phage elements previously identified via PHASTER (Arndt et al 2016) found no resistance genes present upon the DNA elements and screening of mobile genetic elements identified by ICEfinder (Liu et al. 2019b) found no integrative and conjugative or mobile elements.

In summary, there are no mobile elements for AMR and toxin genes, nor the phage elements found. Therefore, the mobility potential via horizontal gene transfer for *Bacillus amyloliquefaciens* BaXXX is low.

Production Process

Systems and processes are in place to ensure production is well controlled to maintain purity and stability of the parent culture as well as to prevent contamination from unwanted microorganisms and their metabolites.

Summary of Results

The supplier is experienced in microbiological management with over 20-years' experience in producing fermentation products. The organism *Bacillus amyloliquefaciens* BaXXX originated from a plant source, and stocks are kept at NCIBM where fresh lyophilised seeds can be requested when needed.

Microbiologists play a key role in the process by:

- overseeing the creation of stored seeds whilst checking purity at the same time. These pure cultures are stored close to the production facility for access/reference.
- preparation of the microorganism for production, by visually checking colony morphology, subculturing to inoculate vessels of increasing size until the production vessel is prepared.
- Taking samples to ensure no contamination is taking place during production.

Good manufacturing practices (GMP) procedures are in place as well as Risk Analysis by Hazard Analysis and Critical Control Point (HACCP). A flowchart and a description of the production process is also available, which indicates good management and quality control.

The growth in the reactor vessel is monitored using a variety of methods including Optical Density (OD600), dissolved oxygen and phase contrast microscopy and towards the end of fermentation the cells undergo sporulation. Dissolved oxygen and biomass cell density are used as quantitative markers for the end point of a run. This is combined with qualitative visualisation methods to ensure that the vast majority of cells have sporulated and that any aggregates or clumps have broken up. A pasteurisation step follows of 65 °C for 30 minutes to eliminate viable vegetative cells (including potential contaminants), followed by centrifugation and spray drying at 125°C.

The final material from each batch has a microbial specification, which is set at nondetectable using appropriate methods of analysis for the following species: Gram negative bacteria, *Bacillus cereus*, *Listeria*, Yeasts and Moulds, *Salmonella/Shigella*, *Staphylococcus aureus* and *Escherichia coli*.

In summary, the audit of the supplier demonstrated satisfactory management procedures in place to ensure control of the integrity of production strain *Bacillus amyloliquefaciens* BaXXX.

CONCLUSION

In this case study, *Bacillus amyloliquefaciens* BaXXX was characterized for hazards relevant to 'ingestion' as a route of exposure from its use in a cleaning product intended for food contact surfaces.

In summary, the strain was found to be low risk based on the following:

- It is non-pathogenic (from body of knowledge survey)
- It is non-toxicogenic (absence of active toxin gene)
- It is not resistant to therapeutic antimicrobials
- It does not harbour any 'acquired' antimicrobial resistance genes
- It lacks the ability to spread antimicrobial resistance genes and toxins to other microorganisms via horizontal transfer
- It is manufactured in a production process that has been designed and controlled to protect specific strain characteristics and prevent microbiological contamination
- There is a reference of the strain available in a standard culture collection repository, NCIMB.

A cleaning product containing *Bacillus amyloliquefaciens* BaXXX (MBCP) is intended for use around the home, including on food contact surfaces in the kitchen; given the use of the product in this way, this microorganism could be ingested via residues passing from hand to mouth and/or eating food which has been in contact with these cleaned surfaces.

Note that this assessment does not obviate the need for a risk assessment covering other health effects that may be necessary considering the product type, its manner of use and the other ingredients contained within it.

However given the hazard characterisation in this case study, the risk of infection or intoxication when exposed to *Bacillus amyloliquefaciens* BaXXX via ingestion is considered low

REFERENCES

1. ACDP List [The Approved List of biological agents: Advisory Committee on Dangerous Pathogens \(hse.gov.uk\)](https://hse.gov.uk/pathogens/)
2. Alcock BP, et al. CARD 2020: antibiotic resistance surveillance with the comprehensive antibiotic resistance database. *Nucleic Acids Res.* 2020 Jan 8;48(D1):D517-D525. doi: 10.1093/nar/gkz935. PMID: 31665441; PMCID: PMC7145624.
3. Altschul SF, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* 1997 Sep 1;25(17):3389-402. doi: 10.1093/nar/25.17.3389. PMID: 9254694; PMCID: PMC146917.
4. Apweiler R, et al. The InterPro database, an integrated documentation resource for protein families, domains and functional sites. *Nucleic Acids Res.* 2001 Jan 1;29(1):37-40. doi: 10.1093/nar/29.1.37. PMID: 11125043; PMCID: PMC29841.
5. Arndt D, et al. PHAST, PHASTER and PHASTEST: Tools for finding prophage in bacterial genomes. *Brief Bioinform.* 2019 Jul 19;20(4):1560-1567. doi: 10.1093/bib/bbx121. PMID: 29028989; PMCID: PMC6781593.
6. ASTM (American Society for Testing and Materials) [F895 Standard Test Method for Agar Diffusion Cell Culture Screening for Cytotoxicity \(astm.org\)](https://www.astm.org/standards/F895/)
7. Clinical Laboratory Standards Institute (CLSI) [Clinical & Laboratory Standards Institute: CLSI Guidelines](https://www.clinical-laboratory-standards.org/standards-guidelines)
8. EUCAST (The European Committee on Antimicrobial Susceptibility Testing) [eucast: Clinical breakpoints and dosing of antibiotics](https://www.eucast.org/clinical breakpoints and dosing of antibiotics)
9. EFSA (2012) Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. *EFSA Journal* 10(6), 2740.
10. EFSA (2018) Guidance on the characterisation of microorganisms used as feed additives or as production organisms. *EFSA Journal*, 16(3), 5206.
11. EFSA (2024) [Update of the list of qualified presumption of safety \(QPS\) recommended microbiological agents intentionally added to food or feed as notified to EFSA 20: Suitability of taxonomic units notified to EFSA until March 2024 | EFSA \(europa.eu\)](https://www.efsa.europa.eu/en/press/news/24-05-01)
12. European Committee on Antimicrobial Susceptibility Testing (EUCAST) methodologies [eucast: AST of bacteria](https://www.eucast.org/AST of bacteria)
13. FSAI (2024) [Assessment of the safety of “probiotics” in food supplements | Food Safety Authority of Ireland \(fsai.ie\)](https://www.fsai.ie/assessments/assessment-of-the-safety-of-probiotics-in-food-supplements)
14. Glenwright H, et al. The Identification of Intrinsic Chloramphenicol and Tetracycline Resistance Genes in Members of the *Bacillus cereus* Group (*sensu lato*). *Front Microbiol.* 2017 Jan 4;7:2122. doi: 10.3389/fmicb.2016.02122. PMID: 28101085; PMCID: PMC5209696.
15. Kim, J., Boesenberg, D., & Van Trump, I. (2025). Risk Analysis Approaches for Microbial Ingredients in Microbial Based Cleaning Products. *Risk Analysis*, 45(2), 123-145. <https://doi.org/10.1111/risa.17707>
16. Liu B, Zheng D, Jin Q, Chen L, Yang J. VFDB 2019: a comparative pathogenomic platform with an interactive web interface. *Nucleic Acids Res.* 2019 Jan 8;47(D1): D687-D692. doi: 10.1093/nar/gky1080. PMID: 30395255; PMCID: PMC6324032.
17. Liu M, Li X, Xie Y, Bi D, Sun J, Li J, Tai C, Deng Z, Ou HY. ICEberg 2.0: an updated database of bacterial integrative and conjugative elements. *Nucleic Acids Res.* 2019 Jan 8;47(D1):D660-D665. doi: 10.1093/nar/gky1123. PMID: 30407568; PMCID: PMC6323972.

18. Martínez JL, Coque TM, Baquero F. What is a resistance gene? Ranking risk in resistomes. *Nat Rev Microbiol*. 2015 Feb;13(2):116-23. doi: 10.1038/nrmicro3399. Epub 2014 Dec 15. PMID: 25534811.
19. Massilamany C, et al. Whole genomic sequence analysis of *Bacillus infantis*: defining the genetic blueprint of strain NRRL B-14911, an emerging cardiopathogenic microbe. *BMC Genomics*. 2016 Aug 22;17 Suppl 7(Suppl 7):511. doi: 10.1186/s12864-016-2900-2. PMID: 27557119; PMCID: PMC5001198.
20. Mathur S, Singh R. Antibiotic resistance in food lactic acid bacteria--a review. *Int J Food Microbiol*. 2005 Dec 15;105(3):281-95. doi: 10.1016/j.ijfoodmicro.2005.03.008. Epub 2005 Nov 8. PMID: 16289406.
21. Merenstein, D., Pot, B., Leyer, G., Ouwehand, A. C., Preidis, G. A., Elkins, C. A., ... Sanders, M. E. (2023). Emerging issues in probiotic safety: 2023 perspectives. *Gut Microbes*, 15(1). <https://doi.org/10.1080/19490976.2023.2185034>. [from ISAPP meeting- International Scientific Association for Probiotics and Prebiotics]
22. [oriTfinder \(sjtu.edu.cn\)](http://oriTfinder.sjtu.edu.cn)
23. Partridge SR, et al. Mobile Genetic Elements Associated with Antimicrobial Resistance. *Clin Microbiol Rev*. 2018 Aug 1;31(4):e00088-17. doi: 10.1128/CMR.00088-17. PMID: 30068738; PMCID: PMC6148190.
24. Roe, A.L., Boyte, M.E., Elkins, C.A. et al. (2022) Considerations for determining safety of probiotics: A USP perspective. *Regulatory Toxicology and Pharmacology* 136, 105266
25. VERO ATCC CCL-81 African Green Monkey kidney cells [Vero - CCL-81 | ATCC](https://atcc.ccr.cit.nih.gov/cell-lines/cell-line-detail.aspx?cellLine=VERO)
26. World Health Organization. Laboratory biosafety manual. – 3rd ed (2004) [9241546506 eng.pdf \(who.int\)](https://www.who.int/publications-detail/9241546506-eng.pdf)
27. World Health Organisation (2017) [Critically Important Antimicrobials for Human Medicine : 5th Revision \(who.int\)](https://www.who.int/publications-detail/critically-important-antimicrobials-for-human-medicine-5th-revision)
28. Zankari, et al. Identification of acquired antimicrobial resistance genes, *Journal of Antimicrobial Chemotherapy*, Volume 67, Issue 11, November 2012, Pages 2640–2644, <https://doi.org/10.1093/jac/dks261>