Australian Government
Department of Health
Therapeutic Goods Administration

Compositional Guideline for *Streptococcus salivarius* M18

Name of the ingredient

Streptococcus salivarius

Synonym

Streptococcus salivarius M18, BLIS M18™

Definition of the ingredient

Streptococcus salivarius is a bacterium found as part of the normal human oral microflora, predominately colonising the oral cavity (teeth, tongue). They are Gram positive, cocci which grow in chains or pairs, are catalase negative and nonmotile. On blood agar they form non-haemolytic colonies. They form distinctive mucoid colonies when grown on sucrose agar, due to the production of extracellular polysaccharides.

BLIS M18™ is a freeze-dried culture of *Streptococcus salivarius* M18 (NLT 1 x 10^{11} CFU/g) in a protective matrix of food-grade Maltodextrin, Trehalose Dihydrate and Lactitol.

S. salivarius M18 has been deposited in the American Type Culture Collection (ATCC) as ATCC BAA 2593. It also has been lodged with the German Collection of Microorganisms and Cell Cultures Deutsche Sammlung von Mikroorganismen und Zellkuturen GmbH) under the accession number DSM 14685. The strain is also patented for the prevention of dental caries (Patent number: US 7,226,590 B2) in the following territories: New Zealand, United States, European Union, Canada, Australia, China, India, Denmark, Finland, France, Germany, Italy, Netherlands, Spain, Sweden, Switzerland and the United Kingdom.

Table 1. Ingredient specific requirements

Test	Method reference	Acceptance criteria
Description		
Appearance	Visual evaluation	Free flowing off-white powder
Odour/ taste	Organoleptic evaluation	Proteinaceuous taste

Test	Method reference	Acceptance criteria
Characteristics		
Particle size	Sieve analysis	90 % NMT 500 µm
Water activity (aw)	USP <1112>	NMT 0.25
Identification		
Microscopic morphology	USP <1113> Gram staining	Gram reaction: positive Cellular shape: cocci in chains or pairs
Macroscopic morphology	Visual examination of growth on Mitis salivarius agar at 37°C in 5 % CO_2 in air after 24-48 hr	Colony size: 1-2 mm in diameter Colony shape: · Form: round · Margin : entire · Elevation: convex Colony colour: blue Colony texture: mucoid
	Visual examination of growth on Blood agar [Columbia Agar Base with 5 % human blood] at 37°C in 5 % CO_2 in air after 24-48 hr	Colony size: NMT 1 mm in diameter Colony shape: · Form: round · Margin: entire · Elevation: convex Colony Colour: white Colony Texture: butyrous Haemolysis: None
Biochemical profile	USP <1113> biochemical tests and/ or Automated microbial identification test (e.g. API 20 Strep test system)	Negative for: catalase Positive for: acetoin production, β-glucosidase, α- galactosidase, β-galactosidase leucine aminopeptidase, D- lactose, D-trehalose, inulin, and D-raffinose.

Test	Method reference	Acceptance criteria
Molecular identification of strain	Heng NC, (2011)[1]	Matches the sequence for *S. salivarius* M18
Deferred antagonism, P-typing producer	Tagg and Bannister (1979)[2], Burton (2013)[3]	Matches the fingerprint for *S. salivarius* M18 (677)
Assay		
Streptococcus salivarius M18	Viable plate count on CAB K12 agar cultured at 37°C in 5 % CO_2 in air after 24-48 hr per Ishijama (2012)[4], Burton (2011)[5] modified to enumerate M18	NLT 1 x 10^{11} CFU/g

Table 2. Incidental constituents

Test	Method reference	Acceptance criteria
Microbiology		
Total aerobic microbial count @35˚C	Compendium 5th Edition 2015, Chapter 8 (excluding *S. salivarius* M18)	NMT 10,000 CFU/g
Coliforms	Compendium 5th Edition 2015 chapter 9 modified	Not detected/g
E. coli	Compendium 5th Edition 2015 chapter 9 modified	Not detected/g
Salmonella	ISO 6579:2002 (E)	Not detected/25g

[1] Heng NC, (**2011**). Genome sequence of the bacteriocin-producing oral probiotic Streptococcus salivarius strain M18. *J Bacteriol 193(22)*, 6402-3.
[2] Tagg, J. R. & Bannister, L. V. (1979). "Fingerprinting" B-Haemolytic Streptococci by their Production of and Sensitivity to Bacteriocine-Like inhibitors. The Journal of Medical Microbiology, 12(4), 397-411
[3] Burton JP, W. P. (2013). Persistence of the oral probiotic Streptococcus salivarius M18 is dose dependent and mega plasmid transfer can augment their bacteriocin production and adhesion characteristics. *PLoS One*, Jun 13;(8(6)), e65991
[4] Ishijima SA, H.K. (2012). Effect of Streptococcus salivarius K12 on the in vitro growth of Candida albicans and its protective effect in an oral candidiasis model. *Appl Environ Microbiol*., 78(7), 2190 – 2199
[5] Burton JP, C.S. (2011). Evaluation of safety and human tolerance of the oral probiotic Streptococcus salivarius K12: a randomized, placebo-controlled, double-blind study. *Food Chem Toxicol*., 49(9), 2356-2364

Test	Method reference	Acceptance criteria
Mesophilic aerobic spore count @ 35°C	Compendium 5th Edition 2015 chapter 23	NMT 200 CFU/g
Coagulase producing Staphylococcus aureus	ISO 6888-3:2003	Not detected/g
Moulds	BP	NMT 50 CFU/g
Yeasts	BP	NMT 50 CFU/g
Notes		
While ingredient manufacturers are encouraged to include limits for incidental metals and non- metals, it is the product into which those substances are formulated that contains the ingredient, alone or in combination with other ingredients, which must comply with the acceptance criteria set in the United States Pharmacopoeia – National Formulary (USP-NF) general chapter '<2232> Elemental Contaminants in Dietary Supplements'.		

Key to abbreviations:

BP = British Pharmacopoeia

CFU = Colony forming units

NLT = Not less than

NMT = Not more than

Ph Eur = European Pharmacopoeia

USP = United States Pharmacopoeia