Yakult

Science for Health

LACTICASEIBACILLUS PARACASEI SHIROTA FUNDAMENTAL RESEARCH BOOKLET

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INTRODUCTION

As healthcare professionals, we are interested in exploring approaches that may help to maintain or restore health. We now know that optimal nutrition for health extends beyond the need for macronutrients, vitamins and minerals. Over the last few decades, the importance of the gut microbiota has become widely recognised. However, the ever-increasing number of diseases that have been 'associated' with a microbiota 'deficiency' has caused some scepticism of the underlying science behind these observations. The complexity and individual diversity of the gut microbiota have added to this scepticism. Still, the progress in our understanding of the interactions between the host and its microbiota has been fascinating. New health concepts have been discovered, and new therapeutic or prophylactic routes are ready for further exploration.

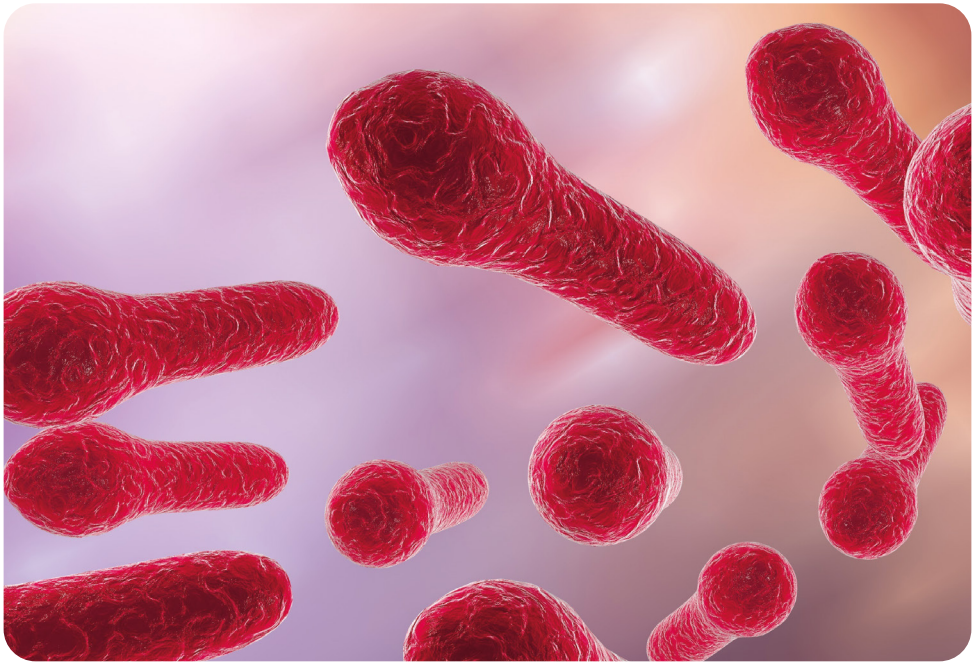
One of these routes, however, is not so new: the probiotic route.

The term 'probiotic' was first coined back in the 1950s, and later formally proposed in 1965 (Lilley & Stillwell 1965). As a result of growing scientific and commercial interest in this sector, the Food & Agriculture Organisation (FAO) and the World Health Organisation (WHO) consulted experts. The resulting working group published guidelines for the evaluation of probiotics in food and agreed on a definition for this term.

Probiotics are defined as: *'Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host.'*

(FAO/WHO Working Group 2002, Hill et al. 2014)

This document contains an overview of fundamental research, including studies that demonstrate survival of *Lacticaseibacillus paracasei* Shirota in the gut, safe consumption for humans, beneficial modulation of the intestinal microbiota and metabolites, strain identification and stability.



SURVIVAL THROUGH THE GASTROINTESTINAL TRACT

Survival through the gut is considered a key characteristic of probiotic strains as the mechanism of activity underlying most health benefits is associated with the transient presence, growth and activity of the live probiotic cells in the gut. The strongest evidence is detection of the strain in the faeces of people who have consumed the probiotic.

In vitro or model studies are not proof of gut survival *in vivo*, however they do provide useful information on factors that can affect the viability of the strains. In a study where *Lacticaseibacillus paracasei* Shirota (*L. paracasei* Shirota) was exposed to physiological levels of digestive juices (namely gastric, bile, and pancreatic juices) for sufficient time, excellent survivability was reported, particularly when embedded in a milk matrix (Lo Curto *et al.* 2011).

The *L. paracasei* Shirota strain was selected because of its ability to survive the harsh conditions of the gut. Several research papers describing human studies encompassing a mix of population groups and a variety of ages, geographical locations and health conditions have demonstrated the survival of *L. paracasei* Shirota.

SURVIVAL OF *L. PARACASEI* SHIROTA THROUGH THE GUT

Example Study: Spanhaak *et al.* (1998) Eur J Clin Nutr 52:899-907.

Method: This double-blind, placebo-controlled, randomised trial in the Netherlands involved 20 healthy adult men drinking either 3 x 100ml a day of fermented milk containing 10^9 colony forming units (CFU) *L. paracasei* Shirota per ml, or the same quantity of an unfermented milk placebo, for 4-weeks. Faecal samples were collected throughout the trial to assess the human gut survival of *L. paracasei* Shirota.

Results: *L. paracasei* Shirota was detected at significantly increased levels in the faeces of subjects in the treatment group during the intervention period, compared to the placebo group ($P<0.01$).

In the treatment group, *L. paracasei* Shirota was detected at levels reaching 10^7 CFU/g of wet faeces (see Figure 1). Once ingestion *L. paracasei* Shirota had ceased, levels decreased and reverted back to their pre-intervention state. Additionally, in only those consuming *L. paracasei* Shirota, there was a significant increase in *Bifidobacterium* ($P<0.05$).

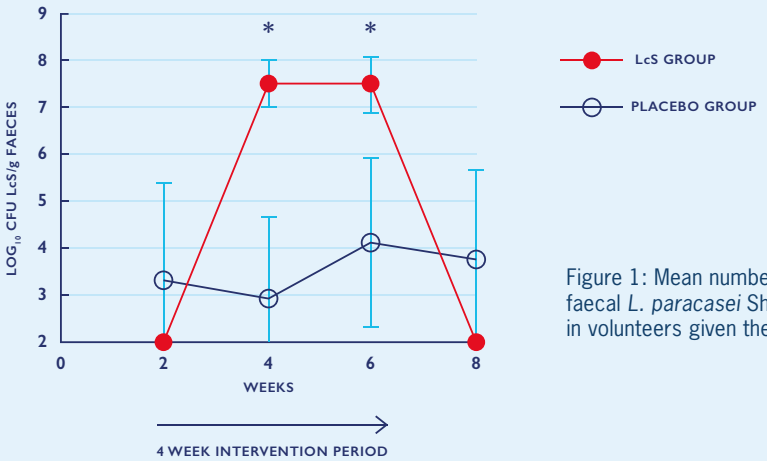


Figure 1: Mean numbers of faecal *L. paracasei* Shirota (LcS) in volunteers given the probiotic.

Other examples of studies that have shown survival of *L. paracasei* Shirota include, but are not limited to, studies conducted in healthy adults within European populations (Tilley *et al.* 2014, Tuohy *et al.* 2007) as well as several Asian populations (Mai *et al.* 2017, Utami *et al.* 2015, Wang et al. 2015a), elderly populations (Bian *et al.* 2011), pre-school and school-age children (Wang *et al.* 2015b) and critically ill children (Srinivasan *et al.* 2006).

PRODUCT QUALITY

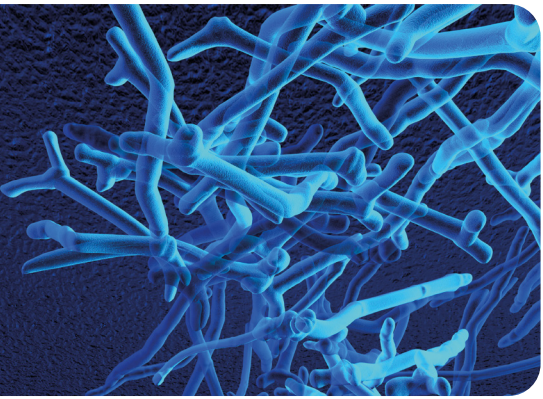
Quality assurance programmes are in place to ensure consistent product quality. For example, each batch is tested at the beginning, during and end of shelf life to check the numbers of viable probiotic bacteria are as stated on the label.

GUT MODULATION

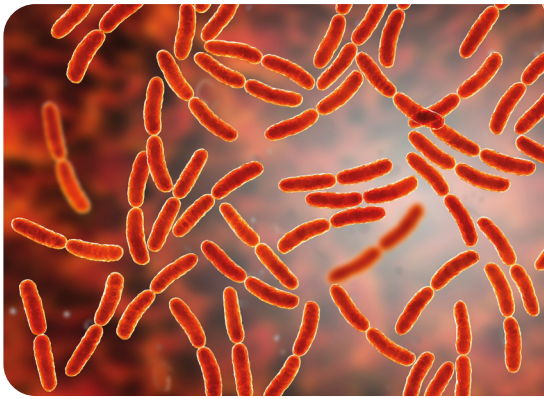
The gut microbiota is a dynamic intestinal ecosystem that is constantly shifting throughout our lifetime, in response to factors such as diet, medication, exercise and the external environment. Moreover, the composition of our gut microbiota varies along the course of our gastrointestinal tract. There is such a wide variation in composition within and between individuals that there cannot be a defined profile of a 'healthy' microbiota that is common to all. Instead a 'healthy' gut microbiota is characterised by high diversity, stability and resilience (McBurney *et al.* 2019).

Bifidobacterium: Gram-positive anaerobes, often with a branched (bifurcated) appearance. Produce lactic acid but generally not considered 'real' lactic acid bacteria due to phylogenetic and metabolic differences. Early colonisers of the gut; breast feeding promotes colonisation. Major constituents of the adult colonic microbiota, detected at about 10^8 to 10^{10} cells per gram of faeces (wet weight).

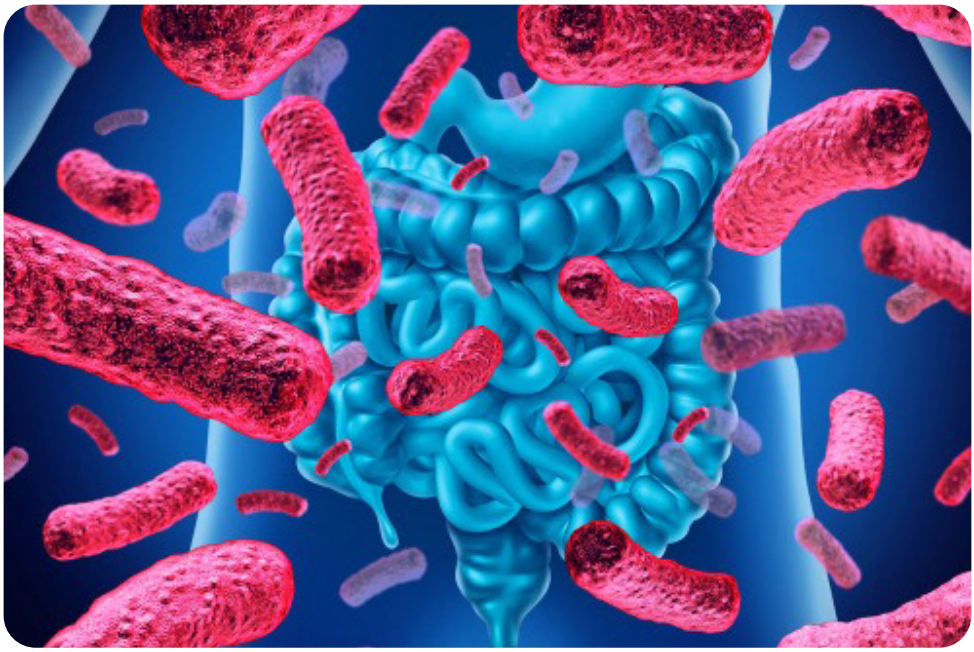
Lactobacillus: Gram-positive facultative anaerobic rods found widely in nature, and first described in 1901. Often used to make fermented foods such as cheese, yoghurt, pickles, salami etc. Considered part of the normal commensal intestinal microbiota; detected in adults at about 10^6 to 10^8 cells per gram of faeces (wet weight). Recently, this genus has been reclassified into 25 genera, including 23 novel genera (Zheng *et al.* 2020).



Bifidobacterium



Lactobacillus



Lactobacilli and bifidobacteria are generally considered to be beneficial in the gut. These genera have consistently shown positive health benefits when their numbers are restored or maintained. Possible underlying mechanisms for these observations include the production of beneficial metabolites (i.e., short chain fatty acids, monosaccharides, vitamins) and promotion of acidic conditions in the colon, thereby inhibiting the growth of harmful bacteria.

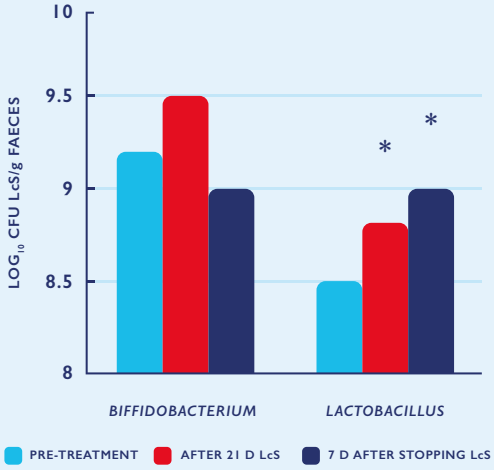
The ability for *L. paracasei* Shirota to modulate the gut microbiota has been investigated in a number of studies (Bian *et al.* 2011, Kato-Kataoka *et al.* 2016, Motoori *et al.* 2017, Nagata *et al.* 2011, Pirker *et al.* 2012, Rao *et al.* 2009, Yamagishi *et al.* 1974). For example, over a 6 month period a group of twenty-three Japanese children consumed daily a fermented milk containing $4x10^{10}$ CFU of *L. paracasei* Shirota. Stool samples were collected at baseline and months (m) 1, 3 and 6 during, and 6 after the intervention. During the ingestion period, *L. paracasei* Shirota was detected at levels reaching 10^7 cells/g. Additionally, there were significant increases in total *Bifidobacterium* (3m $P<0.05$, 6m $P<0.01$) and *Lactobacillus* (1m, 3m, 6m, $P<0.01$). Six months after the intervention these increases had returned back to their baseline levels (Wang *et al.* 2015b).

INCREASE IN LACTOBACILLI AND BIFIDOBACTERIA IN THE GUT (HEALTHY ADULTS)

Example Study: Tuohy *et al.* (2007) J Appl Microbiol 102(4):1026-1032.

Method: This double-blind, placebo-controlled study at the University of Reading involved 20 healthy volunteers who for 21 days consumed either *L. paracasei* Shirota as a fermented milk drink (at least 13 x 10^9 CFU) or placebo. Stool samples were collected at days 0, 7, 14, 21, and 28 to determine survival of *L. paracasei* Shirota and to determine changes to the faecal bacteria.

Results: Seven days after subjects started to take the probiotic, *L. paracasei* Shirota was recovered at a mean level of 1.1 x 10^7 CFU/g of faeces, and was maintained at this level over the course of probiotic feeding, but decreased after cessation of the feeding regime. Concurrently, in subjects consuming *L. paracasei* Shirota, an increase in total Lactobacilli was observed, which persisted even after the regime had stopped. Bifidobacteria were also found to increase during the regime, but this increase was not sustained once consumption of *L. paracasei* Shirota had stopped (see Figure 2).



The intestinal microbiota comprises a range of bacteria: some beneficial, some neutral for health, and some that are pathogenic or harmful. In the latter case, this may be due to the production of toxins, carcinogens or other substances that, over a period of time, may have a negative association with our health.

Figure 2: Mean bacterial numbers in volunteers fed *L. paracasei* Shirota (LcS), determined by FISH.

In an early study by Dr Shirota, infants who were fed *L. paracasei* Shirota fermented milk for 14 days had decreased levels of Enterobacteriaceae and Streptococci, compared to infants who were fed a placebo fermented milk drink (heat-treated to kill the *L. paracasei* Shirota) (Shirota *et al.* 1966). Since then, several studies with *L. paracasei* Shirota have reported that its consumption is associated with the reduction of harmful bacterial species in the gut (Kato-Kataoka *et al.* 2016, Nagata *et al.* 2011, Nagata *et al.* 2016, Tsuji *et al.* 2014). For example, the faecal microbiota of some female workers at Yakult in Japan, who regularly consume *L. paracasei* Shirota had increased levels of *Bifidobacterium* and *Lactobacillus* ($P<0.05$) and decreased levels of *Prevotella* and *Staphylococcus* ($P<0.05$) compared to a similar cohort of women who do not regularly consume *L. paracasei* Shirota (Tsuji *et al.* 2014).

REDUCTION OF *CLOSTRIDIUM DIFFICILE, CLOSTRIDIUM PERFRINGENS,* AND ENTEROBACTERIACEAE IN THE GUT

Example Study: Nagata *et al.* (2016) Ann Nutr Metab 68(1):51–59.

Method: A double-blind, placebo-controlled randomised trial of residents (n=72) and staff (n=20) at a facility for the aged, consumed either an *L. paracasei* Shirota fermented milk drink (4×10^{10} CFU) or placebo daily for 6 months. Faecal samples were collected from all subjects at baseline and at months 1, 3 and 6.

Results: Those who consumed *L. paracasei* Shirota, had a significant increase in bifidobacteria, and a significant decrease in *Clostridium difficile*, *Clostridium perfringens*, and Enterobacteriaceae in their fecal samples. Additionally, the elderly residents who had consumed *L. paracasei* Shirota, had significantly lower levels of *Staphylococcus* and *Pseudomonas*. Interestingly, residents who had consumed *L. paracasei* Shirota, had significantly lower incidence of diarrhoea and constipation, and fewer days with a fever, compared to placebo ($P<0.05$).

There is no consensus of 'an ideal intestinal microbiota', but it is generally considered healthier for it to be predominantly saccharolytic, resulting in the production of short chain fatty acids such as butyrate, acetate, and propionate. These metabolites increase gut motility, decrease pH, provide energy for the commensal bacteria and help absorb minerals. In contrast, proteolytic fermentation results in potentially toxic and carcinogenic metabolites including ammonia, phenols, indoles and amines.

DECREASE IN TOXIC BACTERIAL METABOLITES IN THE GUT (HEALTHY ADULTS)

Example Study: De Preter *et al.* (2004) Brit J Nutr 92:439-446.

Method: Researchers in Belgium investigated the effects of *L. paracasei* Shirota on toxic fermentation metabolites (NH_3 and *p*-cresol) in the gut. In this crossover study, healthy subjects (n=19) consumed either an *L. paracasei* Shirota fermented milk drink (6.5 x10^9 CFU), a prebiotic or the respective placebo twice a day for two weeks, with a two week washout period in-between. A test meal was consumed at the end of weeks 2, 4, and 6, which contained stable isotope-labelled biomarkers (a ^2H and a ^{15}N marker). Urine samples were collected before each test meal, and for the following 48-hours after the meal, to determine phenolic compounds, total nitrogen, and ^{15}N.

Results: The data indicated a reduction in the production of the toxic fermentation metabolites NH_3 (^{15}N biomarker, P=0.047) and *p*-cresol (^2H biomarker, P=0.032) among the probiotic group, which was significantly different to the placebo group (P=0.016 and P=0.042, respectively).

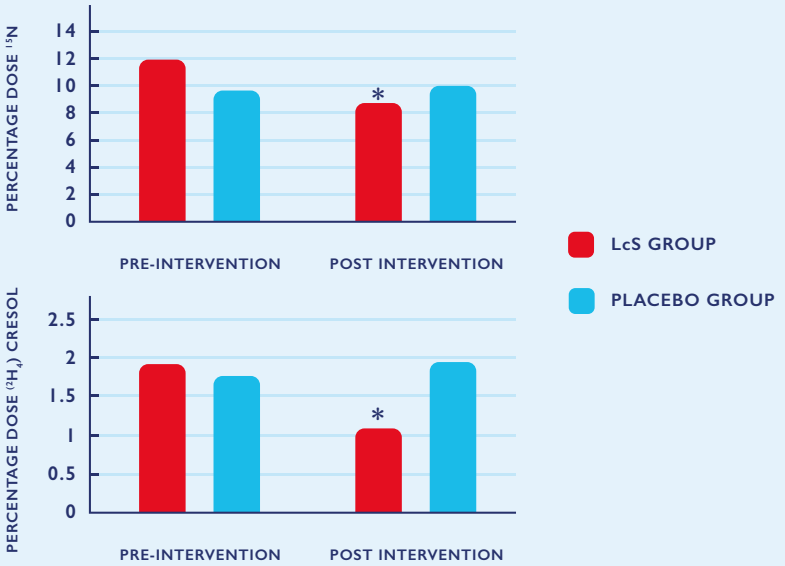


Figure 3: Influence of *L. paracasei* Shirota consumption on faecal levels of ^{15}N and *p*-cresol.

SAFETY

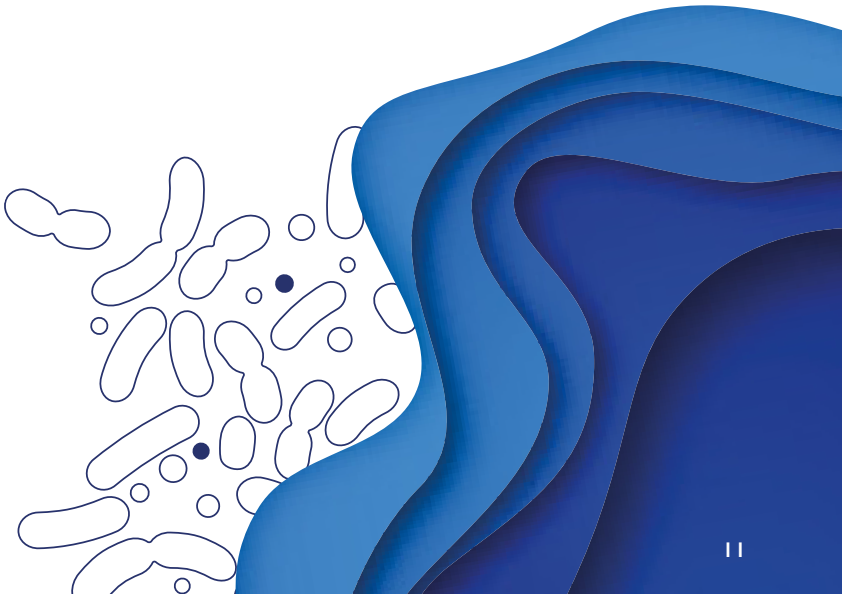
Lactic acid bacteria have been consumed for centuries in fermented foods. Regarding probiotics, *L. paracasei* Shirota (and its fermented milk product) has an unparalleled history of safe use, having been consumed by the general public on a very wide scale for more than 85 years, as well as being used by independent researchers and clinicians across a range of patients. Amongst a wide range of studies published to date, there have been no reports of serious side effects or adverse events. Similarly, there has never been any report on the uptake of transferrable antibiotic resistance genes by *L. paracasei* Shirota.

CRITICALLY ILL PATIENTS

The *L. paracasei* Shirota strain has been used in clinical trials conducted in seriously ill patients with conditions such as alcoholic cirrhosis (Stadlbauer *et al.* 2008), severe systemic inflammatory response (Shimizu *et al.* 2009), on long-term mechanical ventilation (Hayakawa *et al.* 2012), elective liver donor patients (Eguchi *et al.* 2010), bladder, biliary and colorectal cancer patients (Kanazawa *et al.* 2005, Naito *et al.* 2008, Sugawara *et al.* 2006), very-low birth weight preterm infants (Braga *et al.* 2011) and critically ill children in intensive care (Srinivasan *et al.* 2006) as detailed further. Case reports have also reported the use of *L. paracasei* Shirota in patients with severe respiratory distress (Kanamori *et al.* 2006) and short bowel syndrome (Candy *et al.* 2001, Kanamori *et al.* 2001, Uchida *et al.* 2007).

ENTERAL NUTRITION SUPPORT

There are also clinical trials and case reports on delivery of *L. paracasei* Shirota via enteral feeding tubes including nasogastric, nasojejunal, and gastrostomy (Kanamori *et al.* 2010, Kanazawa *et al.* 2005, Shimizu *et al.* 2009, Srinivasan *et al.* 2006, Sugawara *et al.* 2006). The primary objective of this study was to assess safety using *L. paracasei* Shirota delivered via enteral feeding tubes in critically ill children. If you are considering using probiotics with adult or paediatric patients on enteral feeding regimes, please see further considerations below.



CLINICAL SAFETY IN CRITICALLY ILL PATIENTS

Example study: Srinivasan *et al.* (2006) J Ped Gastroenterol Nutr 42:171-173.

Method: The objective of this study was to establish clinical safety of *L. paracasei* Shirota used as a probiotic in critically ill children. *L. paracasei* Shirota was administered three times a day at a dosage of 10^7 CFU/day via an indwelling nasogastric tube for five days to children admitted to a paediatric intensive care unit in the UK. Safety was assessed by bacteriologic surveillance for the strain in surface swabs, endotracheal aspirates and blood, urine and sterile body fluid samples.

Results: From the 28 patients with available safety data, there was no evidence of either colonisation or bacteraemia with *L. paracasei* Shirota from this testing. The *L. paracasei* Shirota was well tolerated with no apparent side effects or adverse reactions, supporting the conclusion that *L. paracasei* Shirota as a probiotic in critically ill children fed via a nasogastric tube appears safe.

SAFETY CONSIDERATIONS

Although many of the case reports, intervention studies and meta-analyses that describe probiotic use in seriously ill patients report evidence of benefit, most also caution that more research is needed. If considering administration of a high dose of probiotics via a nasojejunal tube, alongside enteral nutrition containing a high concentration of fermentable sugars, utmost caution and close monitoring should be undertaken. This is especially important in patients with non-occlusive mesenteric ischemia, common in critically ill patients such as acute pancreatitis patients for whom this regime would not be advised (Besselink *et al.* 2008). When considering the use of probiotics for a specific patient where you may have safety concerns, it is important to consider (i) the quality and safety record of the particular probiotic; (ii) the administration mode and (iii) the patient's condition.

CONCLUSION

Researchers are continuing to explore the intricate and complex relationship between the host and microbiota and this includes further examination of the effect of regular probiotic consumption on day to day health and its efficacy in the management of a range of conditions. When recommending any particular probiotic, healthcare professionals should carefully consider its research evidence, namely survival through the gastrointestinal tract, modulation of the intestinal microbiota and safety.

THE PROBIOTIC PIONEER

Lacticaseibacillus paracasei Shirota, the strain unique to Yakult, was selected and cultivated in 1930 by the scientist Dr Minoru Shirota in Japan. Inspired by Professor Elie Metchnikoff's theory that there are health benefits in replacing harmful proteolytic microbes in the colon with beneficial saccharolytic lactic acid bacteria, Dr Shirota spent years screening a collection of lactic acid bacteria to look for one with the ability to resist exposure to gastric acid and bile salts. After selecting and cultivating this strain, he used it to develop a probiotic fermented milk drink.

A COMPANY FOCUSED ON SCIENCE

As well as having Yakult research institutes in Japan and Belgium, the Yakult company sponsors and supports studies by independent researchers in hospitals, universities and institutes worldwide. New papers are constantly being published, with over 470 papers published to date. Furthermore, Yakult organises national and international scientific symposia on the latest scientific developments relating to probiotics, such as the International Yakult Symposium. In Europe, the company also sponsors awards for healthcare professionals and researchers (including students), and supports digestive health charities.

REFERENCES

1. Bian *et al.* (2011) Int J Probiotics Prebiotics 6(2):123-132
2. Braga *et al.* (2011) Am J Clin Nutr 93(1):81-6
3. Candy *et al.* (2001) J Pediatr Gastroenterol Nutr 32:506-508
4. De Preter *et al.* (2004) Brit J Nutr 92:439-446. [highlighted below]
5. Eguchi *et al.* (2010) Am J Surg 201(4):498-502
6. FAO/WHO Working Group (2002) Report on Drafting Guidelines for the Evaluation of Probiotics in Food. London, Ontario, Canada, April 30 and May 1, 2002
7. Hayakawa *et al.* (2012) Dig Dis Sci 57:2642–2649
8. Hill *et al.* (2014) Nat Rev Gastroenterol Hepato 11(8):506-14.
9. Kanamori *et al.* (2001) Dig Dis Sci 46(9):2010-2016
10. Kanamori *et al.* (2006) Int J Probiotics Prebiotics 1(3/4) :161-168
11. Kanamori *et al.* (2010) Pediatr Int 52(3):362-367
12. Kanazawa *et al.* (2005) Langenbecks Arch Surg 390 :104-113
13. Kato-kataoka *et al.* (2016) Benef Microbes 7(2):153-156
14. Lilley & Stillwell (1965) Science 147:747-748.
15. Mai *et al.* (2017) Asia Pacific Journal of Clinical Nutrition 26(1):72-77
16. McBurney *et al.* (2019) J Nutr 11(149): 1882-1895
17. Motoori *et al.* (2017) Clin Nutr 36(1):93-99
18. Nagata *et al.* (2011) Br J Nutr 106(4):549-56
19. Nagata *et al.* (2016) Ann Nutr Metab 68(1):51–59
20. Naito *et al.* (2008) Int J Probiotics Prebiotics 3(3) :163-164
21. Pirker *et al.* (2012) Food Agr Immunol 23(3):315-330
22. Rao *et al.* (2009) Gut Pathog 1(1):6
23. Shimizu *et al.* (2009) Dig Dis Sci 54 :1071-1078
24. Shirota *et al.* (1966) Japan J Bacteriol 21(5) :274-283
25. Srinivasan *et al.* (2006) J Ped Gastroenterol Nutr 42:171-173.
26. Stadlbauer *et al.* (2008) Int J Probiotics Prebiotics 3(3) :188
27. Sugawara *et al.* (2006) Ann Surg 244: 706–714
28. Tilley *et al.* (2014) Int J Probiotics Prebiotics 9(1/2):23-30
29. Tsuji *et al.* (2014) Int J Probiotics Prebiotics 9(1/2) :31-38
30. Tuohy *et al.* (2007) J Appl Microbiol 102(4):1026-1032
31. Uchida *et al.* (2007) Pediatr Surg Int 23:243–248
32. Utami *et al.* (2015) Int J Probiotics Prebiotics 10(2/3):77-84
33. Wang *et al.* (2015a) Microbiol Immunol 59(5):268-276
34. Wang *et al.* (2015b) Ann Nutr & Metab 67:257-266
35. Yamagishi *et al.* (1974) Japan J Microbiol 18(3):211-216
36. Zheng *et al.* (2020) Int J Syst Evol Microbiol 70 :2782-2858

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