

2010

Responders and non-responders to probiotic interventions

Gregor Reid

Canadian Research and Development Centre for Probiotics; Lawson Health Research Institute, gregor@uwo.ca

Estelle Gaudier

Unilever Research & Development Vlaardingen; The Netherlands

Francisco Guarner

Digestive System Research Unit; Hospital Vall d'Hebron; CIBEREHD; Barcelona, Spain

Gary B. Huffnagle

The University Of Michigan

Jean M. Macklaim

Canadian Research and Development Centre for Probiotics; Lawson Health Research Institute

See next page for additional authors

Follow this and additional works at: <http://digitalcommons.unl.edu/foodsciefacpub>



Part of the [Food Science Commons](#)

Reid, Gregor; Gaudier, Estelle; Guarner, Francisco; Huffnagle, Gary B.; Macklaim, Jean M.; Munoz, Alicia Murcia; Martini, Margaret; Ringel-Kulka, Tamar; Sartor, Balfor R.; Unal, Robert R.; Verbeke, Kristin; and Walter, Jens, "Responders and non-responders to probiotic interventions" (2010). *Faculty Publications in Food Science and Technology*. 168.
<http://digitalcommons.unl.edu/foodsciefacpub/168>

This Article is brought to you for free and open access by the Food Science and Technology Department at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Faculty Publications in Food Science and Technology by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Authors

Gregor Reid, Estelle Gaudier, Francisco Guarner, Gary B. Huffnagle, Jean M. Macklaim, Alicia Murcia Munoz, Margaret Martini, Tamar Ringel-Kulka, Balfor R. Sartor, Robert R. Unal, Kristin Verbeke, and Jens Walter

Responders and non-responders to probiotic interventions

How can we improve the odds?

Gregor Reid,^{1,2,*} Estelle Gaudier,³ Francisco Guarner,⁴ Gary B. Huffnagle,⁵ Jean M. Macklaim,¹ Alicia Murcia Munoz,⁴ Margaret Martini,⁶ Tamar Ringel-Kulka,⁷ Balfor R. Sartor,⁸ Robert R. Unal,⁹ Kristin Verbeke¹⁰ and Jens Walter¹¹

¹Canadian Research and Development Centre for Probiotics; Lawson Health Research Institute; ²Departments of Microbiology, Immunology and Surgery; The University of Western Ontario; London, Canada; ³Unilever Research & Development Vlaardingen; The Netherlands; ⁴Digestive System Research Unit; Hospital Vall d'Hebron; CIBEREHD; Barcelona, Spain; ⁵Division of Pulmonary & Critical Care Medicine; Department of Internal Medicine; University of Michigan; USA; ⁶Kraft Foods; Glenview, IL USA; ⁷UNC Gillings School of Global Public Health; The University of North Carolina at Chapel Hill; Chapel Hill, NC USA; ⁸UNC Department of Medicine/Division of Gastroenterology and Hepatology; and Department of Microbiology and Immunology; Chapel Hill, NC USA; ⁹Yakult USA, Inc.; Torrance, CA USA; ¹⁰Department of Gastroenterology and Leuven Food Science and Nutrition Research Centre (LForCe); Katholieke Universiteit Leuven; Leuven, Belgium; ¹¹Department of Food Science and Technology; University of Nebraska; Lincoln, USA

Key words: responders, non-responders, probiotics, prebiotics, clinical trial design

As with many clinical studies, trials using probiotics have shown clearly that some patients benefit from the treatment while others do not. For example if treatment with probiotics leads to 36% cure rate of diarrhea, why did the other 64% not have the same result? The issue is important for human and indeed experimental animal studies for two main reasons: (i) Would changing the design of the study result in more subjects responding to treatment? (ii) If a subject does not respond what are the mechanistic reasons? In order to tackle the issue of responders and non-responders to therapy, a workshop was held by the International Scientific Association for Probiotics and Prebiotics (ISAPP). The outcome was four recommendations.

(1) **Clearly define the end goal:** this could be supporting a health claim or having the highest clinical effect and impact.

(2) **Design the study** to maximize the chance of a positive response by identifying precise parameters and defining the level of response that will be tested.

(3) **Base the selection of the intervention on scientific investigations:** which strain(s) and/or product formulation should be used and why.

(4) **Carefully select the study cohort:** use biological or genetic markers when available to stratify the patient population before enrollment and decide at what point intervention will provide the best outcome (for example, in acute phase of disease, or during remission, with or without use of pharmaceutical agents).

By following these recommendations and selecting an appropriate primary outcome, it is hoped that clinical data will emerge in the future that expands our knowledge of which probiotics benefits which subjects and by what mechanism.

Introduction

At time of writing, there were 5,729 citations on the topic of 'responders and non-responders' in the PubMed database (<http://www.ncbi.nlm.nih.gov>). This would suggest that the topic is of interest to many people, but in truth no studies have explored why some subjects respond to probiotic or prebiotic intervention and others do not. The issue is important for human and indeed experimental animal studies for two main reasons: (i) Would changing the design of the study result in more subjects responding to treatment, and thereby increase the clinical data showing that these interventions can provide added value to patient/animal care? (ii) If a subject does not respond what are the mechanistic reasons?

The term responder refers primarily to a subject who reacts favorably to the therapy, but clearly there are degrees of responsiveness and different parameters by which the response could be measured. For example, in the one study pertaining to responders and non-responders to probiotic therapy, the primary measurement of response was based upon the Crohn's disease activity index and the International Organization for the Study of Inflammatory Bowel Disease score.¹ The conclusion was that the scores were significantly reduced after therapy (255–136, $p = 0.009$; 3.5–2.1, $p = 0.03$, respectively), based upon six patients having a complete response, one a partial response and three not responding. If the primary measurement of response had been decreased or discontinuance of prednisone use, then one of the 'non-responders' and the 'partial responder' would have been categorized as 'responders,' and four original responders would have been classified as 'non-responders'. This case illustrates some of the issues involved in how response and lack of response to probiotics is defined.

A second example derives from a study of HIV-positive men treated with anti-retroviral therapy plus a so-called probiotic. Although the primary outcome was not clear, diarrhea was completely resolved in 36% (10/28) subjects,² raising the question

*Correspondence to: Gregor Reid; Email: gregor@uwo.ca
Submitted: 12/18/09; Revised: 03/27/10; Accepted: 04/12/10
Previously published online:
www.landesbioscience.com/journals/gutmicrobes/article/12013

what happened to the other 64%? In fact, 15 of the remaining 18 subjects had fewer stool passages per day ($p < 0.05$), which could be regarded as a response to therapy, albeit not the primary response. This illustrates how a response rate is influenced by the defined endpoint(s) of interest, as well as which is deemed to be most clinically relevant. This latter point is often dependent upon what the company making the product wants to claim in its regulatory documentation. In some countries, claiming that diarrhea is cured by a probiotic would be regarded as a drug claim, while reduction in stool frequency would not. Since probiotics and prebiotics are food additives and can also be medical therapies, research in this arena presents unique challenges for the scientific community.

Predicting the Outcome

It is sometimes possible to predict who will respond to a specific treatment and who is less likely to do so. This can be achieved if biomarkers for disease response are identified. For example, using a rat model of galactosamine-induced hepatitis, there are clearly responders and non-responders to this hepatitis-inducing agent. However, the presence of fecal galN-pyrazines in non-responders, but not responders, allows the separation of the two groups by a fecal biomarker.³ In terms of probiotics, if a Crohn disease patient has defective nucleotide-binding oligomerization domain (Nod)2 receptors, it is likely that they would not respond to lactobacilli probiotic treatment.⁴ This should be investigated before a clinical study is performed to determine if any patients have Nod2 mutations and subject enrollment criteria modified appropriately. However, in the study cited above,¹ no Nod2 status was reported, and therefore its role in non-responses cannot be assessed.

Another issue is extent to which the disease being treated progresses or does not progress. Some HIV-infected patients respond to highly active antiretroviral therapy (HAART), while others do not. In the latter group, a study has shown that an increase in apoptotic CD8⁺ T cells and decrease in Treg cells is associated with this failure, and leads to progression of the disease to AIDS.⁵ The implication is that a therapy, such as probiotic lactobacilli known to increase Treg cell numbers⁶ could help the efficacy of HAART. In terms of maximizing the response rate, in this case the difficulty comes from not easily identifying subjects prior to enrollment whose disease will progress and whose will not.

A practical problem comes when there is a 10% difference between results from active and placebo treatments. In the case of preventing diarrhea or respiratory infections, how do we find the 10% of subjects who did not get sick but were 'exposed' to the virus or pathogen? Without stringent assessment of all enrolled subjects, this is a difficult goal to achieve.

The actual condition being examined may also differ between countries, regions and subject populations. For example if setting up a study to determine if probiotics increase time to onset of diabetes, a US cohort might be obese 'healthy' children while in France these subjects may be not be defined as healthy. Thus, in order to optimize the usefulness of the study's conclusions, the subjects would need to be defined by a range of factors including

body mass index, cholesterol levels, pre-diabetic markers, and other surrogate markers such as weight, glucose levels, and bile salt hydrolase. Precise stratification of the disease or health state of the enrolled subjects is critical.

Timing of the Intervention

The time at which an intervention can have a greater chance or being effective is important for several reasons. The multi-strain probiotic VSL#3 has been used to help patients stay in remission from mild pouchitis during maintenance treatment.⁷ In this open-label study, 16 (69%) of the 23 patients went into remission while 7 showed no change. Notably, the entrance criterion was mild pouchitis, and potentially had they chosen a more severe status fewer subjects would have responded. The intervention was timed for when the subjects were already in remission rather than when they were being treated for active disease. Although there are some mechanistic rationales for using probiotics to treat active colitis, such as normalizing epithelial ion transport function,⁸ until clinical studies are done, the effectiveness of this approach will not be known.

A randomized, placebo-controlled trial of 327 patients with quiescent UC treated with *E. coli* Nissle 1917 showed equivalence in relapse rate (34–36%) to treatment with mesalazine.⁹ This again illustrates that timing (quiescent phase of the disease) is important in conjunction with design (comparison with a drug), outcome (number of relapses) and an understanding of mechanism (anti-inflammatory effect).

An argument has been made that probiotic use immediately after intestinal surgery or following relapse diagnosis, might help prevent or delay IBD recurrence.¹⁰ However, a study of 70 Crohn's disease patients showed that use of *Lactobacillus johnsonii* LA1 for 12 weeks following ileo-caecal resection, did not prevent early endoscopic recurrence.¹¹ One explanation could be that not all probiotic strains are able to have an effect in this disease, as also shown by failure of *L. rhamnosus* GG along with standard drug therapy to prolong time to relapse in children with Crohn's disease.¹² Given the fact that probiotic effects are strain-specific, it is rather unlikely that we will ever be able to state that probiotics (in general) are efficient in one or another disease. Rather, we should confine statements to the specific strain and product for which the effect has been shown.

Selecting the Best Probiotic Based on Mechanistic, Scientific Criteria

The selection of a probiotic that has the highest chance of response in the host is far from easy. A mechanistic understanding about the effect of the probiotic should be achieved. However, while in vitro experiments can help describe a strain, and animal models may suggest efficacy, only when the human studies are performed can the definitive answer be obtained. Even then, phase one safety trials do not necessarily predict efficacy as has been found with recombinant *Lactococcus lactis* expressing human IL-10 which showed initial promise,¹³ but in phase two studies in Crohn patients has so far failed.

One philosophy for selection of certain strains is whether they are naturally occurring in target site, and the predominant member of that species. This is the rationale for selecting *L. crispatus* CTV05 for implantation into the vagina,¹⁴ albeit *L. iners* is arguably more commonly found there.¹⁵ Another approach is to select a strain that has a set of characteristics deemed best suited for application to the target site. This was the reason for choosing *L. rhamnosus* GR-1 and *L. reuteri* RC-14 for improving vaginal health.¹⁶ This was perhaps fortuitous, given that in vitro data cannot fully predict in vivo utility.

If we were to take the same approach to improving intestinal health in patients with IBD, using a strain of *Faecalibacterium prausnitzii* could be worthwhile given that it can downregulate inflammation,¹⁷ and is an extremely common member of the healthy gut microbiota, and absent or in low counts in patients with colitis.¹⁸ A strain of this species has so far not been propagated for use in humans, and it may prove difficult to achieve given its anaerobic requirements. Nevertheless, if it was available, a key element of its application would be to use it in patients with depleted *F. prausnitzii* numbers in the gut, and to be able to detect the probiotic strain and its affect on inflammation after use. The latter is difficult given the accessibility of the sites where anti-inflammatory activity might occur, and that even if the strain was found associated with previously inflamed and now normal sites, it would still be difficult to prove cause and effect.

It has long been assumed that the probiotic strain(s) is responsible directly for the clinical effect, but it is also feasible that the strain alters the micro-environment in such a way as to alter the microbiota, and this then indirectly changes the host's disease status. In the multi-species oral cavity, inter-bacterial communication and cooperative interactions have been known for some time,¹⁹ yet no probiotic has so far been selected based upon its activity within a dynamic multi-species biofilm. Rather, multiple strains of bacteria have been chosen, apparently at random, and put into a formulation that is used as a so-called probiotic. Unfortunately, these formulations have been created without evidence that the additional strains augment the effects of the single strain formulation, or more importantly, that the multiple constituents do not counteract each other's activity. The repeated failure of products such as Ecologic 641,^{20,21} may be explained by this poor selection process.

Dealing with Confounders

Once a study cohort has been selected and a clinical trial design established using a well-documented probiotic, it is important to consider confounding factors that may or may not influence the host's response. For example, if a probiotic has been selected that degrades certain carcinogens, downregulates chronic inflammation and affects apoptosis and there is a cancer patient cohort just having completed surgical removal of a tumor and chemotherapy, how will factors like family history and genetics play a role in recurrences? Similarly, how will previous history of the disease, body mass index, use of common prescription and OTC medications such as non-steroidal anti-inflammatory agents, and

potential exposure to carcinogens in the workplace affect the recurrence rate? Some of these confounders can be dealt with by sub-analysis of the clinical trial, as long as care was given in selecting a large enough study group, and some consideration was given to matching factors at enrolment. In addition, the trial should be designed to increase the chance of detecting an independent effect associated with the probiotic. For example, if the probiotic was shown to adhere to polyps and directly reduce their size or eradicate them, then the outcome of fewer or smaller polyps at follow-up or longer time to next occurrence of a polyp could more easily be attributed to the probiotic than to other confounders. The use of cluster models for clinical trials²² has many advantages including being better suited to account for confounders, even at the end of the trial through regression analysis. Clustered data are collected from subjects who are members of a group and who may be presumed, by virtue of the fact that they are members of that group, to have a greater similarity to those within the group than to individuals outside it.

Acquiring Relevant Data that Might Explain Differences between Responders and Non-Responders

To date, there are few reliable biomarkers available to help measure the outcome of probiotic or prebiotic trials. Nevertheless, there are ways to gather volumes of data within which may be indicators of change associated with the therapy. For example, the gut microbiota of humans is highly individual, and compositional differences might define who is a responder and who not. Sequencing analytical tools are now available to obtain a readout of the composition and semi-quantitative count of the microbiota of the gut and vagina.^{23,24} With bar-coding sequencing techniques insight can be provided such as presence of drug resistance mutations,²⁵ and traced back to the samples. Likewise, multiplex immunological assays and genomic arrays can identify host factors that change as a result of exposure to the treatment.^{6,26} Such information can help answer questions such as what role do the microbiota and the functions of the microbiota, and what role do the host's response to the microbes play in the eventual response or non-response to the therapy? The use of metabolomic analyses can be daunting given the requirement for expensive equipment and novel expertise. However, if accessible, it can provide valuable data, such as the use of Ion Cyclotron Resonance Fourier Transform Mass Spectrometry (ICR-FT/MS) to interpret the thousands of metabolites present in fecal samples from healthy individuals and those with Crohn disease.²⁷ In twins, metabolites can be positively or negatively correlated to the disease phenotype (such as obese or lean) and to specific microbes recovered from the stool.²⁸ The combined use of metabolomics and microbial genomics can reveal novel metabolites associated with disease and potentially useful for diagnosis and treatment. Detecting metabolites such as butyrate can on one hand correlate with a health benefit,²⁹ and on the other hand suggest a potential adverse outcome.³⁰

Lastly, the value of quality of life (QoL) and treatment compliance assessments should be considered when acquiring

data about who responds to a treatment and how this response is perceived, as well as why no effect is detected. Relatively few probiotic clinical trials have used QoL tools, but one recent study of 120 subjects with ulcerative colitis showed changes in emotional, bowel and social functioning with various treatments.³¹ In the end, how the person feels is a major determinant of being a responder to treatment, but this depends on whether or not QoL is used to define “responder.” If QoL is measured along with other data such as microbiota composition, dietary profile, anthropometrics, ethnicity, physical activity and other potential confounders, the findings can not only be better put into context, but they will allow a better understanding of how and why some subjects do or do not respond.

Recommendations

The workshop participants made the following four recommendations with respect to increasing the number of subjects responding to the administration of probiotic and/or prebiotic use:

(1) **Clearly define the end goal**—this could be supporting a health claim or consumer benefit, or having the highest clinical effect and impact.

(2) **Design the study** to maximize the chance of a positive response by using clearly defined parameters for the level of response that will be tested: from quality of life or days off work,

to reduced use of pharmaceutical agents, longer time to disease recurrence, changes in bowel habits, up or downregulation of specific immunological or microbiologic parameters, or changes in other specific biomarkers.

(3) **Base the selection of the intervention on scientific investigations:** which strain(s) and/or product formulation should be used and why; can we expect and measure the same mechanism of action in humans; for example using *Faecalibacterium prausnitzii* because it can downregulate inflammation.

(4) **Carefully select the study cohort**, by using biological or genetic markers to stratify the patient population before enrollment. For example inflammatory bowel disease patients (children, adults, Crohn disease or ulcerative colitis) whose *F. prausnitzii* levels are low; decide at what point intervention will provide the best outcome (for example in acute phase of disease, or during remission, with or without use of pharmaceutical agents); and try to obtain as much sample data as possible (microbiota sequencing, fecal water and blood metabolomics, immunological parameters, subject parameters such as demographics, lifestyle or anthropometric variables, and quality of life questionnaire) so as to increase understanding of what happened and why.

Acknowledgements

Valuable input from Linda Duffy, Brian Feagan, Niklas Larsson and Eveliina Myllyluoma is gratefully acknowledged.

References

- Fujimori S, Tatsuguchi A, Gudis K, Kishida T, Mitsui K, Ehara A, et al. High dose probiotic and prebiotic cotherapy for remission induction of active Crohn's disease. *J Gastroenterol Hepatol* 2007; 22:1199-204.
- Heiser CR, Ernst JA, Barrett JT, French N, Schutz M, Dube MP. Probiotics, soluble fiber, and L-Glutamine (GLN) reduce nelfinavir (NFV)- or lopinavir/ritonavir (LPV/r)-related diarrhea. *J Int Assoc Physicians AIDS Care* 2004; 3:121-9.
- Coen M, Want EJ, Clayton TA, Rhode CM, Hong YS, Keun HC, et al. Mechanistic aspects and novel biomarkers of responder and non-responder phenotypes in galactosamine-induced hepatitis. *J Proteome Res* 2009; 8:5175-87.
- Yamamoto S, Ma X. Role of Nod2 in the development of Crohn's disease. *Microbes Infect* 2009; 11:912-8.
- Jiao Y, Fu J, Xing S, Fu B, Zhang Z, Shi M, et al. The decrease of regulatory T cells correlates with excessive activation and apoptosis of CD8⁺ T cells in HIV-1-infected typical progressors, but not in long-term non-progressors. *Immunology* 2009; 128:366-75.
- Baroja ML, Kirjavainen PV, Hekmat S, Reid G. Anti-inflammatory effects of probiotic-yogurt in inflammatory bowel disease patients. *Clin Experimental Immunol* 2007; 149:470-9.
- Gionchetti P, Rizzello F, Morselli C, Poggioli G, Tambasco R, Calabrese C, et al. High-dose probiotics for the treatment of active pouchitis. *Dis Colon Rectum* 2007; 50:2075-82.
- Resta-Lenert SC, Barrett KE. Modulation of intestinal barrier properties by probiotics: role in reversing colitis. *Ann N Y Acad Sci* 2009; 1165:175-82.
- Kruis W, Frick P, Pokrotnieks J, Lukás M, Fixa B, Kascák M, et al. Maintaining remission of ulcerative colitis with the probiotic *Escherichia coli* Nissle 1917 is as effective as with standard mesalazine. *Gut* 2004; 53:1617-23.
- Rutgeerts P. Modern therapy for inflammatory bowel disease. *Scand J Gastroenterol Suppl* 2003; 237:30-3.
- Van Gossum A, Dewit O, Louis E, de Hertogh G, Baert F, Fontaine E, et al. Multicenter randomized-controlled clinical trial of probiotics (*Lactobacillus johnsonii* LA1) on early endoscopic recurrence of Crohn's disease after ileo-caecal resection. *Inflamm Bowel Dis* 2007; 13:135-42.
- Bousvaros A, Guandalini S, Baldassano RN, Botelho C, Evans J, Ferry GD, et al. A randomized, double-blind trial of Lactobacillus GG versus placebo in addition to standard maintenance therapy for children with Crohn's disease. *Inflamm Bowel Dis* 2005; 11:833-9.
- Braat H, Rottiers P, Hommes DW, Huyghebaert N, Remaut E, Remon JP, et al. A phase I trial with transgenic bacteria expressing interleukin-10 in Crohn's disease. *Clin Gastroenterol Hepatol* 2006; 4:754-9.
- Antonio MA, Meyn LA, Murray PJ, Busse B, Hillier SL. Vaginal colonization by probiotic *Lactobacillus crispatus* CTV-05 is decreased by sexual activity and endogenous lactobacilli. *J Infect Dis* 2009; 199:1506-13.
- Burton JP, Cadieux PA, Reid G. Improved understanding of the bacterial vaginal microbiota of women before and after probiotic instillation. *Appl Environ Microbiol* 2003; 69:97-101.
- Reid G. Probiotic lactobacilli for urogenital health in women. *J Clin Gastroenterol* 2008; 42:234-6.
- Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermúdez-Humarán LG, Gratadoux JJ, et al. *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proc Natl Acad Sci USA* 2008; 105:16731-6.
- Sokol H, Seksik P, Furet JP, Firmesse O, Nion-Larmurier I, Beaugerie L, et al. Low counts of *Faecalibacterium prausnitzii* in colitis microbiota. *Inflamm Bowel Dis* 2009; 15:1183-9.
- Hojo K, Nagaoka S, Ohshima T, Maeda N. Bacterial interactions in dental biofilm development. *J Dent Res* 2009; 88:982-90.
- Besselink MG, van Santvoort HC, Buskens E, Boermeester MA, van Goor H, Timmerman HM, et al. Dutch Acute Pancreatitis Study Group. Probiotic prophylaxis in predicted severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial. *Lancet* 2008; 371:651-9.
- Vleggaar FR, Monkelbaan JF, van Erpecum KJ. Probiotics in primary sclerosing cholangitis: a randomized placebo-controlled crossover pilot study. *Eur J Gastroenterol Hepatol* 2008; 20:688-92.
- Christie J, O'Halloran P, Stevenson M. Planning a cluster randomized controlled trial: methodological issues. *Nurs Res* 2009; 58:128-34.
- Hill JE, Goh SH, Money DM, Doyle M, Li A, Crosby WL, et al. Characterization of vaginal microflora of healthy, nonpregnant women by chaperonin-60 sequence-based methods. *Am J Obstet Gynecol* 2005; 193:682-92.
- Zhang H, DiBaise JK, Zuccolo A, Kudrna D, Braidotti M, Yu Y, et al. Human gut microbiota in obesity and after gastric bypass. *Proc Natl Acad Sci USA* 2009; 106:2365-70.
- Hoffmann C, Minkah N, Leipzig J, Wang G, Arens MQ, Tebas P, et al. DNA bar coding and pyrosequencing to identify rare HIV drug resistance mutations. *Nucleic Acids Res* 2007; 35:91.
- Kirjavainen PK, Laine RM, Carter D, Hammond J-A, Reid G. Expression of anti-microbial defense factors in vaginal mucosa following exposure to *Lactobacillus rhamnosus* GR-1. *Int J Probiotics* 2008; 3:99-106.
- Jansson J, Willing B, Lucio M, Fekete A, Dicksved J, Halfvarson J, et al. Metabolomics reveals metabolic biomarkers of Crohn's disease. *PLoS One* 2009; 4:6386.
- Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, et al. A core gut microbiome in obese and lean twins. *Nature* 2009; 457:480-4.
- Louis P, Scott KP, Duncan SH, Flint HJ. Understanding the effects of diet on bacterial metabolism in the large intestine. *J Appl Microbiol* 2007; 102:1197-208.

-
30. Marquet P, Duncan SH, Chassard C, Bernalier-Donadille A, Flint HJ. Lactate has the potential to promote hydrogen sulphide formation in the human colon. *FEMS Microbiol Lett* 2009; 299:128-34.
 31. Fujimori S, Gudis K, Mitsui K, Seo T, Yonezawa M, Tanaka S, et al. A randomized controlled trial on the efficacy of synbiotic versus probiotic or prebiotic treatment to improve the quality of life in patients with ulcerative colitis. *Nutrition* 2009; 25:520-5.

©2010 Landes Bioscience.
Do not distribute.