FOOD STANDARDS Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

21 May 2021
156-21

Supporting document 2

Microbiology risk assessment: L-lactic acid producing microorganisms

Safety & food technology Consultation paper
Proposal P1028—Review of the regulation of infant formula products

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Executive summary

Human breast milk naturally contains a variety of lactic acid producing bacteria, including lactobaccili, enterococci, streptococci and bifidobacteria. Standard 2.9.1—Infant formula—of the Australia New Zealand Food Standards Code (the Code) currently contains an unconditional permission for the optional use of L(+) lactic acid producing microorganisms (hereafter referred to as L-lactic acid producing microorganisms) in infant formula products. This permission (in common with other permissions for lactic acid producing microorganisms in the Code) was originally intended to enable their use as food additives—i.e. as acidity regulators and for pH adjustment—but has unintentionally permitted their use for other purposes (e.g. as probiotics, to confer a health benefit) without a requirement to undergo pre-market safety assessment.

FSANZ has, therefore, assessed the risk to the health and safety of infants—healthy, as well as preterm, low birth weight and immunocompromised—from the addition to infant formula products of any L-lactic acid producing microorganisms, and whether any risk applies specifically to the use of L-lactic acid producing microorganisms for preterm, low birth weight and immunocompromised infants. The risk from DL-lactic acid producing microorganisms of the order Lactobacillales was also assessed, as a large number of studies were identified that investigated probiotic supplementation of infants with DL-lactic acid bacteria.

This assessment has identified relevant, appropriately designed studies, including clinical trials, case reports, other relevant epidemiological studies and studies evaluating safety. These studies assessed the addition of lactic acid producing bacteria to infant formula in a viable form; supplementation through means other than infant formula; and fermentation of infant formula where no viable bacteria remain in the final product.

From published clinical trial data on the safety of a range of L-lactic acid producing microorganisms—including species of *Bifidobacterium*, *Propionibacterium* and *Lactobacillus*—FSANZ has not identified any risks for healthy, full term infants. Infant formulas supplemented with L-lactic acid producing bacteria were well tolerated, and no adverse events associated with the lactic acid producing bacteria were noted in the clinical trials assessed. FSANZ concludes that infant formula supplemented with non-pathogenic,

non-toxigenic L-lactic acid producing microorganisms does not present a risk to public health and safety for healthy, full term infants.

The published clinical trials on the safety of a number of DL-lactic acid producing bacteria—alone or in combination with L-lactic acid producing bacteria—did not identify any risks for healthy full term and preterm infants. Infant formulas supplemented with DL-lactic acid producing bacteria were well tolerated, and no adverse events associated with the lactic acid producing bacteria were noted in the clinical trials assessed. FSANZ concludes that infant formula supplemented with non-pathogenic, non-toxigenic DL-lactic acid producing microorganisms does not present a risk to public health and safety for healthy, full term and preterm infants.

The intent of the original permission in the Code was for the addition of *non-pathogenic* lactic acid producing microorganisms. However, certain genera of lactic acid producing bacteria—such as *Enterococcus* and some spore-forming bacilli—are known to include pathogenic or toxigenic species. Therefore, FSANZ also assessed safety aspects of these potentially pathogenic genera.

Enterococci are ubiquitous in nature and are a normal component of the healthy intestinal microflora of humans and animals. The two most prominent species—*E. faecium* and *E. faecalis*—are opportunistic human pathogens which may also be used to produce foods (e.g. cheese and fermented meats) and which are also increasingly being developed for use as probiotics. Enterococci are often resistant to a wide range of clinically important antimicrobials. Hospital-associated *E. faecium* and *E. faecalis* strains also typically harbour virulence genes that promote colonisation, biofilm formation and pathogenesis. Since there are very few clinical trials assessing the safety of enterococci, establishing safety for the addition of lactic acid producing enterococci to infant formula would require assessment on a case-by-case basis.

Spore forming *Bacillus* spp. are amongst a number of bacilli used in the food industry to produce enzymes and, increasingly, as probiotics. Production of L-lactic acid is strain specific—it is not uniformly distributed across the *Bacillus* genus or within species groups such as *B. subtilis* or *B. cereus*. The principal safety concern for infants is the capacity for toxin production. Since the potential for production of toxins or other toxic metabolites by lactic acid producing *Bacillus* spp. is unevenly distributed and must be conclusively excluded, and since there are very few clinical trials assessing their safety, establishing safety for their addition to infant formula would require assessment on a case-by-case basis.

For infants with underlying clinical complications—including preterm, low birth weight and immunocompromised infants—there are case reports of sepsis and bloodstream infections associated with dietary supplementation with non-pathogenic L- and DL-lactic acid producing bacteria. However, due to a lack of sufficient data on infectivity and exposure, FSANZ is unable to assess the level of the risk in these circumstances.

There is limited published data on the safety of fermented formulas, but no potential risks to public health and safety have been identified for healthy full term infants. Therefore, FSANZ concludes that the use of non-toxigenic L-lactic acid producing bacteria in the production of fermented infant formula—where no viable bacteria are present in the final product—does not present a risk to public health and safety.

Very limited data is available on the safety of fermented formulas for preterm infants and other vulnerable groups. However, no potential risks to public health and safety have been identified for preterm infants. FSANZ therefore concludes that formula fermented with L-lactic acid producing bacteria is unlikely to present a risk to public health and safety in healthy preterm infants.

Table of contents

1 Introduction

Human breast milk naturally contains a variety of L- and DL-lactic acid producing bacteria—including lactobaccili, enterococci, streptococci and bifidobacteria—which appear to play a role in early colonisation of the neonatal gastrointestinal tract (Martín et al. 2003; López-Huertas 2015; Asan-Ozusaglam & Gunyakti 2018). Some strains—e.g. *L. fermentum* CECT5716—have been studied for their ability to confer health benefits, with a view to their development as probiotics (López-Huertas 2015; Asan-Ozusaglam & Gunyakti 2018).

Standard 2.9.1—Infant formula—of the *Australia New Zealand Food Standards Code* (the Code) currently contains a permission for the optional use of L(+) lactic acid producing microorganisms (hereafter referred to as L-lactic acid producing microorganisms) in infant formula products without conditions or criteria stipulated. The original intent of this permission—in common with other permissions for lactic acid producing microorganisms in the Code[1]—was to enable their use as fermentative agents for acidity regulation / pH adjustment. However, as that intention was not specified in the Code, the permission has unintentionally facilitated the use of L-lactic acid producing microorganisms as optional ingredients for other purposes (e.g. as probiotics[2]). The current permission in Standard 2.9.1 means that there is no requirement for pre-market assessment of L-lactic acid producing microorganisms added to infant formula.

2 Scope of microbiology risk assessment

2.1 Assessment questions

1. Does the addition of L- and/or DL-lactic acid producing bacteria to infant formula products pose a risk of harm to the health and safety of:
    (i) healthy infants
    (ii) preterm and low birth weight infants
    (iii) immunocompromised infants.
2. If yes to question 1, is there any risk to public health and safety associated with the use of non-pathogenic L- and/or DL-lactic acid producing bacteria for preterm, low birth weight and immunocompromised infants?

2.2 Scope and method of assessment

This assessment included a search of the scientific literature (up to 2019) for L-lactic acid producing bacteria, including members of the traditionally defined lactic acid bacteria (LAB) belonging to the order Lactobacillales; bifidobacteria belonging to the genus *Bifidobacterium*; and spore forming L-lactic acid producing bacteria belonging to the genus *Bacillus*.

A search of the NCBI and EBSCO databases was conducted using key terms to identify studies related to the addition of lactic acid producing bacteria to infant formula in a viable form to confer a health benefit; supplementation through means other than infant formula; and fermentation of infant formula where no viable bacteria remain in the final product[3].

[1] Definitions in the Code (see www.foodstandards.gov.au/code/Pages/default.aspx) for butter, cheese and edible oil spreads permit the addition of lactic acid producing microorganisms to those foods. The Code also permits food for infants to contain lactic acid producing microorganisms (see Standard 2.9.2). The definition of yoghurt in the Code requires fermentation to have been carried out with lactic acid producing microorganisms.
[2] Probiotics are defined as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” (Hill et al. 2014).
[3] Search terms included: lactic acid, lactobacill*, lactococcus, enterococc*, bifidobacteri*, streptococc*, leuconostoc, bacillus, probiotic, neonate, pre-term, premature, low birth weight, infant, sepsis, adverse,

Primary studies were identified from the searches or through referencing in review articles. Appropriately designed studies were included in the body of evidence, which included clinical trials as well as case reports and other relevant epidemiological studies or studies evaluating safety.

DL-lactic acid producing bacteria belonging to the order Lactobacillales were also included in the scope of this assessment, as a large number of studies were identified that supplemented infants with DL-lactic acid bacteria to confer a health benefit.

3 Hazard assessment

3.1 L-lactic acid producing bacteria safety in healthy term infants

Seven studies were identified that assessed the short-term (<1 year) safety of feeding infant formula supplemented with L-lactic acid producing bacteria (either *Bifidobacteria* spp., *Lactobacillus* spp. or *Propionibacterium freundenreichii* subsp. *shermanii*) to healthy, full term newborn infants using anthropometric variables as either primary or secondary outcomes (Baglatzi et al. 2016; Chouraqui et al. 2008; Gibson et al. 2009; Holscher et al. 2012; Kukkonen et al. 2008; Radke et al. 2017; Scalabrin et al. 2009; Table 3.1). In total, 1260 infants consumed test formula, 813 unsupplemented control formula, and 44 breast milk as the reference group, with no significant differences in anthropometric measures observed by the study authors.

Two studies were identified that assessed the long-term (≥2 years) safety of feeding infant formula supplemented with LAB to healthy, full term newborn infants (Kukkonen et al. 2008; Scalabrin et al. 2017; Table 3.1). In total, 514 infants consumed the test formula and 488 the control formula, with no significant differences in anthropometric measures observed by the authors after two years and five years follow-up, respectively.

Baglatzi et al. (2016) compared infants fed two probiotic supplemented formulas provided by the study sponsor (Nestlé) with breast fed infants. Healthy full term infants less than 4 days old were recruited from mothers who had elected not to breast feed or had stopped breast feeding within 24 hours after delivery. Infants from mothers who had elected to breast feed for at least 4 months were recruited as the reference group. The study was exploratory in nature, and no formal power calculation was used to determine study size.

Chouraqui et al. (2008) compared three treatment formulas with control formula. The test formulas were the same as the control (Nan; Nestec SA, Switzerland), except for supplementation with LAB with or without galactooligosaccharide (GOS) and short-chain fructooligosaccharide (SCFOS; Table 3.1). Healthy, full term C-section delivered infants ≤14 days old were recruited from mothers who elected not to breast feed, and the study was powered to detect a 3.9 g/day or greater change in body weight using a 2-sided test.

Gibson et al. (2009) compared whey-predominant infant formula (Nestlé; Switzerland) with the same formula supplemented with fish docosahexaenoic acid (DHA), arachidonic acid (AA) and *Bifidobacterium lactis* CNCM I-3446 (Table 3.1). Healthy full term infants ≤10 days old were recruited from mothers who elected not to breast feed, and the study was powered to detect a 3.9 g/day or greater change in body weight using a 2-sided test.

Holscher et al. (2012) compared breast fed infants with infants fed partially hydrolysed whey infant formula (Nestlé, USA) with and without *B. lactis* Bb12 supplementation (Table 3.1).

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septic[a]emia, blood stream infection, bacter[a]emia, endocarditis, meningitis, encephalitis, clinical trial, and fermented formula.

Healthy full term infants were recruited to receive control and test formula from 6 weeks of age for 6 weeks. The self-selected breastfed reference group was exclusively breastfed and followed for 6 weeks, starting at 6 weeks of age. Anthropometric and tolerance measures were secondary outcomes, and the study design was not specifically powered to detect clinically relevant differences in weight gain. Rather, sample size calculations were based on detecting significant changes in the level of faecal secretory IgA.

Kukkonen et al. (2008) recruited mothers whose infants were at increased risk of developing allergy. Supplementation of mothers commenced from 35 weeks gestation once daily in a capsule and in their infant using the same bacterial mix plus 0.8g GOS, administered once daily in a sugar syrup (Table 3.1). Anthropometric and tolerance measures were secondary outcomes, and the study design was not specifically powered to detect clinically relevant differences in weight gain. Rather, sample-size calculation was based on detecting a 10% absolute reduction in incidence of allergic diseases at age 5 years. Three additional papers report on long-term outcomes of this study (Korpela et al. 2018; Kuitunen et al. 2009; Kukkonen et al. 2007), with no safety related adverse events identified.

Radke et al. (2017) compared test formula containing bovine milk-derived oligosaccharides (BMOs) and *B. lactis* CNCM I-3446 with identical formula (Nestlé; Switzerland) without BMOs and *B. lactis*. Healthy full term infants ≤14 days old were recruited from mothers who elected not to breast feed, and the study was powered to detect ≥20% difference between the two groups in rates of diarrhoea and overall infections during the first 6 and 12 months of life. The authors stated that the sample size was adequate for demonstrating non-inferiority in daily weight gain, where the non-inferiority margin was 3 g/day and the estimated standard deviation 6.1 g/day.

Scalabrin et al. (2009) compared extensively hydrolysed control formula (Nutramigen LIPIL, Mead Johnson & Company, USA) with the same formula containing *L. rhamnosus* GG and a partially hydrolysed formula containing *L. rhamnosus* GG. Healthy full term infants who were exclusively formula fed were enrolled at 14 days old, and the study was powered to detect a clinically relevant difference of 3 g/d in weight gain from 14 to 120 days of age (80% power, 1-tailed).

Scalabrin et al. (2017) was a continuation of the Scalabrin et al. (2009) study, where a subset of infants continued on the test and control formulas until 1 year of age, and anthropometric and other secondary measures were taken at 3 and 5 years of age (Table 3.1).

A further study by Simeoni et al. (2016) specifically examined the influence of prebiotic[4] and probiotic supplementation on infant microbiota development. The study compared test formula containing BMOs and *B. lactis* CNCM I-3446 with identical formula (Nestlé; Switzerland) without BMOs and *B. lactis* (Table 3.1). Healthy full term infants ≤14 days old were recruited from mothers who elected not to breast feed. Infants from mothers who had elected to breast feed were recruited as the reference group. No formal power calculation was used to determine study size.

The published clinical trial data on the safety of L-lactic acid producing bacteria has not identified any potential risks to public health and safety for healthy full term infants consuming up to $5x10^9$ cfu/day. Therefore, FSANZ concludes that infant formula supplemented with non-pathogenic L-lactic acid producing bacteria does not present a risk to public health and safety for healthy full term infants.

[4] A prebiotics is defined as “a substrate that is selectively utilized by host microorganisms conferring a health benefit” (Gibson et al. 2017).

Table 3.1 Clinical trials in healthy newborn infants receiving one or more L-lactic acid producing bacteria

Reference Country	ITT Study size (T/C)[1]	L-lactic acid producing bacteria and synbiotic added[2]	Concentration and frequency[3]	Age[4] (treatment duration)	Intervention and adverse event outcomes
Baglatzi et al. (2016) Greece	77/44	*B. lactis CNCM I-3446*	$3.1x10^7$ cfu/g formula powder	<4 days (6 months)	No significant differences observed for anthropometric measures weight-for-age, length-for-age, BMI-for-age and head circumference-for-age at 1 and 4 months of age or at follow-up at 12 month of age. No significant differences observed for the two treatment arms and breast fed infants for other outcomes including prevention of diarrhoea, bifidobacteria counts or immune responses to vaccination. No adverse events associated with probiotic were reported.
	77/44	*B. lactis CNCM I-3446*	$3.7x10^4$ cfu/g formula powder		
Chouraqui et al. (2008) France	60/53	*B. longum* BL999 *L. rhamnosus* LPR	$1.29x10^6$ cfu/ml formula $6.45x10^6$ cfu/ml formula Ad libitum	≤14 days (4 months)	Anthropometric measures were equivalent among infants in the different formula groups during the treatment period (0–4 months) and observation period (4–12 months). Stool frequency was greater in the treatment arms containing FOS/SCGOS compared to control and LAB only groups. Frequency of flatulence, vomiting, colic and spitting up were not significantly different between the groups. No adverse events associated with probiotics were observed.
	54/53	*B. longum* BL999 *L. rhamnosus* LPR +GOS/SCFOS	$1.29x10^6$ cfu/ml formula $6.45x10^6$ cfu/ml formula 4mg/ml formula Ad libitum	≤14 days (4 months)	
	60/53	*B. longum* BL999 *L. paracasei* ST11 +GOS/SCFOS	$2.58x10^6$ cfu/ml formula $2.58x10^6$ cfu/ml formula 4mg/ml formula Ad libitum	≤14 days (4 months)	

Reference Country	ITT Study size (T/C)[1]	L-lactic acid producing bacteria and synbiotic added[2]	Concentration and frequency[3]	Age[4] (treatment duration)	Intervention and adverse event outcomes
Gibson et al. (2009) Australia	55/43	*B. lactis* CNCM I-3446 +Fish DHA +Arachidonic acid	$3.85x10^8$ cfu/100kcal 0.24% of total fatty acid 0.24% of total fatty acid Ad libitum (min. 500ml /day)	≤10 days (7 months)	No significant difference in anthropometric measures were observed between control and treatment arms at 4 months of age. Stools, colic, spitting up, vomiting and restlessness occurred at similar frequencies in the two groups. No adverse events associated with probiotic were observed.
Holscher et al. (2012) USA	41/34 (40 breast fed infants)	*B. lactis* Bb12	$1x10^6$ cfu/g formula Ad libitum	6 weeks (6 weeks)	"Mean weight percentiles generated from the World Health Organization growth charts did not differ among the three groups". No differences among the groups in frequency of flatulence, spit up or vomiting. No adverse events associated with probiotic were reported.
Kukkonen et al. (2008) Finland	446/456	*B. breve* Bb99 *P. freundenreichii* subsp. *shermanii* *L. rhamnosus* Lc705 *L. rhamnosus* GG +GOS	$2x10^8$ cfu/dose daily $2x10^9$ cfu/dose daily $5x10^9$ cfu/dose daily $5x10^9$ cfu/dose daily 0.8g/dose daily Once daily in sugar syrup	Birth (6 months)	Anthropometric measures were equivalent for test and control groups at the end of treatment (6 months) and observation (24 months) periods. Frequency of vomiting, constipation, excessive crying and abdominal discomfort were similar between groups. No adverse events associated with probiotic were observed.

Reference Country	ITT Study size (T/C)[1]	L-lactic acid producing bacteria and synbiotic added[2]	Concentration and frequency[3]	Age[4] (treatment duration)	Intervention and adverse event outcomes
Radke et al. (2017) Germany, France, Netherlands	179/180 (59 breast fed infants reference group)	*B. lactis* CNCM I-3446 BMOs	$1x10^7$ cfu/g formula 5.8g/100g formula Ad libitum	≤14 days (6 months)	Anthropometric measures were not significantly different for the test, control and breast fed groups at the end of the treatment period (6 months) and observation period (12 months). In the first 3 months, the test group had higher daily stool frequency and higher proportion of liquid stools as compared to control formula, but was most similar to the breast fed reference group. Other measures of tolerance were similar for the formula groups. No adverse events associated with probiotic were observed.
Scalabrin et al. (2009) USA	94/94	*L. rhamnosus* GG	$1x10^8$ cfu/g EH formula Ad libitum	14 days (106 days)	No relevant differences in formula tolerance, adverse events, or allergic and immune markers between groups. Both formulas supplemented with LGG were well tolerated and safe. No significant differences in growth rates to 150 days of age.
	94/94	*L. rhamnosus* GG	$1x10^8$ cfu/g PH formula Ad libitum	14 days (106 days)	
Scalabrin et al. (2017) USA	69/32	*L. rhamnosus* GG	$1x10^8$ cfu/g formula Ad libitum	14 days (12 months)	Anthropometric measures were not significantly different for test and control formula groups at 3 years and 5 years. No significant adverse events were observed for secondary measures of allergy or infectious disease at 5 years follow up.

Reference Country	ITT Study size (T/C)[1]	L-lactic acid producing bacteria and synbiotic added[2]	Concentration and frequency[3]	Age[4] (treatment duration)	Intervention and adverse event outcomes
Simeoni et al. (2016) Poland	39/37 (39 breast fed infants reference group)	*B. lactis* CNCM I-3446 BMOs	$1x10^7$ cfu/g formula 5.7g/100g formula Ad libitum	5 days (12 weeks)	Infants from the test and control groups did not differ in spitting up, vomiting, crying, colic, flatulence and irritability. At six and 12 weeks, infants in the control group had a significantly more diverse microbiota than breast fed infants, but the test group did not. The authors did not report adverse events associated with the probiotic and prebiotic included in the test formula.

[1] ITT, intent to treat; T/C, test formula/control formula; [2] A synbiotic is defined as "a mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host" (Swanson et al. 2020). BMOs, bovine milk-derived oligosaccharides from whey permeate; DHA, docosahexaenoic acid; GOS, galactooligosaccharide; GOS/SC-FOS, 90% galactooligosaccharide/ 10% short-chain fructooligosaccharide (SCFOS); [3] EH, extensively hydrolysed; PH, partially hydrolysed; [4] Age at commencement of feeding, and duration of probiotic supplementation.

3.2 L-lactic acid producing bacteria safety in preterm, low birth weight and immune-compromised infants

3.2.1 Clinical trials to assess safety or efficacy of probiotics supplementation preventing clinical complications associated with prematurity

Three studies were identified that examined the safety of L-lactic acid producing bacteria in preterm and very low birth weight infants where the primary outcome was improved growth and tolerance (Al-Hosni et al. 2012; Hays et al. 2016; van Niekerk et al. 2014; Table 3.2). A further two studies reported on a primary outcome of time to achieve full enteral feeding in preterm very low birth weight infants (Rougé et al. 2009; Totsu et al. 2014; Table 3.2). Lactic acid producing bacteria were administered in enteral feeding by mixing with expressed breast milk or preterm formula.

Al-Hosni et al. (2012) recruited premature infants with a birth weight from 501–1000 g, with the sample size calculated to detect a 50% reduction in the number of preterm infants discharged with a weight less than the 10th percentile of the corresponding postmenstrual age infants.

Hays et al. (2016) recruited infants with a gestational age of 25–31 weeks and birth weight from 700–1600 g, and the study was powered to detect a 150 g difference in body weight at the end of the intervention.

Van Niekerk et al. (2014) recruited exclusively breast fed, very low birth weight infants born to mothers who were either HIV-positive or HIV-negative, and the sample size was calculated based on live birth statistics for infants born to HIV-positive mothers.

The studies by Rougé et al. (2009) and Totsu et al. (2014) recruited preterm infants with a birth weight less than 1500 g and gestational age less than 33 and 32 weeks, respectively. The Rougé et al. (2009) study was powered to detect a 20% difference in the number of infants receiving >50% of their nutritional needs by enteral feeds by postnatal age 14 days. Totsu et al. (2014) was powered to detect a decrease of 2 days to reach full feeding—defined as 100 mL/(kg/day)—in the probiotic treatment group.

Eleven studies were identified that examined the efficacy of L-lactic acid bacteria in reducing the rate of necrotising enterocolitis (NEC), sepsis or infection as the primary outcome (Bin-Nun et al. 2005; Braga et al. 2011; Costeloe et al. 2016; Dani et al. 2002; Dilli et al. 2015; Hikaru et al. 2010; Jacobs et al. 2013; Manzoni et al. 2009; Manzoni et al. 2014; Mihatsch et al. 2010; Sari et al. 2011; Table 3.2). Very low birth weight infants were recruited, and L-lactic acid producing bacteria were administered in enteral feeding by mixing with expressed breast milk or preterm formula. Sample size calculations were based on reduction in incidence of NEC, sepsis or infection and, where noted in Table 3.2, below, secondary outcomes of growth, tolerance and time to enteral feeding were reported.

Ten studies were identified that examined the effect of L-lactic acid bacteria supplementation in modulating the intestinal microflora of very low birth weight infants to promote probiotic colonisation, prevent fungal colonisation, or improve digestion, immune function or intestinal permeability (Chrzanowska-Liszewska et al. 2012; Fujii et al. 2006; Kitajima et al. 1997; Li et al. 2004; Manzoni et al. 2006; Millar et al. 1993; Mohan et al. 2006; Pärtty et al. 2013; Patole et al. 2014; Stratiki et al. 2007; Table 3.2).

Table 3.2 Clinical trials in preterm and low birth weight infants receiving one or more L-lactic acid producing bacteria

Reference Country	ITT Study size (T/C)[1]	L-lactic acid bacteria and synbiotic added	Concentration and frequency	Age[2] (duration)	Intervention and adverse event outcomes[3]
Al-Hosni et al. (2012) USA	50/51	*L. rhamnosus* GG *B. infantis*	$5x10^9$ cfu once daily $5x10^9$ cfu once daily	4d (63d)	There was no difference in the proportion of infants with weight below 10th percentile between the treatment and control arms at 34 weeks postmenstrual age. Growth velocity and average daily weight gain trended higher in treated arm. No adverse or significant event related to probiotic supplementation was observed in the study population.
Bin-Nun et al. (2005) Israel	72/73	*B. bifidus* *S. thermophiles* *B. infantis*	$3.5x10^8$ cfu once daily $3.5x10^8$ cfu once daily $3.5x10^8$ cfu once daily	2–3d (42d)	No difference between treatment and control arms in incidence of feeding intolerance including diarrhoea, vomiting or abdominal distension. Non-significant trend towards increased weight gain after six weeks. Combined NEC and mortality was significantly greater in control group (17/73) then treatment group (6/72; RR=0.358, 95% CI=0.150–0.856). No drop-outs or adverse events associated with probiotics were reported.
Braga et al. (2011) Brazil	119/112	*L. casei* *B. breve*	$3.5x10^7$ cfu once daily $3.5x10^9$ cfu once daily	2–3d (28d)	No difference in the relative risk of sepsis or death between treatment and control arms. 4 infants developed NEC in control group vs none in the treatment group. Significant reduction in time (days) to reach full enteral feeding in the treatment arm. No drop outs or adverse events associated with probiotics were reported.

Reference Country	ITT Study size (T/C)[1]	L-lactic acid bacteria and synbiotic added	Concentration and frequency	Age[2] (duration)	Intervention and adverse event outcomes[3]
Chrzanowska-Liszewska et al. (2012) Poland	21/26	*L. rhamnosus* GG ATCC 53103	$6x10^9$ cfu once daily	NR (42d)	LGG was increased in stools at day 7 and day 14 but was at background levels on day 42 (end of study). Greater proportion of stool samples from treatment arm were positive for *Enterobacteriaceae*, enterococci and staphylococci compared with control. No drop outs or adverse events associated with probiotics were reported.
Costeloe et al. (2016) UK	650/660	*B. breve* BBG-001	$6.7x10^7$ to $6.7x10^9$ cfu once daily	1–2d (to 36w PMA)	No difference in incidence or severity of NEC, late onset sepsis or death between treatment and control arms. No difference between groups in time taken to reach full enteral feeds of 150 ml/kg/day. No adverse events associated with probiotic were observed and *B. breve* was not isolated from any normally sterile site.
Dani et al. (2002) Italy	295/290	*L. rhamnosus* GG	$6x10^9$ cfu once daily	3–4d (47d)	No reduction in the incidence of urinary tract infection, NEC or sepsis was observed in the treatment arm compared to the control arm. No adverse events associated with probiotic supplementation were observed.
Dilli et al. (2015) Turkey	100/100 100/100 100/100	*B. lactis* *B. Lactis* + inulin Inulin	$5x10^9$ cfu once daily $5x10^9$ cfu/900 mg once daily 900 mg once daily	3–4d (≤56d)	NEC rate lower in probiotic and synbiotic c.f. control and prebiotic arms. Lower sepsis and mortality, shorter stays in intensive care and shorter time to full enteral feeding in probiotic, synbiotic and prebiotic arms c.f. control. No adverse events associated with probiotic.

Reference Country	ITT Study size (T/C)[1]	L-lactic acid bacteria and synbiotic added	Concentration and frequency	Age[2] (duration)	Intervention and adverse event outcomes[3]
Fujii et al. (2006) Japan	11/8	*B. breve* M-16V	$1x10^9$ cfu twice daily	<24h (59d)	No adverse effects were observed after *B. breve* supplementation.
Hays et al. (2016) France	50/52 48/52 47/52	*B. lactis* *B. longum* *B. lactis* *B. longum*	$1x10^9$ cfu once daily $1x10^9$ cfu once daily $1x10^9$ cfu once daily $1x10^9$ cfu once daily	≤7d (28d if GA≥29w; 42d if GA≤28d)	Similar weight gain across treatment arms. The same incidence of NEC, use of antibiotics and feeding tolerance across treatment arms. Significantly higher proportion of infants with stool positive for *Bifidobacteria* spp. in treatment arms containing *B. lactis*. No adverse effects were associated with probiotic administration.
Hikaru et al. (2010) Japan	108/100	*B. breve* M-16V	$1x10^9$ cfu once daily	<24h (until 2300g or CA=37w)	Rates of infection and culture proven sepsis were significantly lower in the probiotic group compared to control. Time to full enteral feeding of 100 ml/kg bw/day was significantly shorter, and weight at expected delivery date was significantly greater, in the probiotic group compared to control. No drop outs or adverse events associated with probiotics were reported.
Jacobs et al. (2013) Australia and New Zealand	548/551	*B. infantis* BB–02 *S. thermophiles* TH–4 350 *B. lactis* BB-12	$3x10^8$ cfu once daily $3.5x10^8$ cfu once daily $3.5x10^8$ cfu once daily	4–7d	Rate of NEC was significantly lower in probiotic treatment arm compared to control. No difference in rates of late-onset sepsis or all-cause mortality in probiotic and control arms. No significant adverse effects of the probiotics were observed and no episodes of definite late onset sepsis were due to probiotics.
Kitajima et al.	45/46	*B. breve* YIT4010	$0.5x10^9$ cfu once daily	<24h	Aspirated air volume from the stomach of

Reference Country	ITT Study size (T/C)[1]	L-lactic acid bacteria and synbiotic added	Concentration and frequency	Age[2] (duration)	Intervention and adverse event outcomes[3]
(1997) Japan				(28d)	infants born at 25–28 gestation was significantly lower in the probiotic arm during the first four weeks compared to control. In a subset of infants, feeding volume and weight gain were greater in infants colonised with *B. breve* compared to infants not colonised. No adverse events associated with probiotic were reported.
Li et al. (2004) Japan	10/10 10/10	*B. breve*	$1.6x10^8$ cfu twice daily	<24h (ns) >24h (ns)	A *Bifidobacteria* spp.-predominant microflora was formed on average after 2 weeks when administration commenced within a few hours of birth compared to 4 weeks if administration commenced after 24 hours. Eight out of ten infants in control period did not have detectable *Bifidobacteria* spp. after the 7 week observation period. No differences in rates of NEC or sepsis between the three arms. No adverse events associated with probiotic were observed.
Manzoni et al. (2006) Italy	39/41	*L. rhamnosus GG*	$6x10^9$ cfu once daily	3d (42d)	Incidence of fungal enteric colonisation was significantly lower in the probiotic arm compared to control. No adverse effects potentially associated with the probiotic were recorded.
Manzoni et al.	151/168	*L. rhamnosus GG*	$6x10^9$ cfu once daily	3d	Incidence of late-onset sepsis was significantly lower in the probiotic group compared to

Reference Country	ITT Study size (T/C)[1]	L-lactic acid bacteria and synbiotic added	Concentration and frequency	Age[2] (duration)	Intervention and adverse event outcomes[3]
(2009) Italy		+ bovine lactoferrin	+100mg once daily	(28d if BW≥1000g 42d if BW<1000g)	control. No adverse effects or intolerances to treatment occurred.
Manzoni et al. (2014) Italy	238/258	*L. rhamnosus GG* + bovine lactoferrin	$6x10^9$ cfu once daily +100mg once daily	3d (30d if BW≥1000g 45d if BW<1000g)	Incidence of NEC or death-and/or-NEC was significantly lower in the probiotic group compared to control. No adverse effects or intolerances to treatment occurred.
Mihatsch et al. (2010) Germany	91/89	*B. lactis BB12*	$2x10^9$ cfu/kg body weight six times per day	11–12d (until 42d)	No differences in incidence of nosocomial infections or NEC were observed between treatment and control groups. No adverse effect of *B. lactis* and no blood cultures positive for *B. lactis*.
Millar et al. (1993) United Kingdom	10/10	*L. rhamnosus GG*	$1x10^8$ cfu twice daily	From initiation of milk feeds (14d)	LGG was present in stool from week 1 and declined in number at 5 weeks. Colonisation with probiotic strain did not reduce the reservoir of potential pathogens in the intestine. No difference in antibiotic use and no clinical benefit was observed in the probiotic group compared to control. No adverse events were observed and no infections were attributed to LGG.
Mohan et al.	37/32	*B. lactis* BB12	$1.6x10^9$ cells (d1–3)	<48h	Bifidobacterial numbers were significantly higher in the probiotic group compared to

Reference Country	ITT Study size (T/C)[1]	L-lactic acid bacteria and synbiotic added	Concentration and frequency	Age[2] (duration)	Intervention and adverse event outcomes[3]
(2006) Germany			$4.8x10^9$ cells (from d4)	(21d)	control. Infants supplemented with BB12 had significantly lower viable counts of *Enterobacteriaceae* and *Clostridium* spp. No adverse effect were observed in any of the infants supplemented with BB12.
Patole et al. (2014) Australia	77/77	*B. breve* M-16V	$1.5x10^9$ cfu once daily (GA≤27w and milk feeds <50ml/kg bw/d)[2] $1.5x10^9$ cfu twice daily (milk feeds >50ml/kg bw/d)	From enteral feeding for <12h (PMA=37w or until discharge)	No difference between probiotic and control groups in incidence of NEC, early and late onset sepsis, discharge weight, time to full enteral feeding and length of hospital stay. No adverse effects observed, including probiotic sepsis and no deaths.
Pärtty et al. (2013) Finland	31/32	*L. rhamnosus* GG	$1x10^9$ cfu once daily (d1–30) $1x10^9$ cfu twice daily (d31–60)	<24h (60 days)	Frequency of fussing and crying was significantly less in the probiotic group compared to control. No adverse events related to probiotic supplementation.
Rougé et al. (2009) France	45/49	*B. longum* BB536 *L. rhamnosus* GG	$1x10^8$ cfu four times daily $1x10^8$ cfu four times daily	3–5d (Until discharge; 28–86d)	No difference between probiotic and control groups in incidence of nosocomial infections, NEC, sepsis, enteral feeding measures, antibiotic use, death and length of hospital stay. No adverse effects associated with probiotic.
Sari et al. (2011)	110/111	*Bacillus coagulans* (referred to as	$3.5x10^8$ cfu once daily	2d	There was no significant difference in the incidence of death or NEC between the groups.

Reference Country	ITT Study size (T/C)[1]	L-lactic acid bacteria and synbiotic added	Concentration and frequency	Age[2] (duration)	Intervention and adverse event outcomes[3]
Turkey		*Lactobacillus sporogenes*)		(32d)	Feeding intolerance was significantly lower in the probiotics group than in the control group. No adverse events associated with probiotic and blood cultures did not grow *B. coagulans*.
Stratiki et al. (2007) Greece	41/34	*B. lactis*	$2x10^7$ cfu/g dry milk powder (bolus feeding every 2h up to d2; milk feeds increased by 20 ml/kg bw/day until 150 ml/kg bw/day)	<24h (30d)	Lactulose/mannitol ratio significantly lower in probiotic group c.f. control at day 30. No significant difference in NEC or sepsis incidence or time to full enteral feeding. No significant difference in somatic growth except for head growth. No drop outs or adverse events associated with probiotics were reported.
Totsu et al. (2014) Japan	153/130	*B. bifidum*	$1.25x10^9$ cfu twice daily	<48h (until reaching 2000 g bw)	Enteral feeding was established significantly earlier in the probiotic group and incidence of late-onset sepsis was significantly lower in the probiotic group compared to controls. No adverse events related to probiotic.
van Niekerk et al. (2014) South Africa	37/37 (HIV exposed infants) 54/56 (HIV unexposed)	*L. rhamnosus* GG *B. infantis* *L. rhamnosus* GG *B. infantis*	$0.35x10^9$ cfu once daily $0.35x10^9$ cfu once daily $0.35x10^9$ cfu once daily $0.35x10^9$ cfu once daily	3d (28d) 3–4d (28d)	No difference between probiotic and control groups for feeding tolerance, time to enteral feeding and somatic growth for both HIV exposed and unexposed arms. No drop outs or adverse events associated with probiotics were reported.

[1] ITT, intent to treat; T/C, test formula/control formula; [2] Age at commencement of feeding, and duration of probiotic supplementation; NR, not reported; PMA, postmenstrual age; GA, gestational age; CA, conceptional age; BW, birth weight; [3] LGG, *L. rhamnosus* GG; NEC, necrotising enterocolitis.

3.2.2 Infectious complications with lactic acid producing bacteria supplementation

Outside of the clinical trials summarised in the preceding sections, infectious complications have been associated with the use of L- and DL-lactic acid producing bacteria in preterm infants. Nineteen case studies of sepsis or bloodstream infection associated with the use of lactic acid producing bacteria in infants from 2004 to 2019 were identified, of which 16 were associated with preterm infants with complications including intestinal perforations and surgery, short gut syndrome and respiratory distress. For the three case studies of clinically unwell full term infants, surgical complications were associated with impaired gut barrier function.

Six of the case studies reporting sepsis or bloodstream infections were associated with *L. rhamnosus* GG; two were associated with untyped *Lactobacillus* spp.; and one was associated with *L. reuteri* (Table 3.3). Ten of the case reports were associated with the administration of *B. longum* subsp. *infantis* in combination with *L. acidophilus* (Infloran*)*, and all but one of these reports isolated *B. longum* subsp. *infantis* from blood cultures. The remaining case isolated *L. rhamnosus* from blood cultures, and it was also cultured from the probiotic capsule listed as containing only *B. longum* subsp. *infantis* and *L. acidophilus* (Brecht et al. 2016).

3.2.3 Key findings on safety of L-lactic acid producing bacteria for preterm, low birth weight and immune-compromised infants

The published clinical trial data on the safety of L-lactic acid producing bacteria has not identified any potential risks to public health and safety for preterm, low birth weight and immune-compromised infants. The formulas supplemented with lactic acid producing bacteria were well tolerated, and no adverse events associated with the lactic acid bacteria were noted in the clinical trials assessed.

FSANZ did, however, identify 19 case reports of sepsis or bloodstream infection linked to the administration of lactic acid producing bacteria to preterm infants and term infants with clinical complications. FSANZ concludes that, in preterm, low birth weight and immunocompromised infants, predisposing clinical complications can increase the likelihood that infant formula supplemented with non-pathogenic L- and DL-lactic acid producing bacteria can cause opportunistic sepsis or bloodstream infections. However, due to a lack of sufficient data on infectivity and exposure, FSANZ is unable to assess the level of the risk in these circumstances.

Table 3.3 Summary of case studies detailing sepsis and bloodstream infections associated with administration of lactic acid producing bacteria

Author Country	Commencement of Supplementation[1]	Bacterial species	Onset of symptoms and positive culture[1]	Blood culture isolate	Background[2]
Kunz et al. (2004) USA	DOL 95	*L. rhamnosus* GG	DOL 108	*Lactobacillus* spp. (no typing results reported)	Preterm GA 36 weeks. Short gut syndrome secondary to surgery to correct congenital intestinal abnormality
Kunz et al. (2004) USA	DOL 17	*L. rhamnosus* GG	DOL186	*L. rhamnosus* GG (identical to probiotic strain by DNA finger printing)	Preterm GA 34 weeks. Severe bowel infarction and corrective surgery. Gastrostomy and jejunostomy shortly after birth and dependent on total parenteral nutrition
Land et al. (2005) USA	Day 79 post hospitalisation	*L. rhamnosus* GG	Day 99 post hospitalisation	*Lactobacillus* spp. (no typing results reported)	Full term (BW 3200 g). Admitted for scheduled cardiac surgery at six weeks of age with post-operative complications. Probiotic therapy commenced to resolve antibiotic associated non-bloody diarrhoea
Groote et al. (2005) USA	9.5 mo	*L. rhamnosus* GG	11 mo	*L. rhamnosus* GG *Candida albicans* (ribosomal gene analysis)	Short gut syndrome. Probiotic supplementation for treatment and prevention of rotavirus related diarrhea; *C. albicans* primarily responsible for clinical symptoms
Ohishi et al. (2010) Japan	DOL 2	*B. breve* BBG-01	DOL 10	*B. breve* BBG-01 (rapid amplified polymorphic DNA (RAPD) analysis)	Full term GA 37/2 weeks; BW 2060 g; omphalocele[2], intestinal resection

Author Country	Commencement of Supplementation[1]	Bacterial species	Onset of symptoms and positive culture[1]	Blood culture isolate	Background[2]
Jenke et al. (2012) Germany	DOL 9	*B. longum* subsp. *infantis* ATCC 15697 *L. acidophilus* (Infloran)	DOL 18	*B. longum* *B. longum* subsp. *infantis* (*B. infantis* identical to probiotic strain by strain-specific PCR)	Preterm GA 27/5 weeks; BW 600 g; twin-to-twin transfusion syndrome *in utero* treated at 16 weeks GA
Doern et al. (2014) USA	8 mo	*L. rhamnosus* GG	11 mo	*L. rhamnosus* GG (aspiration pneumonia) (repetitive sequence PCR fingerprinting)	Down syndrome. Hospital admission at 11 months with febrile illness and aspiration pneumonia positive for respiratory syncytial virus (RSV) and probiotic *L. rhamnosus* GG; oesophageal atresia, surgery, ongoing difficulty swallowing and gastrostomy tube dependent from 1 mo.
Zbinden et al. (2015) Switzerland	DOL 1	*B. longum* subsp. *infantis* ATCC 15697 *L. acidophilus* (Infloran, Italy)	DOL 20	*B. longum* subsp. *infantis* (16s rRNA gene analysis)	Preterm GA 30 weeks; BW 1200 g; Mild respiratory distress requiring breathing assistance to DOL 9
Zbinden et al. (2015) Switzerland	DOL 3	As above	DOL 20	As above	Preterm GA 28 weeks; BW 850 g; Mechanical breathing and supplemental oxygen with development of chronic lung disease
Zbinden et al. (2015) Switzerland	DOL 1	As above	DOL 11	As above	Preterm GA 29 weeks; BW 1230 g; rapid onset of NEC on DOL 11 with surgery and small bowel resection.
Bertelli et al. (2015)	DOL 5	*B. longum* subsp. *infantis*	DOL 14	*B. longum* subsp.	Preterm GA 26/2 weeks; BW 867 g;

Author Country	Commencement of Supplementation[1]	Bacterial species	Onset of symptoms and positive culture[1]	Blood culture isolate	Background[2]
Switzerland		ATCC 15697 *L. acidophilus* (Infloran, Italy)		*infantis* (Whole genome comparative genomics analysis)	respiratory distress
Bertelli et al. (2015) Switzerland	DOL 5	As above	DOL 10	As above	Preterm GA 28/6 weeks; BW 1090 g; respiratory distress
Brecht et al. (2016) Australia	DOL 18	*B. longum bifidum* *L. acidophilus* *(Infloran)* *(+L. rhamnosus contaminant)*	DOL63	*L. rhamnosus* (RiboPrint analysis)	Preterm GA 25/6 weeks; BW 970 g; Multiple intestinal perforations, followed by resection surgery
Esaiassen et al. (2016) Norway	DOL 1	*B. longum* subsp. *infantis* ATCC 15697 *L. acidophilus* (Infloran, Italy)	DOL 8	*B. longum* subsp. *infantis* (no typing results reported)	Preterm GA 24 weeks; BW 730 g; respiratory distress syndrome and received mechanical ventilation; multiple gut perforations and necrosis identified on DOL 12.
Esaiassen et al. (2016) Norway	DOL 1	As above	DOL 12	As above	Preterm GA 23 weeks; BW 500 g; respiratory distress syndrome and received mechanical ventilation
Esaiassen et al. (2016) Norway	DOL 1	As above	DOL 46	As above	Preterm GA 24 weeks; BW 697 g; respiratory distress syndrome and received mechanical ventilation; Multiple gut perforations; *Enterococcus faecalis* associated sepsis on DOL 9
Molinaro et al. (2016)	DOL 2	*L. rhamnosus* GG (ATCC 53103)	DOL 20	*L. rhamnosus* GG (no typing results	Preterm GA 23 weeks

Author Country	Commencement of Supplementation[1]	Bacterial species	Onset of symptoms and positive culture[1]	Blood culture isolate	Background[2]
Italy				reported)	
Celis Castañeda et al. (2019) Columbia	DOL 1	*L. reuteri*	DOL 3	*L. reuteri* (Blood cultures positive for *L. reuteri*. No typing results reported)	Preterm GA 27 weeks; BW 840 g; respiratory distress. Patient died on DOL 3.
Cavicchiolo et al. (2019) Italy	DOL 3	*L. rhamnosus* GG	DOL 18	*L. rhamnosus* GG (RAPD analysis)	Preterm GA25/6 weeks; BW 770 g; peripherally inserted central catheter

[1] DOL, day of life; mo, months old; [2] GA, gestational age; BW, birth weight; NEC, necrotising enterocolitis; omphalocele, infant born with intestine or other abdominal organs outside the body.

3.3 DL-lactic acid producing bacteria

Lactobacillus reuteri and *L. fermentum* are both DL-lactic acid producing bacteria commonly used as probiotics in infant formula. Other common probiotics that are DL-lactic acid producing bacteria include *L. acidophilus, L. johnsonii*, and *L. helveticus*. These DL-lactic acid producing bacteria have been assessed in clinical trials in healthy full term and preterm infants either alone or in combination with L-lactic acid producing bacteria, administered in infant formula or in oil emulsion. A summary of the clinical trials are detailed in Tables 3.4 and 3.5, below.

In healthy full term infants, five studies were identified that examined the safety of DL-lactic acid producing bacteria where growth was the primary outcome (Gil-Campos et al. 2012; Le Lee et al. 2015; Maldonado et al. 2019; Maldonado-Lobón et al. 2015; Manzano et al. 2017).

Gil-Campos et al. (2012) and Maldonado-Lobón et al. (2015) compared infant formula containing GOS (0.3 g/100 ml) and *L. fermentum* CECT5716 with an identical formula without *L. fermentum*. Healthy, full term one-month old infants were recruited from mothers who had elected to exclusively formula-feed. The study was powered to detect a difference in weight gain equal to 0.5 standard deviations from baseline to 120 days of age.

Le Lee et al. (2015) compared infant formula containing *L. reuteri* DSM 17938 with and without concurrent addition of the oligosaccharides GOS (5.5 g/L) and FOS 0.36 g/L. Healthy, full term newborn infants were recruited from mothers who had elected not to breast feed after discharge, and the sample size was calculated based on the primary outcome of showing non-inferiority in weight gain. The non-inferiority margin was set at −0.5 standard deviations (SD) based on the WHO child growth standards[5].

Maldonado et al. (2019) compared standard formula with identical formula containing either *L. fermentum* CECT5716 Lc40 or *B. breve* CECT7263. Healthy, full term one-month old infants were recruited from mothers who had elected to exclusively formula-feed. The study was powered to detect a difference in weight gain equal to 0.5 standard deviations from baseline to 120 days of age.

Manzano et al. (2017) compared infants supplemented with either *B. infantis* R0033, L*. helveticus* R0052 or *B. bifidum* R0071 in potato starch as the excipient and reconstituted in 10 ml of water, breast milk or infant formula. The control group were given the excipient only. Healthy, full term infants aged 3 to 12 months were recruited, and the study was powered to assess equivalence with a minimum weight gain of 6 g/day. Urinary D- and L-lactate concentrations were also compared between the test and control groups.

Eight other studies were identified that examined the efficacy of DL-lactic acid bacteria in treating or preventing functional gastrointestinal disorders—such as colic, regurgitation or constipation—in healthy full term infants (Chau et al. 2015; Indrio et al. 2014; Savino et al. 2007; Savino et al. 2010; Savino et al. 2015; Sung et al. 2014; Szajewska et al. 2013). A further three studies were identified that examined safety associated with urinary or blood D-lactate concentration (Connolly et al. 2005; Haschke-Becher et al. 2008; Papagaroufalis et al. 2014). Two related studies were identified that examined the use of DL-lactic acid producing bacteria to prevent allergy (Abrahamsson et al. 2007; Abrahamsson et al. 2013; Table 3.4). Sample size calculations were based on primary outcome measures and, where noted in Table 3.4 below, anthropometric measures were also reported.

[5] https://www.who.int/toolkits/child-growth-standards/standards

In preterm infants, 13 studies were identified that assessed the efficacy of DL-lactic acid producing bacteria—either alone or in combination with L-lactic acid producing bacteria—to prevent NEC, sepsis, infection, feeding intolerance or fungal colonisation (Chowdhury et al. 2016; Fernández-Carrocera et al. 2013; Indrio et al. 2008; Indrio et al. 2017; Kanic et al. 2015; Lin et al. 2005; Lin et al. 2008; Oncel et al. 2014; Rojas et al. 2012; Romeo et al. 2011; Roy et al. 2014; Saengtawesin et al. 2014; Samanta et al. 2009; Table 3.5). Dutta et al. (2015) assessed the effect of probiotic supplementation on gastrointestinal colonisation in preterm infants. Sample size calculations were based on primary outcome measures and, where noted in Table 3.5 below, anthropometric measures were also reported.

The published clinical trials on the safety of DL-lactic acid producing bacteria—alone or in combination with L-lactic acid producing bacteria—did not identify any risks for healthy full term and preterm infants. Infant formulas supplemented with DL-lactic acid producing bacteria were well tolerated, and no adverse events associated with the lactic acid bacteria were noted in the clinical trials assessed.

FSANZ concludes that infant formula supplemented with non-pathogenic DL-lactic acid producing microorganisms does not present a risk to public health and safety for healthy, full term and preterm infants.

As noted above (Section 3.2.3), predisposing clinical complications can increase the likelihood that infant formula supplemented with non-pathogenic DL-lactic acid producing bacteria can cause opportunistic sepsis or bloodstream infections in infants with underlying clinical complications—including preterm, low birth weight and immunocompromised infants. However, due to a lack of sufficient data on infectivity and exposure, FSANZ is unable to assess the level of the risk in these circumstances

Table 3.4 Clinical trials in healthy term infants receiving DL-lactic acid producing bacteria supplementation

Reference Country	ITT Study size (T/C)[1]	Lactic acid bacteria and synbiotic added	Concentration and frequency	Age (treatment duration)	Intervention and adverse event outcomes
Abrahamsson et al. (2007) Sweden	97/93	*L. reuteri* ATCC 55730	$1x10^8$ cfu once daily	<1d (12m)	Cumulative incidence of eczema was the same in each group. However, incidence of IgE associated eczema was significantly lower in the *L. reuteri* group at 2 year follow up. No other differences for allergy related outcomes. Infants significantly heavier in the *L. reuteri* than the placebo group at 3 months (6.4 vs 6.1 kg), but not at other time points. No differences for measures of tolerance—including spitting-up, colic—and constipation to 12 months of age. No severe adverse events were reported.
Abrahamsson et al. (2013) Sweden	94/90	*L. reuteri* ATCC 55730	$1x10^8$ cfu once daily	<1d (12m)	Follow-up from Abrahamsson et al. (2007), the prevalence of asthma, allergic rhinoconjunctivitis, eczema and skin prick test reactivity was similar in the probiotic and placebo group after 7 years. No differences in anthropometric measurements between groups. No severe adverse events were reported.
Chau et al. (2015) Canada	27/28	*L. reuteri DSM 17938*	$1x10^8$ cfu once daily	41–42d (21d)	Significant reduction in crying time in the treatment group compared to control. Tolerance and growth parameters equivalent for treatment and control groups. No adverse events associated with the probiotic supplementation were reported.
Coccorullo et al. (2010) Italy	22/22	*L. reuteri DSM 17938*	$1x10^8$ cfu once daily	>6m (8w)	Significant increase in frequency of bowel movements in the probiotic group, but no difference in stool consistency or inconsolable crying compared to control formula. No adverse effects such as vomiting, bloating or increased flatulence were reported.

Reference Country	ITT Study size (T/C)[1]	Lactic acid bacteria and synbiotic added	Concentration and frequency	Age (treatment duration)	Intervention and adverse event outcomes
Connolly et al. (2005) Sweden	14/10	*L. reuteri ATCC 55730*	$1x10^8$ cfu once daily	<24h (12m)	No differences in height and weight for infants in the treatment and control groups after 12 months supplementation. D-lactic acid levels in the blood of infants was very low in both groups and no symptoms associated with lactic acidosis were observed. No adverse events were observed after long-term dietary supplementation in newborn infants.
Gil-Campos et al. (2012) Spain	66/71	*L. fermentum* CECT5716 +GOS	$1x10^7$ cfu/g formula 0.3 g/100 mL	1m (5m)	No significant difference in anthropometric measures of weight and head circumference at 6 months of age. Mean length of infants in the probiotic group (68.1cm) was significantly greater than the control group (66.6cm), but no difference was observed for length gain per day. This difference was not observed on long term follow up (Maldonado-Lobón et al. 2015). No adverse effects associated with probiotic supplementation were observed and tolerance was the same between groups.
Haschke-Becher et al. (2008) Chile	19/26 (+26 breast fed infant reference group)	*L. johnsonii La1*	$1x10^8$ cfu/g formula (ca. $0.8–1.1x10^{10}$ cfu daily)	16w (4w)	No significant difference in urinary D-lactate excretion between the two formula groups. Both formula groups significantly higher than breast fed infants. No difference for breast fed and formula fed infants for urinary L-lactate excretion. No differences in weight and length gain at end of 4 week trial. Authors did not report on adverse effects.
Indrio et al. (2014) Italy	276/278	*L. reuteri DSM 17938*	$1x10^8$ cfu once daily	<1w (90d)	At 90 days, probiotic group had significantly decreased crying time and regurgitation frequency and increased frequency of bowel movements compared to controls. No adverse events reported that were related to the trial.

Reference Country	ITT Study size (T/C)[1]	Lactic acid bacteria and synbiotic added	Concentration and frequency	Age (treatment duration)	Intervention and adverse event outcomes
Le Lee et al. (2015) Singapore	72/68 (control group was *L. reuteri*)	*L. reuteri* DSM 17938 +GOS/FOS	$1x10^8$ cfu per day in formula (ad libitum) 5.5–0.36 g/L	≤14d (to 6mo)	Test formula containing *L. reuteri* and GOS/FOS was not inferior to the WHO standards nor to that of infants fed a control formula containing only *L. reuteri*. Other anthropometric measures at 4 months were equivalent between groups. Urinary D-lactate concentrations were not different between the two groups. Relative abundance of bifidobacteria was significantly greater in test formula as was higher frequency of liquid stools compared to formula containing only *L. reuteri*. Other measures of tolerance were not different and no adverse effects associated with formula or probiotic supplementation were observed in either group.
Maldonado et al. (2012) Spain	110/98	*L. fermentum* CECT5716 + GOS	$2x10^8$ cfu per day average dose 0.4g/100 ml	6m (6m)	No differences were found for weight, length, head circumference and growth rate between the study groups. No adverse effects related to the formulas and probiotic were reported.
Maldonado-Lobón et al. (2015) Spain	55/55	*L. fermentum* CECT5716 +GOS	$1x10^7$ cfu/g formula 0.3 g/100 mL	1m (5m)	See Gil-Campos et al. (2012) for short-term safety outcomes. After 3 years of follow-up, no significant differences were observed between the control and probiotic groups for anthropometric measures, gastrointestinal or respiratory illness, allergy or stool consistency and frequency.
Maldonado et al. (2019) Spain	83/77 76/77	*L. fermentum* CECT5716 Lc40 *B. breve* CECT7263	$1x10^7$ cfu/g formula $1x10^7$ cfu/g formula	1m (11m) 1m (11m)	No significant differences in anthropometric measurements for weight, length and head circumference at 2, 4, 6, 9 and 12 months of age for the three groups. No adverse effects associated to supplementation with probiotics were detected during the study.

Reference Country	ITT Study size (T/C)[1]	Lactic acid bacteria and synbiotic added	Concentration and frequency	Age (treatment duration)	Intervention and adverse event outcomes
Manzano et al. (2017) Spain	53/52 51/52 52/52	*B. infantis* R0033 *B. bifidum* R0071 *L. helveticus* R0052	$3x10^9$ cfu once daily $3x10^9$ cfu once daily $3x10^9$ cfu once daily	3–12m (8w)	Anthropometric measures showed equivalence for each of the 3 treatment groups when compared to the placebo. Probiotics were well tolerated and no differences were observed in sleeping and crying, stool characteristics, diarrhoea, fever, rash or unscheduled doctor visits between the groups. Urinary D-lactic acid levels were below the quantification limit for the test used for all groups and tested samples. No severe adverse effects associated with the probiotics were observed.
Papagaroufalis et al. (2014) Greece	44/44	*L. reuteri* DSM 17938	$1.2x10^6$ cfu/ml formula	<3d (25–28d)	Anthropometric measurements were not significantly between the two groups at 7, 14, 28 and 112 days of follow-up. Urinary D-lactate concentration was greater at day 7 and 14 but not at day 28 and 112. Blood acid and pH was not significantly different between the two groups at day 14. No adverse effects associated with the probiotic were observed.
Savino et al. (2007) Italy	45/45[1]	*L. reuteri* ATCC 55730	$1x10^8$ cfu once daily	11–80d (28d)	Probiotic treatment significantly reduced crying time in colicky infants compared to simethicone[3] treatment.
Savino et al. (2010) Italy	25/25	*L. reuteri* DSM 17938	$1x10^8$ cfu once daily	2–16w (21d)	Probiotic treatment significantly reduced crying time in colicky infants at day 21 compared to placebo control. There were no differences in weight gain, stool frequency, constipation or regurgitation between groups. No adverse events related to the supplementation were observed.

Reference Country	ITT Study size (T/C)[1]	Lactic acid bacteria and synbiotic added	Concentration and frequency	Age (treatment duration)	Intervention and adverse event outcomes
Savino et al. (2015) Italy	55/58	*L. reuteri* DSM 17938 + Vitamin D3	$1x10^8$ cfu once daily + 400 IU once daily	<10d (12w)	Probiotic treatment significantly reduced the use of medications for the management of pain associated with colic compared to control. Adverse effects were not reported by the authors, but no drop-outs associated with the probiotics were reported.
Sung et al. (2014) Australia	85/82	*L. reuteri* DSM 17938	$1x10^8$ cfu once daily	<13w (1m)	The probiotic group cried or fussed significantly more than the placebo group at 1 month but not at 6 months, especially in formula fed infants. Adherence was the same in both groups and no study related adverse events occurred.
Szajewska et al. (2013) Poland	42/40	*L. reuteri* DSM 17938	$1x10^8$ cfu once daily	3–12w (21d)	Probiotic treatment significantly reduced crying time in colicky infants at day 14, 21 and 28. No adverse events associated with the probiotic therapy or with the use of the placebo were reported.

[1] ITT, intent to treat; T/C, test formula/control formula; [2] GOS, galactooligosaccharide; GOS/FOS, 90% galactooligosaccharide/ 10% short-chain fructooligosaccharide (FOS); [3] Breast fed infants and control group received 60 mg/day simethicone.

Table 3.5 Clinical trials in preterm and low birth weight infants receiving a combination of L- and DL-lactic acid bacteria supplementation

Reference Country	ITT Study size (T/C)[1]	Lactic acid bacteria added	Isomer	Concentration and frequency	Age[2] (treatment duration)	Intervention and adverse event outcomes[3]
Chowdhury et al. (2016) Bangladesh	52/50	*B. bifidum* *B. infantis* *B. longum* *L. acidophilus* *L. lactis*	L L L DL L	$3x10^9$ cfu once daily $3x10^9$ cfu once daily $3x10^9$ cfu once daily $3x10^9$ cfu once daily $3x10^9$ cfu once daily	72h (≤ 10d)	Rate and severity of NEC was reported to be reduced with test formula (T1/52 vs C6/50). Age of full feed and length of hospital stay was reduced in the test formula group. No adverse events were reported. No drop-outs due to intolerance.
Dutta et al. (2015) India	38/35	*L. acidophilus* *L rhamnosus* *B longum* *S. boulardii*	DL L L N/A	$5.3x10^9$ cfu twice daily $2.9x10^9$ cfu twice daily $0.7x10^9$ cfu twice daily $1.1x10^9$ cfu twice daily	4d (21d)	Colonisation rates with *Lactobacillus* and *Bifidobacterium* was significantly higher in the three probiotic treatment arms compared to control. Authors did not report on adverse effects. No drop-outs due to intolerance.
	38	*L. acidophilus* *L rhamnosus* *B longum* *S. boulardii*	DL L L N/A	$5.3x10^9$ cfu twice daily $2.9x10^9$ cfu twice daily $0.7x10^9$ cfu twice daily $1.1x10^9$ cfu twice daily	4d (14d)	
	38	*L. acidophilus* *L. rhamnosus* *B longum* *S. boulardii*	DL L L N/A	$6.6x10^8$ cfu twice daily $3.6x10^8$ cfu twice daily $0.9x10^8$ cfu twice daily $1.4x10^8$ cfu twice daily	4d (21d)	
Fernández-Carrocera et al. (2013) Mexico	75/75	*B. infantis* *L. acidophilus* *L. casei* *L. plantarum* *L. rhamnosus* *S. thermophilus*	L DL L DL L L	$2.76x10^7$ cfu/day $1x10^8$ cfu/day $1x10^9$ cfu/day $1.76x10^8$ cfu/day $4.4x10^8$ cfu/day $6.6x10^5$ cfu/day	5d (36–38d)	Rate and severity of NEC was not significantly different between groups. No adverse events observed.

Reference Country	ITT Study size (T/C)[1]	Lactic acid bacteria added	Isomer	Concentration and frequency	Age[2] (treatment duration)	Intervention and adverse event outcomes[3]
Indrio et al. (2008) Italy	10/10	*L. reuteri DSM17938*	DL	1x10^8 cfu/day	3–5d (n.s.)	Gastric motility was more similar to breast milk fed infants in test formula group. No adverse events observed.
Indrio et al. (2017) Italy	30/30	*L. reuteri DSM17938*	DL	1x10^8 cfu/day	48h (30d)	Age to reach full feed, length of hospital stay and duration of antibiotic treatment was significantly reduced and gastric motility improved in test formula group. Weight at 30d was greater in test formula group. No adverse events observed.
Kanic et al. (2015) Slovenia	40/40	*B. infantis* PTA-5843 *E. faecium* PTA 5844 *L. acidophilus* PTA-5845	L L DL	1.2x10^7 cfu/day 1.2x10^7 cfu/day 1.2x10^7 cfu/day	n.s. (n.s.)	Rate of late onset sepsis was reduced with test formula. No adverse effects observed.
Lin et al. (2005) Taiwan	180/187	*B. bifidum* *L acidophilus*	L DL	1x10^9 cfu/day 1x10^9 cfu/day	7d (n.s.)	Incidence of NEC, sepsis and death reduced in test formula group. No adverse effects observed.
Lin et al. (2008) Taiwan	222/221	*B. bifidum* *L acidophilus*	L DL	2x10^9 cfu/day 2x10^9 cfu/day	4d (42d)	Incidence of NEC was reduced in test formula group but death attributable to NEC was not different between groups. No adverse effects observed.

Reference Country	ITT Study size (T/C)[1]	Lactic acid bacteria added	Isomer	Concentration and frequency	Age[2] (treatment duration)	Intervention and adverse event outcomes[3]
Oncel et al. (2014) Turkey	200/200	*L. reuteri DSM17938*	DL	$1x10^8$ cfu/day	24h (3m)	Rate and severity of NEC and mortality did not differ between groups. In VLBW group, a reduction in the rate of sepsis, age of full feed and feeding intolerance was observed in the test formula group. No adverse effects observed.
Rojas et al. (2012) Columbia	372/378	*L. reuteri DSM17938*	DL	$1x10^8$ cfu/day	24–48h (2m)	Morbidity and mortality did not differ between groups. In VLBW cohort, feeding intolerance was reduced in test formula group but this did not affect anthropometric measures. Authors did not report on adverse effects. No drop-outs due to intolerance.
Romeo et al. (2011) Italy	83/83	*L. reuteri* DSM17938 *L. rhamnosus* ATCC 53103	DL L	$1x10^8$ cfu/day $6x10^9$ cfu/day	34h (42d) 34h (42d)	Candida colonisation of stool reduced in test formula group. Time on parenteral feed, antibiotic treatment and hospital stay was also reduced in the test formula group. Number of infants with gastrointestinal symptoms and thus infants requiring hydrolysed milk was lower in the test formula groups. No adverse effects observed.
Roy et al. (2014) India	56/56	*B. bifidum* *B. lactis* *B. longum* *L acidophilus*	L L L DL	$1.2x10^9$ cfu/day $1x10^{10}$ cfu/day $1.2x10^9$ cfu/day $1.2x10^{10}$ cfu/day	72h (42d)	Fungal presence in stool did not differ between groups but rate of fungal sepsis was reduced and absence of sepsis was higher in the test formula group. Age of full feed and length of hospital stay was reduced in the test formula group. Comorbidities and mortality did not differ. Authors did not report on adverse effects. No drop-outs due to intolerance.

Reference Country	ITT Study size (T/C)[1]	Lactic acid bacteria added	Isomer	Concentration and frequency	Age[2] (treatment duration)	Intervention and adverse event outcomes[3]
Saengtawesin et al. (2014) Thailand	31/29	*B. bifidum* *L acidophilus*	L DL	$2x10^9$ cfu/day $2x10^9$ cfu/day	24h (42d)	Morbidity, feeding and anthropometric measures did not differ between groups. No adverse effects observed.
Samanta et al. (2009) India	91/95	*B. bifidum* *B. infantis* *B. longum* *L acidophilus*	L L L DL	$5x10^9$ cfu/day $5x10^9$ cfu/day $5x10^9$ cfu/day $5x10^9$ cfu/day	6d (14–21d)	Rate of NEC, culture-proven sepsis and mortality was lower in test formula group although severity of NEC did not differ between groups. Age of full feed and length of hospital stay was also reduced in the test formula group. Authors did not report on adverse effects. No drop-outs due to intolerance.

[1] ITT, intent to treat; T/C, test formula/control formula; [2] n.s., not specified; [3] NEC, necrotising enterocolitis; VLBW, very low birth weight (<1000 g).

3.4 L-lactic acid producing bacteria and fermented infant formulas

Four randomised clinical control trials were identified that investigated benefits associated with fermented infant formula in healthy full-term infants (Indrio et al. 2007; Morisset et al. 2011; Mullié et al. 2004; Thibault et al. 2004—reviewed by Szajewska et al. 2015 and Agostoni et al. 2007). In the four identified studies, viable bacteria were heat inactivated after the initial fermentation and acidification process, to produce an infant formula with no viable lactic acid producing bacteria. Formulas were fermented with the L-lactic acid producing strains *S. thermophilus* O65 and *B. breve* C50 in all identified studies. Urinary or blood D-lactate concentrations were not tested in the studies identified (Łukasik et al. 2018). However, D-lactic acidosis would not be expected, as the fermenting bacteria used were L-lactic acid producers.

Anthropometric measures were not the primary outcome for any of the four identified studies. However, there were no observed differences in growth between fermented formula (intention to treat n=484) and standard formula (intention to treat n=484) for 4 to 6 month old infants who were followed for five months (Thibault et al. 2004). Mullié et al. (2004) recruited 30 vaginally born healthy infants whose parents had elected not to breast feed. Fifteen infants were assigned to either fermented formula or standard formula groups and followed for 5 months, with the primary outcome being intestinal antibody response to polio vaccination. Nine infants in the fermented formula group and 11 in the standard formula group completed the study and were included in the analysis. No differences were observed in anthropometric and tolerance measurements at the 1, 2, 3 and 4 month follow-up time points (Mullié et al. 2004).

An additional study was identified that investigated the safety of fermented preterm formula in preterm infants born between gestational age 30 and 35 weeks (Campeotto et al. 2011). A total of 58 infants from two hospitals in France were recruited to receive either fermented preterm formula (n=24) or the same formula unfermented (n=34). Test formula was fermented with *S. thermophilus* O65 and *B. breve* C50 and heat inactivated to end the process. Fifty two infants completed the study and were followed up until discharge from hospital. No differences in anthropometric data were found between the two groups. Abdominal distension was similar in the two groups during weeks 1 and 2, but abdominal distension was significantly reduced in the fermented formula group during weeks 3 and 4 ($P<0{\cdot}016$). No adverse effects were observed throughout the study.

The published data on the safety of fermented formulas is limited, but no potential risks to public health and safety for healthy full term infants have been identified. FSANZ therefore concludes that formula fermented with L-lactic acid producing bacteria does not present a risk to public health and safety in healthy, full term infants.

Very limited data is available for preterm infants and other vulnerable groups. However, no potential risks to public health and safety have been identified for preterm infants. Therefore, FSANZ concludes that formula fermented with L-lactic acid producing bacteria is unlikely to present a risk to public health and safety in healthy preterm infants.

3.5 Enterococci

Enterococci are L-lactic acid producing bacteria that are ubiquitous in nature and are a normal component of the healthy intestinal microflora of humans and animals. There are more than 20 recognised *Enterococcus* species. *E. faecium* and *E. faecalis* are the most prominent species, as they are important opportunistic human pathogens which may also be used to produce foods such as cheese and fermented meats. They are also increasingly being developed for use as probiotics (Franz 2003; Franz et al. 2011).

In humans, *E. faecalis* and *E. faecium*, are opportunistic pathogens that can cause a range of infections in patients who have undergone surgery or have implanted invasive devices. They rarely cause disease in healthy people, but may cause infections in vulnerable people, such as the very elderly or people who have compromised immunity. They are an important cause of hospital acquired infections, including bloodstream infections, urinary tract infections, intra-abdominal infections—especially those of the biliary tract—and endocarditis (ACSQHC 2017; Selleck et al. 2019).

Enterococci are intrinsically resistant to a wide range of antimicrobials, including cephalosporins, aminoglycosides, lincosamides and streptogramins (Selleck et al. 2019). In addition, resistance determinants to other clinically important antimicrobials, such as vancomycin, tetracyclines and aminoglycosides, are found on mobile genetic elements—e.g. plasmids and transposons—which facilitates dissemination and transmission of resistance through horizontal gene transfer (Hegstad et al. 2010; Selleck et al. 2019). Together with antimicrobial resistance genes, hospital-associated *E. faecium* and *E. faecalis* harbour virulence genes that promote colonisation, biofilm formation and pathogenesis (EFSA 2012; Hanchi et al. 2018; Selleck et al. 2019).

There are two main clades for *E. faecium*—clades A and B—which are distinct and divergent from one another. Most hospital-adapted *E. faecium* isolates belong to clade A, and closely resemble strains isolated from agricultural and companion animals. Non-hospital associated human faecal isolates of *E. faecium* mainly belong to clade B (Selleck et al. 2019). Hospital-acquired infections belonging to clade A are characterised by resistance to ampicillin, which also confers resistance to piperacillin and high-level resistance to cephalosporins. Together with acquired vancomycin resistance, this provides a selective advantage for *E. faecium* in the hospital environment (EFSA 2012).

EFSA recommends that strains belonging to the hospital-associated clade A of *E. faecium* can be excluded from use in animal nutrition by ensuring sensitivity to ampicillin and the absence of the genetic elements IS*16*, hyl_{Efm}, and *esp* (EFSA 2012). However, EFSA have repeatedly excluded *E. faecium* from the list of microorganisms that have a qualified presumption of safety (QPS) due to the taxonomic unit including known human pathogens. Strains to be considered for use in animal nutrition must be assessed on a case-by-case basis (Koutsoumanis et al. 2019). Hanchi et al. (2018) provided recommendations for evaluating enterococci for use in humans—over and above that required by EFSA (2012)—including exclusion of a broader range of resistance and virulence determinants and an absence of production of biogenic amines or toxins.

The population structure of *E. faecalis* is less discrete, and the three dominant lineages—L1, L2, and L3—are fairly closely related (Selleck et al. 2019). Discrimination between pathogenic and non-pathogenic strains is more difficult.

Enterococci have, however, still been developed as probiotics. The most established and researched enterococcal probiotics include *E. faecium* SF68® and *E. faecalis* Symbioflor 1 (Franz et al. 2011). The functional requirements of probiotics include tolerance to human gastric juice and bile; adherence to epithelial surfaces; persistence in the human gastrointestinal tract; immune stimulation activity; antagonistic activity toward intestinal pathogens through bacteriocin production; and the capacity to stabilize and modulate the intestinal microbiota (Ayala et al. 2019; Hanchi et al. 2018).

FSANZ identified one study that assessed *E. faecium* PTA5844 in very low birth weight preterm infants in combination with *B. infantis* and *L. acidophilus* (Linex®)(Kanic et al. 2015). No information was identified that established the safety of *E. faecium* PTA5844 prior to use in this study. However, the study authors claim the probiotic capsule has been on the market

since 1983 with no adverse effects identified. No adverse effects were reported in the relatively small study of 40 preterm infants in the test group (Kanic et al. 2015; Table 3.5).

The principal safety considerations for infants associated with enterococci are colonisation; biofilm formation; antimicrobial resistance; dissemination of resistance and virulence genes; and the production of toxic metabolites. Enterococci probiotic candidate strains commonly harbour virulence and antimicrobial resistance determinants located on mobile genetic elements (Ayala et al. 2019), and these determinants must be excluded prior to addition to infant formula products. These factors are unevenly distributed over the two predominant species, and establishing safety requires assessment on a case-by-case basis.

3.6 Spore forming L-lactic acid producing bacteria

Spore forming bacilli are typically used in the food industry to produce enzymes. The Code currently permits a range of *Bacillus* spp., *Geobacillus* spp., *Anoxybacillus* spp. and *Paenibacillus* spp. for the production of permitted enzymes. The species listed in the Code include, *B. subtilis*, *B. acidopullulyticus*, *B. amyloliquefaciens*, *B. circulans*, *B. coagulans*, *B. halodurans*, *B. licheniformis*, *G. stearothermophilus*, *P. macerans* and *A. caldiproteolyticus*.

Bacillus spp. are not typically considered to be lactic acid producing bacteria. However, there is an emerging trend for the use of L-lactic acid producing *Bacillus* spp. in the commercial production of optically pure L-lactic acid (Poudel et al. 2016) and for use as probiotics (Elshaghabee et al. 2017). Spore forming L-lactic acid producing bacilli, such as *B. coagulans*, have been assessed and used in infants and children (Dutta et al. 2011; Sari et al. 2011). Strains of *B. coagulans* and non-lactic acid producing *B. subtilis* have been developed for use as probiotics in the general population (Cuentas et al. 2017; Elshaghabee et al. 2017; Endres et al. 2009; Endres et al. 2011; Lefevre et al. 2017; Townsend et al. 2018). A new *Bacillus* spp.—DU-106—was recently identified as a L-lactic acid producing probiotic candidate (Li et al. 2018). The new bacillus has not yet been fully taxonomically characterised, but is closely related to toxigenic *B. cereus* (Li et al. 2018)—the latter being an emerging public health threat in China, where toxigenic *B. cereus* strains have been sold as probiotics (Zhu et al. 2016).

One study was identified that assessed *B. coagulans* (DMG ITALIA SRL, Rome, Italy) in preterm low birth weight infants in Turkey. No strain related information was provided and no data was identified establishing the safety of this bacillus in infants. No adverse effects were identified in the 121 preterm infants in the test group (Sari et al. 2011).

The production of L-lactic acid is not uniformly distributed across the *Bacillus* genus or within species groups such as *B. subtilis* or *B. cereus*. The principal safety concern for infants and consumers associated with *Bacillus* spp. is the capacity for toxin production, which is unevenly distributed over the genus (EFSA 2014).

The Australian and New Zealand Advisory Committee on Novel Foods (ACNF)[6]—which is chaired by FSANZ—considers *Bacillus* spp. intended to be added to food on a case-by-case basis (FSANZ 2019), due to safety concerns associated with strain variation. The potential for probiotic *Bacillus* spp. candidates to produce toxins or other toxic metabolites must be excluded prior to addition to infant formula products, and establishing safety requires assessment on a case-by-case basis.

6 http://www.foodstandards.gov.au/industry/novel/novelrecs/Documents/Record%20of%20views%20updated%20November%202019.pdf

4 Conclusions

Supplementation with live microorganisms

The broad and general permission for the addition of any L-lactic acid producing bacteria to infant formula currently includes bacteria with potential to pose a serious risk to the health and safety of infants, especially bacteria belonging to the genera *Enterococcus* and *Bacillus*. There is insufficient evidence to establish the safety of L-lactic acid producing enterococci and spore-forming bacilli, as only two small studies were identified in preterm low birth weight infants—one study each for *E. faecium* and *B. coagulans*. Due to the variability in safety of these L-lactic acid producing bacteria and the known association of some strains with serious and life-threatening illness, safety should be assessed on a case-by-case basis, prior to addition to infant formula products, to provide assurance of public health and safety.

None of the identified clinical trial studies of dietary supplementation of healthy term infants and preterm infants with non-pathogenic L- and DL-lactic acid producing bacteria found a difference in growth, feeding tolerance or severe adverse effects compared to infants fed either control formula or breast milk. The available evidence, from a limited number of studies, indicates that strains of DL-lactic acid producing lactobacilli in healthy term infants does not result in elevated levels of urinary or blood D-lactate, indicating that the addition of non-pathogenic DL-lactic acid producing microorganisms to infant formula are unlikely to cause D-lactic acidosis in healthy infants. Therefore, FSANZ concludes that, in healthy full term infants, infant formula supplemented with non-pathogenic, non-toxigenic L- and DL-lactic acid producing bacteria does to pose a risk to public health and safety.

For infants with underlying clinical complications—including preterm, low birth weight and immunocompromised infants—there are case reports of sepsis and bloodstream infections associated with dietary supplementation with non-pathogenic L- and DL-lactic acid producing bacteria. However, due to a lack of sufficient data on infectivity and exposure, FSANZ is unable to assess the level of the risk in these circumstances.

Fermented formulas

The published data on the safety of fermented formulas is limited, but no potential risks to public health and safety for healthy full term infants have been identified. Therefore, FSANZ concludes that formula fermented with L-lactic acid bacteria—where no viable bacteria are present in the final product—does not present a risk to public health and safety in healthy full term infants.

Very limited data is available for preterm infants and other vulnerable groups. However, no potential risks to public health and safety have been identified for preterm infants. FSANZ therefore concludes that formula fermented with L-lactic acid bacteria is unlikely to present a risk to public health and safety in healthy preterm infants.

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