Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

VKM Report 2016:27

Risk assessment of specific strains of *Bifidobacterium* spp. used as "other substances"

Opinion of the Panel for Biological Hazards of the Norwegian Scientific Committee for Food Safety

Report from the Norwegian Scientific Committee for Food Safety (VKM) 2016: 27
Risk assessment of specific strains of *Bifidobacterium* spp. used as "other substances".

Opinion of the Panel on Biological Hazards of the Norwegian Scientific Committee for Food Safety
15.12.2016

ISBN: 978-82-8259-217-8
Norwegian Scientific Committee for Food Safety (VKM)
Po 4404 Nydalen
N – 0403 Oslo
Norway

Phone: +47 21 62 28 00
Email: vkm@vkm.no

www.vkm.no
www.english.vkm.no

Cover photo: iStock Photo

Suggested citation: VKM. (2016) Risk assessment of specific strains of *Bifidobacterium* spp. used as "other substances". Opinion of the Panel on Biological Hazards of the Norwegian Scientific Committee for Food Safety, ISBN: 978-82-8259-217-8, Oslo, Norway.

Risk assessment of specific strains of *Bifidobacterium* spp. used as "other substances"

Authors preparing the draft opinion

Siamak Yazdankhah, Danica Grahek-Ogden (VKM staff)

Assessed and approved

The opinion has been assessed and approved by Panel on Biological Hazards. Members of the panel are: Yngvild Wasteson (chair), Karl Eckner, Georg Kapperud, Jørgen Lassen, Judith Narvhus, Truls Nesbakken, Lucy Robertson, Jan Thomas Rosnes, Olaug Taran Skjerdal, Eystein Skjerve, Line Vold and Siamak Yazdankhah.

Acknowledgment

The Panel on Biological Hazards has answered the request from the Norwegian Food Safety Authority. Project leader from the VKM secretariat has been Danica Grahek-Ogden. Judith Narvhus is acknowledged for her valuable work on this opinion. Roar Gudding (The Panel on Animal Health and Welfare) and Øivind Bergh (The Panel on Animal Health and Welfare) constituted a reference group and are acknowledged for their valuable comments and suggestions on this opinion.

Competence of VKM experts

Persons working for VKM, either as appointed members of the Committee or as external experts, do this by virtue of their scientific expertise, not as representatives for their employers or third party interests. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

Table of Contents

Summary

The Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) has, at the request of the Norwegian Food Safety Authority (Mattilsynet; NFSA), assessed the risk of "other substances" in food supplements sold in Norway. These risk assessments will provide NFSA with the scientific basis for regulation of the addition of “other substances” to food supplements and other foods.

"Other substances" are described in the food supplement directive 2002/46/EC *as substances other than vitamins or minerals that have a nutritional and/or physiological effect*. It is added mainly to food supplements, but also to other foods. VKM has not in this series of risk assessments of "other substances" evaluated any claimed beneficial effects from these substances, only possible adverse effects.

The present report is a risk assessment of *Bifidobacterium lactis* Bi-07, *Bifidobacterium bifidum* W23, *Bifidobacterium longum* Rosell-175, *Bifidobacterium breve* Rosell-70, and *Bifidobacterium animalis* sub. *lactis* Bb12 based on previous risk assessments and also publications retrieved from literature search.

The risk of the *Bifidobacterium* strains listed above was assessed for the general population. However, in previous assessments of probiotics published by VKM, concerns have been identified for specific groups. Therefore, the risk was assessed for the age group with immature gastro-intestinal microbiota (age group 0-36 months), population with mature gastro-intestinal microbiota (>3 years) and vulnerable groups with mature gastro-intestinal tract. VKM has also assessed the risk of *Bifidobacterium* spp. in food supplements and other foods independent of the dose and have assessed exposure in general terms.

VKM concludes that it is unlikely that *B. lactis* Bi-07, *B. bifidum* W23, *B. longum* Rosell-175, *B. breve* Rosell-70, and *B. animalis* sub. *lactis* Bb12 would cause adverse health effects in the general healthy population with mature gastro-intestinal tract.

However, no data on long-term adverse effects on infants and young children were identified. As evidence is accruing that the early microbial composition of the neonatal gut is important for the development of the gut microbiota and the immune system of the growing child, it is not possible to exclude that a daily supply of a single particular bacterial strain over a prolonged period of time to an immature gastro-intestinal tract may have long-term, although still unknown, adverse effects on that development.

Key words: Adverse health effect, negative health effect, Norwegian Food Safety Authority, Norwegian Scientific Committee for Food Safety, other substances, risk assessment, VKM, *B. lactis* Bi-07, *B. bifidum* W23, *B. longum* Rosell-175, *B. breve* Rosell-70, and *B. animalis* sub. *lactis*, food supplement

Sammendrag på norsk

På oppdrag for Mattilsynet har Vitenskapskomiteen for mattrygghet (VKM) vurdert risiko ved tilsetting av «andre stoffer» i kosttilskudd som selges i Norge. Disse risikovurderingene vil gi Mattilsynet vitenskapelig grunnlag for å regulere andre stoffer.

«Andre stoffer» er beskrevet i kosttilskuddsdirektivet 2002/46/EC som stoffer som har en ernæringsmessig og/eller fysiologisk effekt, og som ikke er vitaminer og mineraler. VKMs oppgave er å utføre risikovurderinger av mulige negative helseeffekter av «andre stoffer». VKM vurderer ikke påståtte gunstige helseffekter av «andre stoffer».

Denne rapporten er en risikovurdering av *Bifidobacterium lactis* Bi-07, *Bidobacterium bifidum* W23, *Bifidobacterium longum* Rosell-175, *Bifidobacterium breve* Rosell-70, og *Bifidobacterium animalis* sub. *lactis* Bb12. Vurderingen er basert på tidligere risikovurderinger og artikler hentet fra litteratursøk.

Risiko for negative helseeffekter av *Bifidobacterium* spp. som er nevnt ovenfor er vurdert med tanke på hele befolkningen. Mulige uheldige virkninger for bestemte befolkningsgrupper er imidlertid blitt identifisert i tidligere risikovurderinger av probiotika utført av VKM. Risiko er derfor spesielt vurdert for aldersgruppen med umoden tarmflora (aldersgruppe 0-36 måneder), befolkning med moden tarmflora (> 3 år) og sårbare grupper uavhengig av alder. VKM har også vurdert risikoen for negative helseeffekter av *Bifidobacterium* spp. i kosttilskudd uavhengig av dose og har vurdert eksponering på generelt grunnlag.

Risikovurderingen inkluderer ikke andre kilder til de *Bifidobacterium* spp. som er listet ovenfor enn kosttilskudd (som for eksempel mat).

VKM konkluderer med at det er usannsynlig at *B. lactis* Bi-07, *B. bifidum* W23, *B. longum* Rosell-175, *B. breve* Rosell-70, og *B. animalis* sub. *lactis* Bb12 forårsaker negative helseeffekter i den generelle friske befolkningen med moden tarmflora.

Det er imidlertid mangel på data om uønskede langtidsvirkninger for spebarn og små barn (0-36 måneder). Det er økende vitenskapelig dokumentasjon som viser at den mikrobielle sammensetningen i neonatal tarm er viktig for utviklingen av en funksjonell tarmflora og et godt fungerende immunsystem hos det voksende barn. Det kan derfor ikke utelukkes at daglig tilførsel av en enkelt spesifikk bakteriestamme over en lengre tidsperiode til barn med en umoden tarmflora, kan ha langvarige negative effekter på utviklingen av en funksjonell tarmflora.

Abbreviations and glossary

Abbreviations

CFU	- Colony Forming Units
DSMZ	- Deutsche Sammlung von Mikroorganismen und Zellkulturen (German Collection of Microorganisms and Cell Cultures GmbH)
EFSA	- European Food Safety Authority
FAO	- Food and Agriculture Organization of the United Nations
GRAS	- Generally Recognized As Safe
IOM	- Institute of Medicine, USA
ISAPP	- International Scientific Association for Probiotics and Prebiotics
MIC	- Minimum inhibitory concentration
MBP	- Microbial Break Point
NFSA	- Norwegian Food Safety Authority [Norw.: Mattilsynet]
SCF	- Scientific Committee on Food
QPS	- Qualified Presumption of Safety
VKM	- Norwegian Scientific Committee for Food Safety [Norw.: Vitenskapskomiteen for Mattrygghet]
WHO	- World Health Organization

Glossary

"Other substances": a substance other than a vitamin or mineral that has a nutritional or physiological effect (EU, 2006.).

“Negative health effect” and “adverse health effect” are broad terms. VKM uses the definition established by EFSA for “adverse effect”: “A change in morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences.”

Probiotics[1]

In 2001, the Food and Agriculture Organisation (FAO) of the United Nations and the World Health Organisation (WHO) defined probiotics as: “Live microorganisms, which when administered in adequate amounts confer a health benefit on the host” (FAO and WHO, 2002).

Alternative term to “probiotic”:

Currently, there are no approved health claims for probiotics. Applications for health claims on probiotics have been submitted for evaluation to EFSA and no application has received a positive opinion. For this reason, the term ‘probiotic’, when used on a food label, is considered to be a health claim (http://ec.europa.eu/nuhclaims/) and should not be used and should be replaced by “microorganism”.

No claims on probiotics are listed on the EU register (EU, 2016) as authorised for use. The probiotic claims that have been fully evaluated and rejected are listed as non-authorised on the EU register.

[1] The International Scientific Association for Probiotics and Prebiotics, ISAPP, proposed that when combined with the specifications outlined by the FAO/WHO Working Group for the Evaluation of Probiotics in Food (2002), the key aspects of this definition should be more precise and in addition include the following aspects:

- A probiotic must be alive when administered,

- A probiotic must have undergone controlled evaluation to document health benefits in the

target host,

- A probiotic must be a taxonomically defined microbe or combination of microbes (genus,

species and strain level),

- A probiotic must be safe for its intended use.

Background as provided by the Norwegian Food Safety Authority

«Other substances» are substances other than vitamins and minerals, with a nutritional and/or physiological effect on the body. “Other substances” are mainly added to food supplements, but these may also be added to other foods and beverages, such as sports products and energy drinks. Ingestion of these substances in high amounts presents a potential risk for consumers.

While at the EU level, these substances fall under the scope of the European Regulation (EC) No. 1925/2006 on the addition of vitamins, minerals and certain other substances to foods and the European Regulation (EC) No 258/97 concerning novel foods and novel food ingredients, “other substances” remain largely unregulated. In order to ensure safe use of “other substances” many countries have regulated their use at a national level. For example, Denmark regulates these substances in a positive list i.e. a list of substances with maximal daily doses, permitted for use in food supplements and other foods.

The Norwegian Food Safety Authority (NFSA) has recommended the Norwegian Ministry for Health and Care Services to regulate the addition of “other substances” to food supplements and other foods at a national level. NFSA has suggested using the Danish regulation as a model while establishing a national regulatory framework in Norway. NFSA has further suggested that the establishment of a list of substances with permitted maximal doses should be based on the products and substances found on the Norwegian market.

In preparation for a regulation, NFSA has requested the Norwegian Scientific Committee for Food Safety (VKM) to assess the safety of “other substances” found on the Norwegian market. NFSA, after consultation with the industry, has compiled a list of “other substances” added to food supplements and foods marketed in Norway. NFSA requests VKM to carry out safety assessments for the microorganisms on the list.

Terms of reference as provided by the Norwegian Food Safety Authority

The Norwegian Food Safety Authority (NFSA) has requested the Norwegian Scientific Committee for Food Safety (VKM) to:

Phase 1

Since risk/safety assessments for some of the substances on the list have already been carried out by competent authorities (such as the European Food Safety Authority, Institute of Medicine - USA and Norwegian Scientific Committee for Food Safety), in phase 1 of the assignment, VKM has been requested to:

- make an overview of existing risk/safety assessments for «other substances» enlisted by NFSA, prepared by a competent risk assessment authority.

If assessments for some of these substances exist, then, VKM is requested to:

- describe data on upper limits (UL), guidance limits (GL) or other safe limits established for the substances in these assessments.

Phase 2:

Prepare a guidance document outlining the methodology to be used for the safety assessments of microorganisms.

Phase 3:

Assess the safety of microorganisms in accordance to the guidance document developed in Phase 2.

Safety assessments of microorganisms added to food supplements and other foods shall be carried out for the general population.

The NFSA requests the VKM to describe risks for vulnerable groups such as, infants and babies, pregnant and breast feeding women or those suffering from certain illnesses, in each of these assessments.

Attachment:

The list of microorganisms to be assessed.

1 Introduction

This risk assessment addresses specified bacterial strains belonging to the genus *Bifidobacterium*.

VKM has, in this series of risk assessments of "other substances", not evaluated documentation of any claimed beneficial effects from these substances.

According to information from the Norwegian Food Safety Authority (NFSA), *B. bifidum* W23, *B. lactis* Bi-07, *B. longum* Rosell-175, *B. breve* Rosell-70, and *B. lactis* Bb12 are ingredients in food supplements and other foods sold in Norway. Exposure to, *B. bifidum* W23, *B. lactis* Bb12 *B. longum* Rosell-175, *B. breve* Rosell-70 from sources other than food supplements, such as food products, is not included in the risk assessment.

The risk of adverse effects from exposure to specified strains of *Bifidobacterium* spp. was assessed for the general population. However, in previous assessments of probiotics published by VKM concerns in specific groups have been identified. Therefore, the risk was estimated for the age group with immature gastro-intestinal microbiota (age group 0-36 months), population with mature gastro-intestinal microbiota (>3 years) and vulnerable groups with mature gastro-intestinal tract. VKM has also assessed the risk of *Bifidobacterium* spp. independent of the dose and possible matrix effect and have assessed exposure in general terms.

The present report is based on previous risk assessments and articles retrieved from a literature search.

2 Literature

The present risk assessment is based on EFSA's QPS assessment (EFSA, 2008a), previous risk assessments *B. lactis* Bb12 (VKM, 2010) and articles retrieved from a literature search.

2.1 Previous risk assessments

As the recommendation for the QPS status is based on broad criteria, extensive literature searches, and transparent expert judgement, VKM has decided to accept the safety status as given by EFSA in the most up-to-date list including possible qualification criteria (EFSA, 2015). Therefore, the literature search for this assessment has been limited to the reports and articles published in 2015-2016.

2.2 Literature search

Ffollowing literature searches were performed in PUBMED:

("Bifidobacterium lactis Bi-07"[MeSH Terms]; ("Bifidobacterium breve Rosell-70"[MeSH Terms]; ("Bifidobacterium animalis spp lactis Bb12"[MeSH Terms]; ("Bifidobacterium bifidum Ww23"[MeSH Terms]; ("Bifidobacterium longum Rosell-175"[MeSH Terms]

The search returned 36 publications.

Other relevant publications, including reports from EFSA (QPS) and FDA (GRAS) are listed in the reference section.

2.3 Relevance screening

The titles of all results were scanned by project group, and for those that were of potential relevance, the abstracts were also inspected. The members of the project group performed the relevance screening, independently. Citations were excluded if they did not relate to the terms of reference. The reference lists in selected citations were scrutinized to identify additional articles or reports, not identified by the PubMed searches.

3 Hazard identification and characterisation

3.1 Hazard identification

1. *Bifidobacterium lactis* Bi-07,
2. *Bifidobacterium bifidum* W23,
3. *Bifidobacterium longum* Rosell-175,
4. *Bifidobacterium breve* Rosell-70, and
5. *Bifidobacterium animalis* sub. *lactis* Bb12

belong to the genus *Bifidobacterium* that are part of the normal gut microbiota of adults and are also one of the first genera to colonise the gut of infants. In addition, they are normal inhabitants of the gut of animals. A limited number of *Bifidobacterium* species have a history of use in dairy products, especially sour milk products like yoghurts and more recently fermented milk drinks (EFSA, 2007).

Identification of the strain

1. *Bifidobacterium lactis* Bi-07,

B. lactis Bi-07 is a strain of human origin and the strain is deposited in American Type Culture Collection (Manassas, VA, USA) as ATCC Number: SD5220.

2. *Bifidobacterium bifidum* W23,

The strain is of human origin (http://probiotic.org/bifidobacterium.htm), but no data regarding deposition in Culture Collection is available.

3. *Bifidobacterium longum* Rosell-175,

According to the producer, the strain is of dairy origin (http://www.optibacprobiotics.co.uk/live-cultures/articles/what-are-human-strains)

4. *Bifidobacterium breve* Rosell-70,

The strain is of human origin (http://www.optibacprobiotics.co.uk/live-cultures/articles/what-are-human-strains) but no data regarding deposition in Culture Collection is available.

5. *Bifidobacterium animalis* sub. *lactis* Bb12

B. lactis Bb12 is deposited at the German Culture Collection (DSMZ) (Braunschweig, Germany) under the number DSM-20215 and was originally designated as *B. bifidium* of human origin. Following new classification in 1997, the Bb12 strain was found to be identical with *B. lactis* (DSM-10140) and thus identified as *B. lactis*.

According to the scientific Opinion on the substantiation of health claims related to non-characterised bacteria and yeasts pursuant to Article 13(1) of Regulation (EC) No 1924/2006[sup]1[/sup], the Panel on Dietetic Products, Nutrition and Allergies of EFSA considers that *B. lactis* Bi-07, *B. animalis* sub. *lactis* Bb12 are not sufficiently characterised (EFSA, 2010). Information regarding, *B. bifidum* W23, *B. longum* Rosell-175, and *B. breve* Rosell-70 was not found on that list.

3.2 Hazard characterisation

3.2.1 QPS/GRAS

QPS

"A wide variety of microbial species are used in food and feed production. Some have a long history of apparent safe use, while others are less well documented and their use may represent a risk of adverse effects for consumers. Experience has shown that there is a need for a tool for setting priorities within the risk assessment of those microorganisms used in food/feed production referred to EFSA and consequently the subject of a formal assessment of safety. To meet this need a system was proposed for a pre-market safety assessment of selected groups of microorganisms leading to a "Qualified Presumption of Safety (QPS)". In essence this proposed that a safety assessment of a defined taxonomic group (e.g. genus or group of related species) could be made based on four pillars (establishing identity, body of knowledge, possible pathogenicity, and end use). If the taxonomic group did not raise safety concerns or, if safety concerns existed but could be defined and excluded (the qualification), the grouping could be granted QPS status. Thereafter, any strain of microorganism the

identity of which could be unambiguously established and assigned to a QPS group would not require further safety assessment other than satisfying any qualifications specified. Microorganisms not considered suitable for QPS would remain subject to a full safety assessment" (EFSA, 2007).

The list of the microorganisms have been (and will be) regularly updated by EFSA.

GRAS

"Any substance that is intentionally added to food is regarded as a food additive and is subject to premarket review and approval by FDA, unless the substance is generally recognized, among qualified experts, as having been adequately shown to be safe under the conditions of its intended use, or unless the use of the substance is otherwise excluded from the definition of a food additive. The use of a food substance may be GRAS either through scientific procedures or, for a substance used in food before 1958, through experience based on common use in food." (FDA, 2016).

The updated list of the microorganisms is published on FDA website http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/

3.2.2 Influence of live microorganisms on the development of gut microbiota

It is now generally recognised that the establishment of the gut microbiota very early in life is a critical stage of development and probably has far-reaching effects on the health of the individual at all ages, including the development of some so-called life-style diseases later in life. Gut colonization begins very early and may in fact even have started before birth (Greenhalgh et al., 2016). Immediately after birth a beneficial microbiota develops following transfer of bacteria from the mother during birth, from the birth canal. There follows a further transfer of the mother's own microbiota during breast-feeding from bacteria resident in the breastmilk-producing glands and canals. Human milk contains components that stimulate the growth of these bacteria and therefore further influences and encourages the establishment of a beneficial microbiota. There is also evidence that both oral and faecal microorganisms may be transferred from mother to child at a very early stage (Greenhalgh et al., 2016).

Colonization of the infant gut mucosa is important in the establishment of the gut mucosal barrier and for maturation of the gut immune system. It is known that infants born by Caesarean section develop a gut microbiota that is more reflective of environmental bacteria. However, several factors can affect this natural progression, including Caesarean delivery, prematurity, use of formula feeds and treatment with antibiotics (Wang et al., 2016).

The use of antibiotics, both to the neonate and to the mother before parturition, has been shown to change the types and/or the comparative ratios of bacteria in the gut of the neonate. It has been suggested that even a temporary diversion from the establishment of a

healthy gut microbiota at this point may cause alterations in the establishment of the adaptive immune system and that this may have many far-reaching effects later in life, such as allergy and autoimmune diseases.

A disturbance in microbiota from what is presently regarded as “normal” is called dysbiosis. However there is at present no “Gold standard” for the composition of the gut microbiota in neonates and very young children. The human host and its gut microbiota have an important relationship whereby the host recognizes members of the gut microbiota and adjusts the immune response to their presence. Thus the intestinal microbiota of the neonate guides the development of the immune system and a tolerance to the host commensal bacteria. It has been suggested that dysbiosis may be the cause of many conditions, including necrotizing enterocolitis, inflammatory bowel disease, irritable bowel syndrome, atopic and allergic disease and metabolic diseases including obesity and diabetes 1. However, dysbiosis may influence these diseases in different ways – by affecting the immune system or by a direct result of the changed microbiota (Wang et al., 2016). Dysbiosis at an early age can predispose to obesity at any age in life. This may be due to the establishment of a different balance of microorganisms in the gut microbiota which are able to extract energy from multiple sources and thus predispose the host to obesity.

Studies of the role of the neonates GIT microbiota indicate a diversity of microorganisms that include, but not exclusively, such bacteria as lactobacilli and bifidobacteria. Present opinion suggests that this diversity in itself is an important factor. The inclusion of large numbers of one particular strain of probiotic bacteria in the diet of a neonate can therefore be questioned. Indeed, Berstad et al (2016) voiced concern that ingestion of probiotics could negatively affect the resident commensal flora and leave an empty ecological niche following cessation of treatment. Some probiotic strains have been shown to have a number of effects on neonate conditions that can be attributed to the gut microbiota. However, long-term studies of the effects of consumption of probiotic cultures have not been done and therefore it has not been possible to evaluate the long-term effects of manipulating the gut microbiota in neonates and very young children. Similarly, it has not been possible to evaluate the safety of the establishment of a less diverse microbiota as a consequence of feeding probiotics to very young children.

3.2.3 Antimicrobial resistance

Previously published assessments have identified that *Bifidobacterium* spp. are intrinsically resistant to gentamicin, sulphamethoxazole and polymyxin B. Susceptibility to trimethoprim, trimethoprim/ sulphamethoxazole, ciprofloxacin, clindamycin, tetracycline and minocycline was variable (Masco et al., 2006). Although there are few studies on the antibiotic resistance of bifidobacteria strains, the presence of the acquired tetracycline resistance gene tet(W) has been reported in *Bifidobacterium animalis* subsp. *lactis* (Kastner et al., 2006; Masco et al., 2006).

Analysis of *B. animalis* subsp. lactis Bi-07, DGCC 2907 (FDA, 2012):

Antibiogram of DGCC 2907 was established using ISO 10932 IDF223 method and VetMIC Lact-1 and 2 micro-dilution plates that include all antibiotics that are recommended by the FEEDAP. Recorded MICs are displayed in the table below. MIC values are below or equal to the Microbial Break Points (MBPs) defined for *Bifidobacterium* (FDA, 2012).

The presented data indicate that *B. animalis* subsp. *lactis* Bi-07 is not susceptible to gentamicin, kanamycin, streptomycin, and tetracycline. The strain is susceptible to erythromycin, clindamycin, chloramphenicol, ampicillin, virginamycin and vancomycin (Table 1).

Table 1. Resistance profile of *B. animalis* subsp. *lactis* Bi-07

	Gentamycin	**Kanamycin**	**Streptomycin**	**Tetracyclin**	**Erythromycin**	**Clindamycin**	**Chloramphenicol**	**Ampicillin**	**Vancomycin**	**Virginiamycin***
	Gm	Km	Sm	Tc	Em	Cl	Ch	Amp	Va	Vi*
DGCC 2907	Max.	Max.	Max.	Max.	Max.	Max.	Max.	Max.	Max.	Max.
MIC *Bifidobacterium animalis*	64	256	64	8	0,12	<0,03	2	0,25	0,5	0,25
MBP for *Bifidobacterium* **	64	NR***	128	8	1	1	4	2	2	1

* Virginiamycin instead of Synercid

** The EFSA Journal (EFSA, 2008b)

*** NR – not required

While resistance against Gm, Km and Sm is considered to be intrinsic, resistance against tetracycline is associated with a putative tetracycline resistance gene.

Tetracycline resistance in *B. animalis* subsp. *lactis* has previously been shown to correlate directly with the presence of a single gene, tetW (Gueimonde et al., 2010). However, no plasmid was detected in these strains. Nine transposases were identified within the genome of each of these strains, one putative transposase, trp, has been identified immediately upstream of the tetW gene. The presence of a tetW gene that is immediately downstream of a transposon (trp) has been identified in these strains. This tetW gene sequence is identical to the previously reported in *B. animalis* subsp. *lactis* that has demonstrated the genes ability to confer the resistance to tetracycline (Gueimonde et al., 2010). The ability of the strains to transfer the tetracycline resistance was evaluated and the authors found that they could not demonstrate any transfer of resistance to other *B. animalis* subps. *lactis* or any of the 3 other species they evaluated in the *in vivo* experiment.

To date, there has not been any evidence that the tetW gene that is co-transcribed in tandem with this transposase has any ability to transfer resistance, and therefore poses no known risk of transfer. Additionally, through comparative genomics of 5 total proprietary and

public genomes of *B. animalis* subsp. *lactis*, analysis finds that the overall genomic plasticity of the species is extremely stable. In fact, a genome-wide comparison of all the strains that have currently been sequenced reveals little diversity, with 47 confirmed single nucleotide polymorphisms (SNPs) and four insertion/deletion (INDELs) events (Barrangou et al., 2009). From this analysis, it is clear that there has not been an observed incidence of transposition between current *B. animalis* subsp. *lactis* genomes to date, otherwise there would be some evidence of polymorphism between the strains as it relates to transposon insertion. Additionally, the individual sequence composition of the tetW gene was analyzed, and no sharp distinction can be made between the overall GC content of the genome and the GC content of the tetW gene. This further highlights the likelihood that the gene is intrinsic to *B. animalis* subsp*. lactis,* because horizontal gene transfer is often marked with different GC content of the genetic material received than the host genetic material. To conclude, the implied risk of *tetW* transfer is deemed insignificant, as transposition has not been demonstrated experimentally, nor has it been observed naturally (FDA, 2012).

Several studies have shown the presence of tetW gene in various species of *Bifidobacterium* (Masco et al., 2006; Scott et al., 2000).

A sequence similarity of >99.9% was reported between the tetW gene of a rumen isolate of *Butyrivibrio fibriosolven* and that of *Bifidobacterium longum* isolated from human, suggesting possible gene transfer between these species from animals and humans (Scott et al., 2000). However, while the tetW gene in *Butyrivibrio fibriosolven* was associated with the conjugative transposons (TnB1230), (Melville et al., 2004), the tetW gene in *B. lactis* was not shown to be linked with any transposable elements (Scott et al., 2000).

The review article of Salyers et al. summarises the literature that shows that transfer of the tetracycline resistance gene between Gram-positive and Gram-negative bacteria can occur in the mammalian colon and in the other environmental sites (Salyers et al., 2004).

The search of PubMed for antimicrobial resistance in *B. bifidum* W23, *B. longum* Rosell-175, and *B. breve* Rosell-70 did not identify any articles.

3.2.4 Safety concerns

Bifidobacterium spp. are commensal bacteria and can occasionally be associated with local infections or severe systemic infections, as has been demonstrated in previous EFSA opinions (EFSA, 2012). Only one new case report of a septicaemia with *Bifidobacterium longum* and *Bifidobacterium infantis* was identified (Jenke et al., 2012). The patient was an extremely low-birthweight infant. The patient was under probiotic therapy with a product containing *Lactobacillus acidophilus* and *Bifidobacterium infantis*. This is another typical case report, which can be found in relation to immunocompromised hosts. These reports do not change the status of bifidobacteria as safe microorganisms in general. Due to the long history of safe use of *B. adolescentis, B. animalis; B. longum, B. breve* and *B. bifidum*, these species currently have QPS status. Other species could be included subsequent to their industrial

application with the exception of those species associated with dental caries (*B. dentium*) (EFSA, 2012).

According to EFSA (2013) regarding QPS status of *Bifidobacterium,* bacterial species belonging to bifidobacteria are considered as safe and there is no need to change the previous QPS recommendation.

Food & Drug Administration (FDA) (www.fda.gov) has also granted GRAS status to *B. lactis* Bi-07, *B. animalis* sub. *lactis* Bb12 and considered the strain as safe and suitable for use in all food products and dietary supplements (FDA, 2012). We are not aware of the GRAS-status of the other *Bifidobacterium* assessed in this risk assessment, neither at species level nor strain level.

There are apparently no specific safety concerns regarding species *B. lactis* Bi-07 and the strain has not been associated with human clinical disease. Although there are few studies on the antibiotic resistance of bifidobacteria strains, the presence of the acquired tetracycline resistance gene tet(W) has been reported in *B. lactis* Bi-07 and in *B. animalis* sub. *lactis* Bb12, and this may be of concern.

3.2.5 Possible adverse effects of a strain in vulnerable groups

Previously published assessments and literature search conducted for this assessment have not identified safety concerns due to use of *B. lactis* Bi-07, *B. bifidum* W23, *B. lactis* sub *infantis*, *B. longum* Rosell-175, *B. breve* Rosell-70, and *B. animalis* sub. *lactis* Bb12 for the vulnerable groups; pregnant women, children, elderly people, immunocompromised, and critically ill patients. It is known that bacteria with extremely low virulence may be able to produce serious disease in immunocompromised patients (Houck et al., 1972). However, no studies on long-term effects on infants and young children were identified in the literature search. As evidence is accruing that the early microbial composition of the neonatal gut is important for the development of the gut microbiota and the immune system of the growing child, it is not possible to exclude that a daily supply of a single particular bacterial strain over a prolonged period of time to an immature gastro-intestinal tract may have long-term, although still unknown, adverse effects on that development.

4 Exposure assessment

As this assessment is concerned with the general safety of *Bifidobacterium* spp. and is not related to a specific product or dose, the exposure assessment is given in general terms.

The dose ingested in the portion of the product usually recommended for daily consumption contains log 9 of at least one strain among those present in the product. The use of different number of microorganism may be allowed when its rationale has been demonstrated by significant scientific studies. The number of cells must be specified on the product label, and

moreover, this number has to be guaranteed until the end of the product shelf-life, at the specified storage conditions, with uncertainty of 0.5 log units. It is emphasized that the analytical method of quantification of living bacterial cells may differ from species to species (Ministero, 2013).

Regarding consumption by infants, Fernandez et al. (2003) extrapolated from the results of several authors that an infant would consume between log 5 and log 7 bacteria daily along with the consumption of 800 ml breast milk. As a comparison, a 100 g serving of commercial probiotic yoghurt would contain approximately log 9-10 CFU. Thus the amount of cells consumed in a serving of yoghurt would be considerably higher than natural milk levels, in fact up to 10 000 x greater (difference between log 5 and log 9).

5 Risk characterisation

The safety aspects of *Bifidobacterium* species assessed in this risk assessment give no reason for concern and *Bifidobacterium* as a genus has been granted QPS status by EFSA and GRAS status by FDA. However, no studies on long-term effects on infants and young children were identified in the literature search. As evidence is accruing that the early microbial composition of the neonatal gut is important for the development of the gut microbiota and the immune system of the growing child, it is not possible to exclude that a daily supply of a single particular bacterial strain over a prolonged period of time to an immature gastro-intestinal tract may have long-term, although still unknown, adverse effects on that development.

The safety aspects of *Bifidobacterium* assessed in this risk assessment for vulnerable groups other than the one with immature gastrointestinal tract give no reason for concern.

6 Uncertainties

Consumption of microorganism *B. lactis* Bi-07, *B. animalis* sub. *lactis* Bb12, *B. bifidum* W23, *B. longum* Rosell-175, and *B. breve* Rosell-70 in a ”normal” dose is considered safe in an adult (> 3 years) ”normal” population. In this assessment, some uncertainties have been identified. Many of these uncertainties may overlap with the data gaps (Section 8).

The uncertainties identified are as follows:

- Long-term effects on infants and young children
- Consumption by vulnerable groups other than the group with immature gastro-intestinal tract
- Horizontal transfer of chromosomally-located tetracycline resistance gene (tetW) from *B. lactis* Bi-07, and *B. animalis* sub. *lactis* Bb12 to other bacterial species

7 Conclusions with answers to the terms of reference

VKM concludes that it is unlikely that *B. bifidum* W23, *B. longum* Rosell-175, *B. breve* Rosell-70, *B. lactis* Bi-07, and *B. animalis* sub. *lactis* Bb12 cause adverse health effects in the population with mature gastro-intestinal tract. However, it is not possible to exclude that a daily supply of a single particular bacterial strain over a prolonged period of time to an immature gastro-intestinal tract may have long-term, although still unknown, adverse effects on that development.

Data are lacking regarding consumption of *B. bifidum* W23, *B. longum* Rosell-175, *B. breve* Rosell-70, *B. lactis* Bi-07, and *B. animalis* sub. *lactis* Bb12 in vulnerable groups (e.g. elderly, immunocompromised patients, critically ill patients and pregnant women) other than those with an immature gastrointestinal tract.

8 Data gaps

- Studies on adverse effects in children and vulnerable groups are lacking.
- Post-marketing safety data are lacking.
- Data regarding human studies on adverse effects after long-term oral exposure are lacking.
- Data on transfer of tetracycline resistance gene tetW to other bacterial species are insufficient.

9 References

Barrangou R., Briczinski E.P., Traeger L.L., Loquasto J.R., Richards M., Horvath P., Coute-Monvoisin A.C., Leyer G., Rendulic S., Steele J.L., Broadbent J.R., Oberg T., Dudley E.G., Schuster S., Romero D.A., Roberts R.F. (2009) Comparison of the complete genome sequences of *Bifidobacterium animalis* subsp. *lactis* DSM 10140 and Bl-04. J Bacteriol 191:4144-51. DOI: 10.1128/jb.00155-09.

Berstad A., Raa J., Midtvedt T., Valeur J. (2016) Probiotic lactic acid bacteria - the fledgling cuckoos of the gut? Microb Ecol Health Dis 27:31557. DOI: 10.3402/mehd.v27.31557.

EFSA. (2007) Opinion of the Scientific Committee on a request from EFSA on the introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA. The EFSA Journal 587: 1-16.

EFSA. (2008a) Scientific Opinion of the Panel on Biological Hazards on a request from EFSA on the maintenance of the QPS list of microorganisms intentionally added to food or feed. . The EFSA Journal 923:1-48

EFSA. (2008b) Update of the criteria used in the assessment of bacterial resistance to antibiotics of human or veterinary importance. The EFSA Journal 732:1-15

EFSA. (2010) Panel on Dietetic Products, Nutrition Allergies. Scientific Opinion on the substantiation of health claims related to non characterised bacteria and yeasts pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal 8:n/a-n/a. DOI: 10.2903/j.efsa.2010.1470.

EFSA. (2012) Panel on Biological Hazards (BIOHAZ); Scientific Opinion on the maintenance of the list of QPS biological agents intentionally added to food and feed (2012 update). EFSA Journal 10. DOI: 10.2903/j.efsa.2012.3020.

EFSA. (2013) BIOHAZ Panel (EFSA Panel on Biological Hazards. Scientific Opinion on the maintenance of the list of QPS biological agents intentionally added to food and feed (2013 update). EFSA Journal 2015 110. DOI: 10.2903/j.efsa.2013.3449

EFSA. (2015) BIOHAZ Panel (EFSA Panel on Biological Hazards). Statement on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 3: Suitability of taxonomic units notified to EFSA until September 2015., EFSA Journal 2015. pp. 25 pp.

EU. (2006.) Regulation (EC) No 1925/2006 of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods. http://eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX%3A32006R1925.

EU. (2016) EU Register of nutrition and health claims made on foods. http://ec.europa.eu/nuhclaims/.

FAO, WHO. (2002) Guidelines for the Evaluation of Probiotics in Food. Report of a Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food., London Ontario, Canada.

FDA. (2012) GRAS Notice-Exemption Claim for isolates of *Bifidobacterium animalis* ssp. *lactis (B. lactis*), including *B. lactis* HNO19, *B. lactis* Bi-07, *B. lactis* BI-04, and *B. lactis* B420

FDA. (2016) Generally Recognized as Safe (GRAS). http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/.

Fernandez M.F., Boris S., Barbes C. (2003) Probiotic properties of human lactobacilli strains to be used in the gastrointestinal tract. J Appl Microbiol 94:449-55.

Greenhalgh K., Meyer K.M., Aagaard K.M., Wilmes P. (2016) The human gut microbiome in health: establishment and resilience of microbiota over a lifetime. Environmental microbiology 18:2103-16.

Gueimonde M., Flórez A.B., van Hoek A.H.A.M., Stuer-Lauridsen B., Strøman P., de los Reyes-Gavilán C.G., Margolles A. (2010) Genetic Basis of Tetracycline Resistance in *Bifidobacterium animalis* subsp. *lactis*. Applied and Environmental Microbiology 76:3364-3369. DOI: 10.1128/aem.03096-09.

Houck P.W., Nelson J.D., Kay J.L. (1972) Fatal septicemia due to staphylococcus aureus 502a: Report of a case and review of the infectious complications of bacterial interference programs. American Journal of Diseases of Children 123:45-48. DOI: 10.1001/archpedi.1972.02110070095012.

Jenke A., Ruf E.M., Hoppe T., Heldmann M., Wirth S. (2012) *Bifidobacterium* septicaemia in an extremely low-birthweight infant under probiotic therapy. Arch Dis Child Fetal Neonatal Ed 97:F217-8. DOI: 10.1136/archdischild-2011-300838.

Kastner S., Perreten V., Bleuler H., Hugenschmidt G., Lacroix C., Meile L. (2006) Antibiotic susceptibility patterns and resistance genes of starter cultures and probiotic bacteria used in food. Syst Appl Microbiol 29:145-55. DOI: 10.1016/j.syapm.2005.07.009.

Masco L., Van Hoorde K., De Brandt E., Swings J., Huys G. (2006) Antimicrobial susceptibility of *Bifidobacterium strains* from humans, animals and probiotic products. Journal of Antimicrobial Chemotherapy 58:85-94. DOI: 10.1093/jac/dkl197.

Melville C.M., Brunel R., Flint H.J., Scott K.P. (2004) The *Butyrivibrio fibrisolvens* tet(W) gene is carried on the novel conjugative transposon TnB1230, which contains duplicated nitroreductase coding sequences. J Bacteriol 186:3656-9. DOI: 10.1128/JB.186.11.3656-3659.2004.

Ministero d.S. (2013) Guideline on probiotics and prebiotics, in: d. S. Ministero (Ed.), Roma, Italy.

Salyers A.A., Gupta A., Wang Y. (2004) Human intestinal bacteria as reservoirs for antibiotic resistance genes. Trends Microbiol 12:412-6. DOI: 10.1016/j.tim.2004.07.004.

Scott K.P., Melville C.M., Barbosa T.M., Flint H.J. (2000) Occurrence of the New Tetracycline Resistance Gene tet(W) in Bacteria from the Human Gut. Antimicrob Agents Chemother 44:775-7.

VKM. (2010) Assessment of benefits and risks of probiotics in baby foods *Bifidobacterium lactis* Bb12, VKM.

Wang M., Monaco M.H., Donovan S.M. (2016) Impact of early gut microbiota on immune and metabolic development and function. Semin Fetal Neonatal Med. DOI: 10.1016/j.siny.2016.04.004.