View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Scholink Journals

Food Science and Nutrition Studies
ISSN 2573-1661 (Print) ISSN 2573-167X (Online)
Vol. 3, No. 2, 2019
www.scholink.org/ojs/index.php/fsns

*Original Paper*

# Selection and Characterization Criteria of Probiotics Intended for Human Use from the Past to the Future

Valeria Sagheddu[1*], Elena Guidesi[1], Serena Galletti[1] & Marina Elli[1]

[1] AAT-Advanced Analytical Technologies, Fiorenzuola d'Arda, Piacenza, Italy

[*] Valeria Sagheddu, AAT-Advanced Analytical Technologies, Fiorenzuola d'Arda, Piacenza, Italy

Received: April 28, 2019 Accepted: May 10, 2019 Online Published: May 20, 2019

doi:10.22158/fsns.v3n2p73 URL: http://dx.doi.org/10.22158/fsns.v3n2p73

***Abstract***

*The probiotic product consumption has recently increased with the prevalent intent to promote human and animal wellbeing. The complex selection process dealing with new-isolated probiotic candidates is the first challenge that has to be faced. From the isolation to the launch on the market, information about safety, tolerance to host physiological conditions, adhesion properties, genetics and interaction with the host has to be collected. Probiotics must be safe, survive to the exposition to bile salts and to gut transit, adhere to intestinal cells lining and colonize the lumen of the tract. The evaluation process of the possible probiotic health benefits is widely supported by in-vitro assays simulating the in-vivo conditions. The aim of this work is to summarize the classical models usually employed for the probiotic screening by underlying strengths and weaknesses of all models and to present some more recent analysis tools used in the probiotic field. The long term goal in new probiotic candidate selection experiencing these combined essays together would lead to the hypothetical assignment acknowledged as one strain-one function.*

***Keywords***

*screening, platform, in-vitro, in-vivo, model*

## 1. Introduction

International Scientific Association for Probiotics and Prebiotics defined probiotics as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (Hill et al., 2014). In the recent years the global probiotic market has faced a massive growth, this expansion should be related to a robust and univocal legislation (Baldi & Arora, 2015). Nevertheless, focusing the attention on the European regulatory framework, it has to be noticed the absence of harmonized regulation. The Food Products Directive and Regulation (Regulation 178/2002/EC; Directive

2000/13/EU) and Herbal Medicinal Products Directive (2004/24/EC) are the main laws supervising probiotic products (Coppens et al., 2006). Furthermore, the European regulatory pressure in the field of probiotics led to a double negative impact by limiting the communication of their beneficial effects to consumers and by adversely impacting the market. After ten years from the enter into force of the Regulation on health claims made on food (EU 1924/2006), the market of probiotics shows a renewed interest about the health improving potential of beneficial microbes. The restored enthusiasm is mainly driven by the big pool of information derived from the omics approach for the study of the gut microbiota (Baugher & Klaenhammer, 2011). Unfortunately, the legislative framework seems to not run with the innovation associated with new analytical approaches. Actually, of the about 300 calls for health claims submitted to EFSA experts' panel on the food area none has been accepted. The only exception was represented to the use of yoghurt for lactose intolerance (Papadimitriou et al., 2015). The implementation of new and comprehensive models, exploiting the newest omics technology could ameliorate the understanding of the probiotics mechanism of action and assessing the beneficial impact on health exerted by the administration of probiotics at a quantifiable level (Rebollar et al., 2016). The integration of the traditional *in-vitro* and *ex-vivo* screening methods of microorganisms with potential probiotic features are associated with omics techniques with the intent to boost the selection process, to deeper understand their functionality and to examine the opportunity to employ cell fractions instead of viable probiotics in finished products (Franzosa et al., 2015; Lahtinen, 2012). Recent market trends concerning probiotic are interestingly targeted to the zootechnic field. For instance, it has been proposed the probiotic use to produce safe and high-quality poultry meat (Park et al., 2016). Other application fields could be retrieved in cereals, dairy products, fermented meat, baked food, and dry food productions (Competitive Market Share & Forecast, 2016). For the application related to human consumption, the validated approach applied to the selection of probiotics firstly includes the safety assessment intended to verify the absence of pathological and virulence traits (Hill et al., 2014). The next step evaluates the potential application of the isolated strain by considering the prophylactic, therapeutic use or its ability to reduce the risk to develop a certain pathological condition. Regrettably, the implication of this validation results strictly dependent on the availability of measurable biomarkers related to a certain physiological condition.

## 2. Classical *in-vitro* Approaches for New Probiotics Screening

Guidelines for the evaluation of the probiotic use in food have been published several years ago (FAO/WHO, 2002) The principal focus identified was defined as a selection scheme for the conventional assessment of probiotic properties and for the characterization of functional bacterial strains as probiotics in food. Despite their time-consuming application, the *in-vitro* assays are routinely used due to their presumed predictive value to predetermine, the putative association between the isolated probiotic candidate strain and a specific health claim, targeting its potential general or specific application (Kumar et al., 2013). The major limitation of these assays is represented by the high

variability in terms of reproducibility even if a certain degree of standardization has been suggested. The natural continuation of these essays would be theoretically represented by *in-vivo* tests that are laborious, expensive and not feasible for ethical reasons.

**3. Persistence and Survival in the Human Gut**

A peculiar required probiotic feature is represented by the ability to survive the passage through the gastrointestinal tract remaining in a viable state and with the sufficient number of cells necessary to exert the probiotic effect. To verify this ability simulated gastric and pancreatic juices (Charteris et al., 1998; Lavermicocca et al., 2008) and porcine or bovine bile have been extensively used, in fact these assays are easy and cheap if compared to more recent approaches including GIT simulators. Unfortunately, those *in-vitro* approaches are affected of some biases dealing with the unrealistic conditions simulated during benchtop assays. Moreover, potential probiotic candidates are tested against severe environmental parameters not really miming the stomach acidity and lower intestine conditions during food consumption (Papadimitriou et al., 2015).

**4. Safety for the Use in Human Nutrition**

The Joint Working Group FAO/WHO (2002) underlined the relevance of some safety issues for the use of probiotics in food. The EFSA panel of experts suggested the precise determination of the minimal inhibitory concentrations (MICs) towards 9 listed antibiotics and also stated the microbiological cutoff values to evaluate a new probiotic candidate as susceptible or resistant to antibiotics and the reference methodology to assess it (EFSA; 2012). Safety aspects usually assessed by *in-vitro* tests also include aggregation to platelets, to fibronectin and to fibrinogen, hemolytic activity, the synthesis of certain enzymes and the production of biogenic amines. The major concern related to these essays is based on the fact that the expression levels of the *in-vivo* traits could significantly vary from the *in-vitro* conditions inducing to an over- or an under-estimation of some vexing features of probiotics (Papadimitriou et al., 2015).

**5. Colonization Ability**

The colonization ability is an indicator of the probiotic cellular features to interact with the host. The adhesion of bacteria to mucus and epithelial cells is an important parameter evaluated with several *in-vitro* assays; this trait could both represent positive connotations associated with pathogens displacement and negative implications for instance the increasing risk of translocation (Sanders et al., 2010). Furthermore, Caco-2, HT-29 epithelial cell adhesion assays often produce weak results due to the low reproducibility maintenance of the tested conditions. In order to circumvent the problem, resected gut tissue or whole tissue models including the mucus layer have been taken into consideration but reliability bias are still present (Vesterlund et al., 2005; van Tassell & Miller, 2011). To evaluate the ability of a strain to interact with the host, some physical features of probiotics have been considered,

for instance, the hydrophobicity of the cell surface (Yadav et al., 2014). Unfortunately, the scientific significance of these tests is only considered partially because of the lack of the peculiarity of this trait. The enzymatic activity expression such as GAPDH (glyceraldeyde-3-phosphate dehydrogenase) promoting the probiotic attachment to the mucus has been evaluated as a potential screening parameter even if with scarce results (Kinoshita et al., 2008).

The assay conditions, even if optimized could not reproduce the complexity of the bacterial community structure and the possible interactions among different microorganisms. The adhesion assays are performed on a single and purified strain without considering different microbial species leading to doubts about the results, significance and their possible extensive transposition to the gut microbiota.

## 6. Antimicrobial Potential

It is known that probiotics could exert competitive inhibition towards pathogens exploiting several mechanisms, for instance the production of metabolites showing antimicrobial activity. This feature has been proven influence other bacteria, viruses and fungi at a large spectrum, taking advantage of different compounds like organic acids or bacteriocins (Denkova et al., 2013). Several *in-vitro* models have been experienced, from the agar diffusion test to more advanced systems targeting the release of antimicrobial substances using indicator strains (Szweda & Szweda, 2016). Probiotics exhibit other features as co-aggregation with pathogens and/or by reinforcing the integrity and functions of the gut inner barrier. Briefly, probiotic cells bind pathogens causing a sort of clumping effect limiting the pathogen interaction with the surfaces of the host and facilitating the excretion of pathogens trough biological fluids (e.g., saliva, feces) (Janković et al., 2012). The gut barrier could exert its function only if the tight junctions integrity is maintained, this condition represents a key issue to partially prevent the bacterial translocation, pathogens included. It has been proved that probiotics may improve the barrier integrity as measured by specific enzymatic activities (Putaala et al., 2008).

The evaluation of probiotics antimicrobial potential is affected by some biases since the laboratory conditions only partially reproduce the *in-vivo* situation. Similarly to what has been observed for the described above *in-vitro* models, the level of expression of the real antimicrobial potential of tested probiotic strains is not completely defined. Substances as exopolysaccharides (EPS), biosurfactants, bacteriocins and organic acids represent the main antimicrobial compounds produced by probiotics (Evivie et al., 2017).

## 7. Immunomodulatory Actions

Probiotics could stimulate the secretion of antibodies by host cells and activate cell-mediated immune responses (Haghighi et al., 2005). Usually, immunomodulatory effects are promoted by M cells from the Payer's patches or dendritic cells (DCs) and the response to the occurrence of bacterial cells is assessed by co-culture of probiotics and immune cells and by the detection and the quantification of cytokines (Gad et al., 2011). These co-incubation models are frequently applied to the selection of

probiotics showing anti-inflammatory features useful in the management of chronic inflammatory diseases (e.g., IBD) (Rutella and Locatelli, 2011) or to be employed as adjuvant in the treatment of allergic symptoms (Ozdemir, 2010). However, the center of the debate still remains the *in-vivo* modality of action of probiotics and on the possible solution to translate experimental findings to the concrete evaluation of the probiotic strains efficacy in the modulation of the gastrointestinal and systemic immunity.

## 8. *In-vitro* Models of Cardiovascular Diseases

Another trait that is required from probiotic candidates is represented by the expression of the bile salt hydrolase activity (BSH). This enzymatic activity allows the deconjugation of bile acids and it is pretty easy and quick to obtain qualitative and quantitative results (Cani & Van Hul, 2015; Zheng et al., 2013; Tomaro-Duchesneau et al., 2014). However, these routinely used models are sometimes considered relatively insufficient to estimate the real probiotics contribution to reduce cardiovascular diseases risks associated (Papadimitriou et al., 2015), albeit the beneficial effects seem to be highlighted by scientific evidences (Ebel et al., 2014).

## 9. *In-vitro* Anti-cancer Models

It has been proven that probiotics reinforce natural host body protection functions by improving metabolic and immunological parameters (Ashraf & Shah, 2014). Furthermore, these beneficial bacteria could exert antigenotoxic and antimutagenic activities and promote nitrosamines and heterocyclic amine degradation whose accumulation within the intestinal ecosystem could lead to potential negative effects (Duangjitcharoen et al., 2014; Faridnia et al., 2010). Excluding the ability of withdrawing dangerous metabolites, the data support the probiotics ability of releasing short chain fatty acids (SCFA) within the intestinal lumen (Table 1). These substances have been proven to exert some anticarcinogenic effects *in-vivo* (Burns & Rowland, 2004; Castro et al., 2010). Nevertheless, despite a supportive background is already available, researchers consider these *in-vitro* tests as not sufficient to provide consistent probiotic anticancer activity information and, for this reason, the *in-vivo* validation is compelled (Chong, 2014).

To easily complete the probiotic profile of beneficial bacteria, a pool of other *in-vitro* model has been proposed, among them the measurement of B-galactosidase activity to monitor lactose intolerance symptoms (Vonk et al., 2012), B-group vitamin production (Leblanc et al., 2011), oxalate-degrading features (Abratt & Reid, 2010) and inhibition of pathogen oral strains responsible for volatile sulfur compounds (VSCs) production (Lee & Baek, 2014).

**Table 1. Short Chain Fatty Acids (SCFA) Produced by Probiotics**

| Probiotic microorganisms | Produced SCFA | Reference |
|---|---|---|
| *L. salivarius* spp *salcinius* JCM 1230, *L. agilis* JCM 1048 | Propionate/butyrate | Meimandipour et al., 2010 |
| *L. acidophilus* CRL 1014 | Acetate/butyrate/propionate | Sivieri et al., 2013 |
| *L. rhamnosus* LGG | Propionate | Le Blanc et al., 2017 |
| *L. gasseri* PA 16/8 | Propionate | Le Blanc et al., 2017 |
| *B. longum* SP 07/3 | Propionate/acetate | Le Blanc et al., 2017 |
| *B bifidum* MF 20/5 | Propionate/acetate | Le Blanc et al., 2017 |
| *Bifidobacteria* | Acetate/lactate | Pessione et al., 2012 |

**10. Development of Innovative Screening Platforms**

The rationale underlying standardized *in-vitro* assays to study several aspects of probiotic effectiveness is driven by the necessity of set reproducible experimental protocols and consequently decrease the percentage of false positive and negative results.

*In-vivo* models should be considered integrative for *in-vitro* ones with the purpose of choosing the appropriate probiotic for a potential application.

Several animal models including invertebrates (e.g., insect, worm) and vertebrates (e.g., rodents, dogs, monkeys and swine), have been tested even though the predictivity for the situation in humans seems to be powerless and for this reason, a critical approach should be applied when results are transposed to humans. In the latest years the conventional screening pipeline for probiotics (*in-vitro* assays—animal model—clinical trial) including mice or rat models has strongly been debated and there was the tendency to directly check the findings from *in-vitro* evidences on small number human cohorts. However, animal models will certainly be exploited, particularly for studying specific pathological conditions, both induced by chemicals administration or using animal with genetic predispositions, or pathogenic infection conditions. Some probiotic positive modulation effects of intestinal inflammation have been successfully studied thanks to animal models. For instance, the co-occurrence of probiotics and carcinogenesis prevention, metabolic disorders control or auto-immunological conditions have been experienced. On the contrary, immune-deficient animals carrying human tissue or genes also known as humanized mice or rat models, are basically used more for pharmaceuticals purpose instead of probiotic validation (Papadimitriou et al., 2015).

The combination of *in-vitro* and *in-vivo* test represents the new frontier, Europe coupled the two tests in some *in-silico* models to examine from a mathematical point of view the interaction between nutrients, epithelial cells, gut communities and host (Tan & Liong, 2014). These applications are supposed to mimic the complex gut environment processes by proposing predictive functions and taking in consideration the possible interactions between probiotics with multiple factors.

The omics approaches with the advent of new molecular methods greatly contributed to the comprehension of the usefulness of probiotic towards hosts, mainly quantifying the expression variation of certain housekeeping genes of probiotic cells in response to specific environmental conditions. The resistance to stressful conditions, modulation of the immune system, adhesion to host tissues, production of antimicrobial or noble nutrient compounds, degradation of prebiotics and quorum sensing are areas of interest investigated with special consideration (Papadimitriou et al., 2015).

Omics technologies are assumed to explicate the complexity of the communication dynamics inside microbiome or between it and host tissues and to provide new insights on the impact of a foreign probiotic strain meeting those structured communities (De Melo Pereira et al., 2018).

## 11. Conclusions

The number of available assays to validate probiotic efficacy *in-vitro* and *in-vivo* is rather complex. The major limitations linked to those approaches are that they consider variables explanatory and not easily reproducible. As a consequence, often the efficacy of new-isolated probiotic candidates could be accomplished only by combining several assays.

At the state of the art, on the one hand, there is an urging need of defining a clear workflow for the probiotics selection in order to obtain reliable and unquestionable proof of efficacy, and on the other, the design of appropriately designed clinical trials. Human studies are often associated with significant costs, consequently probiotics are still screened following the "funnel-like" approach, firstly using *in-vitro* tests and subsequently by expensive and appropriate validations on selected strains.

The objective sought in new probiotic candidate selection would lead to the development of screening platforms, reducing from a large pool of strains to a restricted group intentionally selected for a specific human need to achieve the ideal aim of *one strain-one function.*

## References

Abratt, V. R., & Reid, S. J. (2010). *Oxalate-Degrading Bacteria of the Human Gut as Probiotics in the Management of Kidney Stone Disease* (pp. 63-87).

Ashraf, R., & Shah, N. P. (2014). Immune System Stimulation by Probiotic Microorganisms. *Crit. Rev. Food Sci. Nutr*., *54*, 938-956. https://doi.org/10.1080/10408398.2011.619671

Baldi, A., & Arora, M. (2015). Regulatory categories of probiotics across the globe: A review representing existing and recommended categorization. *Indian J. Med. Microbiol*., *33*, 2. https://doi.org/10.4103/0255-0857.150868

Baugher, J. L., & Klaenhammer, T. R. (2011). Invited review: Application of omics tools to understanding probiotic functionality. *J. Dairy Sci*., *94*, 4753-4765. https://doi.org/10.3168/jds.2011-4384

Burns, A. J., & Rowland, I. R. (2004). Antigenotoxicity of probiotics and prebiotics on faecal water-induced DNA damage in human colon adenocarcinoma cells. *Mutat. Res*., *551*, 233-243.

https://doi.org/10.1016/j.mrfmmm.2004.03.010

Cani, P. D., & van Hul, M. (2015). Novel opportunities for next-generation probiotics targeting metabolic syndrome. *Curr. Opin. Biotechnol*. https://doi.org/10.1016/j.copbio.2014.10.006

Castro, M. S., Molina, M. A., Di Sciullo, P., Azpiroz, M. B., Leocata Nieto, F., Ster ń-Speziale, N. B., ... Manghi, M. A. (2010). Beneficial activity of Enterococcus faecalis CECT7121 in the anti-lymphoma protective response. J. *Appl. Microbiol.*, *109*, 1234-1243. https://doi.org/10.1111/j.1365-2672.2010.04747.x

Charteris, Kelly, Morelli, & Collins. (1998). Development and application of an in vitro methodology to determine the transit tolerance of potentially probiotic Lactobacillus and Bifidobacterium species in the upper human gastrointestinal tract. *J. Appl. Microbiol.*, *84*, 759-768. https://doi.org/10.1046/j.1365-2672.1998.00407.x

Chong, E. S. L. (2014). A potential role of probiotics in colorectal cancer prevention: Review of possible mechanisms of action. *World J. Microbiol. Biotechnol*., *30*, 351-374. https://doi.org/10.1007/s11274-013-1499-6

Coppens, P., Da Silva, M. F., & Pettman, S. (2006). *European regulations on nutraceuticals, dietary supplements and functional foods: A framework based on safety Toxicology*.

De Melo Pereira, G. V., de Oliveira Coelho, B., Magalh ães J únior, A. I., Thomaz-Soccol, V., & Soccol, C. R. (2018). How to select a probiotic? A review and update of methods and criteria. *Biotechnol. Adv*. https://doi.org/10.1016/j.biotechadv.2018.09.003

Denkova, R., Denkova, Z., Yanakieva, V., & Blazheva, D. (2013). *Antimicrobial activity of probiotic lactobacilli, bifidobacteria and propionic acid bacteria, isolated from different sources* (pp. 857-864).

Duangjitcharoen, Y., Kantachote, D., Prasitpuripreecha, C., Peerajan, S., & Chaiyasut, C. (2014). Selection and characterization of probiotic lactic acid bacteria with heterocyclic amine binding and nitrosamine degradation properties. *J. Appl. Pharm. Sci.*, *4*, 14-23. https://doi.org/10.7324/JAPS.2014.40703

Ebel, B., Lemetais, G., Beney, L., Cachon, R., Sokol, H., Langella, P., & Gervais, P. (2014). Impact of Probiotics on Risk Factors for Cardiovascular Diseases. *A Review. Crit. Rev. Food Sci. Nutr., 54*, 175-189. https://doi.org/10.1080/10408398.2011.579361

EFSA. (2012). *Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance*.

Evivie, S. E., Huo, G. C., Igene, J. O., & Bian, X. (2017). Some current applications, limitations and future perspectives of lactic acid bacteria as probiotics. *Food Nutr. Res.*, *61*(1), 1318034. https://doi.org/10.1080/16546628.2017.1318034

FAO/WHO. (2002). *Guidelines for the Evaluation of Probiotics in Food*. Geneva, Switzerland: Food and Agriculture Organization (FAO), World Health Organization (WHO).

Faridnia, F., Hussin, A. S. M., Saari, N., Mustafa, S., Yee, L. Y., & Manap, M. Y. A. (2010). In vitro

binding of mutagenic heterocyclic aromatic amines by Bifidobacterium pseudocatenulatum G4. *Benef. Microbes*, *1*, 149-154. https://doi.org/10.3920/BM2009.0035

Franzosa, E. A., Hsu, T., Sirota-Madi, A., Shafquat, A., Abu-Ali, G., Morgan, X. C., & Huttenhower, C. (2015). Sequencing and beyond: Integrating molecular "omics" for microbial community profiling. *Nat. Rev. Microbiol.*, *13*, 360-372. https://doi.org/10.1038/nrmicro3451

Gad, M., Ravn, P., Søborg, D. A., Lund-Jensen, K., Ouwehand, A. C., & Jensen, S. S. (2011). Regulation of the IL-10/IL-12 axis in human dendritic cells with probiotic bacteria. *FEMS Immunol. Med. Microbiol.*, *63*, 93-107. https://doi.org/10.1111/j.1574-695X.2011.00835.x

Haghighi, H. R., Gong, J., Gyles, C. L., Hayes, M. A., Sanei, B., Parvizi, P., ... Sharif, S. (2005). Modulation of Antibody-Mediated Immune Response by Probiotics in Chickens. *Clin. Vaccine Immunol.*, *12*, 1387-1392. https://doi.org/10.1128/CDLI.12.12.1387-1392.2005

Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., ... Sanders, M. E. (2014). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat. Rev. Gastroenterol. Hepatol.*, *11*, 506-514. https://doi.org/10.1038/nrgastro.2014.66

Janković, T., Frece, J., Abram, M., & Gobin, I. (2012). Aggregation ability of potential probiotic Lactobacillus plantarum strains. *Int. J. Sanit. Eng. Res.*, *6*, 19-24.

Kinoshita, H., Wakahara, N., Watanabe, M., Kawasaki, T., Matsuo, H., Kawai, Y., ... Saito, T. (2008). Cell surface glyceraldehyde-3-phosphate dehydrogenase (GAPDH) of Lactobacillus plantarum LA 318 recognizes human A and B blood group antigens. *Res. Microbiol.*, *159*, 685-691. https://doi.org/10.1016/j.resmic.2008.07.005

Kumar, A., Kumar, M., Ghosh, M., & Ganguli, A. (2013). Modeling in vitro cholesterol reduction in relation to growth of probiotic Lactobacillus casei. *Microbiol. Immunol.*, *57*, 100-110. https://doi.org/10.1111/1348-0421.12008

Lahtinen, S. J. (2012). Probiotic viability—Does it matter? *Microb. Ecol. Heal. Dis.*, *23*, 10-14. https://doi.org/10.3402/mehd.v23i0.18567

Lavermicocca, P., Valerio, F., Lonigro, S. L., Di Leo, A., & Visconti, A. (2008). Antagonistic activity of potential probiotic lactobacilli against the ureolytic pathogen Yersinia enterocolitica. *Curr. Microbiol.*, *56*, 175-181. https://doi.org/10.1007/s00284-007-9069-5

LeBlanc, J. G., Chain, F., Martín, R., Bermúdez-Humarán, L., Courau, S., & Langella, P. (2017). Beneficial effects on host energy metabolism of short-chain fatty acids and vitamins produced by commensal and probiotic bacteria. *Microb. Cell Fact.* https://doi.org/10.1186/s12934-017-0691-z

Leblanc, J. G., Laiño, J. E., del Valle, M. J., Vannini, V., van Sinderen, D., Taranto, M. P., ... Sesma, F. (2011). B-Group vitamin production by lactic acid bacteria—Current knowledge and potential applications. *J. Appl. Microbiol.*, *111*, 1297-1309. https://doi.org/10.1111/j.1365-2672.2011.05157.x

Lee, S.-H., & Baek, D.-H. (2014). Effects of Streptococcus thermophilus on volatile sulfur compounds

produced by Porphyromonas gingivalis. *Arch. Oral Biol., 59*, 1205-1210. https://doi.org/10.1016/j.archoralbio.2014.07.006

Meimandipour, A., Hair-Bejo, M., Shuhaimi, M., Azhar, K., Soleimani, A. F., Rasti, B., & Yazid, A. M. (2010). Gastrointestinal tract morphological alteration by unpleasant physical treatment and modulating role of Lactobacillus in broilers. *Br. Poult. Sci*. https://doi.org/10.1080/00071660903394455

Ozdemir, O. (2010). Various effects of different probiotic strains in allergic disorders: An update from laboratory and clinical data. *Clin. Exp. Immunol., 160*, 295-304. https://doi.org/10.1111/j.1365-2249.2010.04109.x

Papadimitriou, K., Zoumpopoulou, G., Foligné, B., Alexandraki, V., Kazou, M., Pot, B., & Tsakalidou, E. (2015). Discovering probiotic microorganisms: In vitro, in vivo, genetic and omics approaches. *Front. Microbiol*. https://doi.org/10.3389/fmicb.2015.00058

Park, Y. H., Hamidon, F., Rajangan, C., Soh, K. P., Gan, C. Y., Lim, T. S., ... Liong, M. T. (2016). Application of Probiotics for the Production of Safe and High-quality Poultry Meat. *Korean J. Food Sci. An, 36*, 567-576. https://doi.org/10.5851/kosfa.2016.36.5.567

Pessione, E. (2012). Lactic acid bacteria contribution to gut microbiota complexity: Lights and shadows. *Front. Cell. Infect. Microbiol*. https://doi.org/10.3389/fcimb.2012.00086

*Probiotics Market Size By End Use (Human, Animal)*. (n.d.). By Application (Functional Foods & Beverages [Dairy, Non-dairy, Cereals, Baked Goods, Fermented Meat Products, Dry Foods], Dietary Supplements [Food, Nutritional, Specialty, Infant Formula], Animal Feed Probiotics), Industry Analysis Report, Regional Outlook (U.S., Germany, UK, China, Japan, India, Brazil), Application Potential, Price Trends, Competitive Market Share & Forecast, 2016-2023.

Putaala, H., Salusjärvi, T., Nordström, M., Saarinen, M., Ouwehand, A. C., Bech Hansen, E., & Rautonen, N. (2008). Effect of four probiotic strains and Escherichia coli O157:H7 on tight junction integrity and cyclo-oxygenase expression. *Res. Microbiol., 159*, 692-698. https://doi.org/10.1016/j.resmic.2008.08.002

Rebollar, E. A., Antwis, R. E., Becker, M. H., Belden, L. K., Bletz, M. C., Brucker, R. M., ... Harris, R. N. (2016). Using "omics" and integrated multi-omics approaches to guide probiotic selection to mitigate chytridiomycosis and other emerging infectious diseases. *Front. Microbiol., 7*, 1-19. https://doi.org/10.3389/fmicb.2016.00068

Rutella, S., & Locatelli, F. (2011). Intestinal dendritic cells in the pathogenesis of inflammatory bowel disease. *World J. Gastroenterol, 17*, 3761-3775. https://doi.org/10.3748/wjg.v17.i33.3761

Sanders, M. E., Akkermans, L. M. A., Haller, D., Hammerman, C., Heimbach, J., Hörmannsperger, G., ... Vaughan, E. (2010). Safety assessment of probiotics for human use. *Gut Microbes*. https://doi.org/10.4161/gmic.1.3.12127

Sivieri, K., Morales, M. L. V., Adorno, M. A. T., Sakamoto, I. K., Saad, S. M. I., & Rossi, E. A. (2013). Lactobacillus acidophilus CRL 1014 improved "gut health" in the SHIME® reactor. *BMC*

*Gastroenterol*. https://doi.org/10.1186/1471-230X-13-100

Szweda, P., & Szweda, P. (2016). *World's largest Science, Technology & Medicine Open Access book publisher Antimicrobial Activity of Honey Antimicrobial Activity of Honey*.

Tan, P. L., & Liong, M. T. (2014). In silico approaches for probiotic-derived bioactives. *Trends Biotechnol*, *32*(12), 599-601. https://doi.org/10.1016/j.tibtech.2014.09.011

Tomaro-Duchesneau, C., Jones, M. L., Shah, D., Jain, P., Saha, S., & Prakash, S. (2014). Cholesterol assimilation by Lactobacillus probiotic bacteria: An in vitro investigation. *Biomed Res. Int.*, *2014*, 380316. https://doi.org/10.1155/2014/380316

van Tassell, M. L., & Miller, M. J. (2011). Lactobacillus adhesion to mucus. *Nutrients*, *3*(5), 613-636. https://doi.org/10.3390/nu3050613

Vesterlund, S., Paltta, J., Karp, M., & Ouwehand, A. C. (2005). Adhesion of bacteria to resected human colonic tissue: Quantitative analysis of bacterial adhesion and viability. *Res. Microbiol*, *156*, 238-244. https://doi.org/10.1016/j.resmic.2004.08.012

Vonk, R. J., Reckman, G. A. R., Harmsen, H. J. M., & Priebe, M. G. (2012). Probiotics and lactose intolerance. *Probiotics*, 149-160.

Yadav, A. K., Tyagi, A., Kumar, A., Saklani, A. C., Grover, S., & Batish, V. K. (2014). Adhesion of indigenous Lactobacillus plantarum to gut extracellular matrix and its physicochemical characterization. *Arch. Microbiol*, *197*, 155-164. https://doi.org/10.1007/s00203-014-1034-7

Zheng, Y., Lu, Y., Wang, J., Yang, L., Pan, C., & Huang, Y. (2013). Probiotic Properties of Lactobacillus Strains Isolated from Tibetan Kefir Grains. *PLoS One*, *22;8*(7), e69868. https://doi.org/10.1371/journal.pone.0069868