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COSMETIC PRODUCTS CONTAINING MICROBIOME - MODULATING INGREDIENTS – A SAFETY ASSESSMENT APPROACH

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Introduction

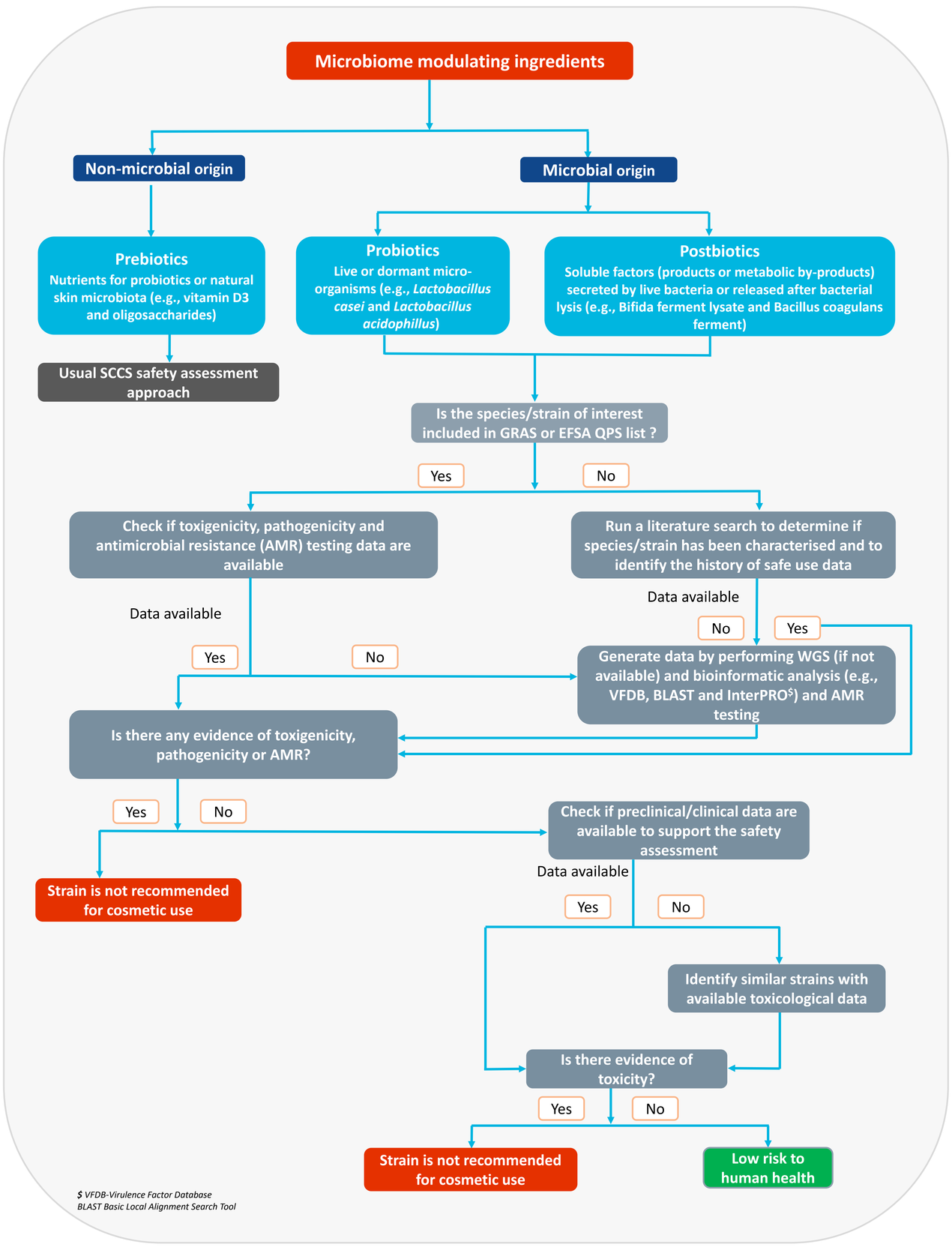
Recently, there has been a growing interest in the launch of cosmetic products containing microbiome-modulating ingredients. These ingredients fall into three categories: pre-, pro-, or postbiotic, depending on factors like their origin, viability, and intended function. Prebiotic ingredients originate from plants, while postbiotics mainly consist of ferments, lysates, extracts, or filtrates from microorganisms. Viable microorganisms, which are added to a cosmetic product to achieve a benefit at the application site, are generally called probiotic ingredients (ICCR, 2021;2022).

Prebiotic ingredients typically do not contain living or non-viable microorganisms, and their safety evaluation follows the usual SCCS assessment practices. On the other hand, for probiotics/postbiotics, the requirements set by the Scientific Committee on Consumer Safety (SCCS) for 'complex substances derived from biotechnology' come into play (SCCS, 2023). Currently, specific regulatory guidelines or a clear framework outlining the requirements and assessment methods for these ingredient types in cosmetics are lacking. Nonetheless, the European Food Safety Authority (EFSA) has established a framework for assessing similar ingredients in food, feed additives and plant protection products (EFSA, 2007). Considering the safety assessment principles outlined by EFSA and the general requirements set by SCCS for cosmetic ingredients, this poster presents a systematic approach to evaluate the safety of cosmetic products containing microbiome-modulating ingredients.

The first step involves a comprehensive literature search to identify the regulatory status of the microbial strain (e.g., biosafety or risk level classification, 'generally recognized as safe' (GRAS) status, EFSA qualified presumption of safety (QPS)), available literature data and history of safe use for the microbial strain. The next step includes the assessment of the available data on toxigenicity, pathogenicity, antimicrobial resistance (AMR), and the preclinical/clinical toxicological data. In the case of a novel strain with no data, whole genome sequencing (WGS) followed by bioinformatic analysis and evaluation of toxicological data on a related strain should be performed. Finally, a weight-of-evidence approach is used to conclude the safety of the microbial strain.

Two case studies are presented to demonstrate the safety assessment approach for data-poor and data-rich microbiome-modulating ingredients for the use in cosmetics.

Workflow for the safety assessment of microbiome-modulating ingredients in cosmetics



Case study I: Data rich micro-organism strain

OBJECTIVE

The safety evaluation of '*Bifidobacterium longum subsp. infantis M-63'* strain was performed for its potential use in a cosmetic formulation.

Steps	Available information
Step 1 Literature search and data gaps	• FDA GRAS: There is a GRAS notification for the strain *'B. longum subsp. infantis M-63'* (Morinaga, 2021), which has been reviewed by US FDA with 'no questions' raised. • Clinical data: Multiple clinical studies of 1 to 6 months duration in adults, children and infants were reported (Morinaga, 2021). • History of use: *B. longum subsp. infantis M-63* containing ingredients for use in infant formulas are being sold in international markets since 2006 (Morinaga, 2021).
Step 2 Evaluation of safety (toxigenicity, pathogenicity, AMR and toxicological data) **Colony Forming Units*	• Toxigenicity and Pathogenicity: – The GRAS notification concluded that *B. longum subsp. infantis* M-63 is non-pathogenic and non-toxigenic (Morinaga, 2021). • AMR: – Based on the results of available studies, *B. longum subsp. infantis M-63* is reported to be resistant to streptomycin. However, the resistance does not pose risks to consumers as it is due to a gene mutation or recombination event rather than due to the presence of an actual streptomycin resistance gene. In addition, the risk of transfer is considered negligible as no plasmids have been found (Morinaga, 2021). • Toxicological data: – *B. longum subsp. Infantis* M-63 was not toxic up to highest tested dose of 4000 mg/kg bw (3.2×10^{11} CFU*/kg bw) in an acute toxicity study in rats (Morinaga, 2021). – It was not toxic up to the highest tested dose of 7.6×10^{10} CFU/kg bw in a subchronic toxicity study in rats (Morinaga, 2021). • Clinical data: – The results of multiple clinical trials (8 weeks in children and 12 weeks in adults) support the safe use of *B. longum subsp. infantis* M-63 in children at doses up to 1.0×10^9 CFU/day, and in adults at doses up to 1.25×10^{10} CFU/day (Morinaga, 2021). – The results of multiple studies in infants showed no adverse effects at doses up to 2.8×10^{10} CFU/day *B. longum subsp. infantis* M-63 (Morinaga, 2021).

CONCLUSION

Based on 1) the GRAS status of the strain, 2) the history of safe use, 3) the absence of toxigenicity, pathogenicity potential, 4) the absence of risk due to AMR and 4) the absence of any toxicity in preclinical/clinical studies, it can be concluded that there is a low risk to human health from the use of '*B. longum subsp. infantis M-63'* strain in the cosmetic formulation.

Case study II: Novel microorganism strain

OBJECTIVE

The safety evaluation of a novel '*Bacillus toyonensis* strain XY#', which belongs to the *Bacillus cereus* group, was performed for potential use in a cosmetic formulation. The strain has not been specified due to confidentiality reasons.

Steps	Available information
Step 1 Literature search and data gaps	• EFSA QPS or GRAS: The novel strain was not listed in either EFSA or GRAS lists. However, B*acillus cereus var. toyoi* species was listed in EFSA QPS (EFSA, 2023). • History of use: A related strain, *B. toyonensis,* BCT-7112^T, is approved as a feed additive for animals (EFSA, 2023). • **No data on toxigenicity, pathogenicity, AMR and/or toxicological data was identified on the strain.**
Step 2 WGS and Bioinformatics data generation	• WGS and bioinformatic analysis using VFDB, BLAST and InterPro were generated. • AMR screening study to determine the minimum inhibitory concentration (MIC) was performed.
Step 3 Evaluation of safety (toxigenicity, pathogenicity, AMR data)	• Toxigenicity and pathogenicity: – Based on improved next-generation WGS sequencing techniques and screening against the BLAST, InterPro and VFDB, the strain did not show the presence of genes encoding thiol-activated and haemolysin BL, cytotoxin K and haemolysin II, anthrax, certhrax and cereulide toxins. – A conventional *in vitro* assay did not show the presence of Bacillus diarrheal enterotoxin. • AMR: – The screening study revealed 3 MICs above the defined breakpoint parameters; the AMR was considered to be intrinsic to the *Bacillus* genus for which has a history of safe use. – An additional screening in PHASTER and ICEfinder, showed no association of mobile elements with AMR or major toxin genes.
Step 4 Evaluation of safety (toxicological data on related strain)	• *'Bacillus toyonensis* BCT-7112', was identified to have 60-70% homology with the novel strain. Therefore, the data from '*B. toyonensis* BCT-7112' was considered for assessing the safety of *'B. toyonensis strain XY#'.* – The BCT-7112^T was not found to be genotoxic in Ames and chromosomal aberration assays (Williams *et al.,* 2009). – It was not toxic in acute and subacute/subchronic/chronic repeated-dose toxicity studies in rats and mice at up to 3×10^{11} spores/kg bw/day (Williams *et al.,* 2009). – In an 8-day human clinical trial, the homologous strain did not cause any adverse effects in healthy male and female subjects at 1×10^9 and 1×10^{10} spores/kg bw/day (Williams *et al.,* 2009). • Since EFSA proposed 10^5 CFU per gram foodstuff for *B. thuringiensis,* which is part of the *B. cereus* group, the same threshold can be proposed for *'B. toyonensis strain XY#'.*

CONCLUSION

Based on 1) the history of use of the *B. cereus* group, 2) the results of the bioinformatic analysis and AMR testing for the novel '*B. toyonensis XY#'* strain, and 3) the toxicological data on the similar '*B. toyonensis BCT-7112' strain*, it can be concluded that there is a low risk to human health related to the use of the '*B. toyonensis XY#'* strain in cosmetic formulation.

References

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