Title: Evaluation of the bifidobacterial microbe-host crosstalk

Scientific quality, interdisciplinary and multidisciplinary aspects of the proposal

Various bifidobacterial strains are intensely exploited as health-promoting microorganisms. However, so far little is known about the molecular mechanisms underlying their capabilities to interact with the human host as well as with the other members of the gut microbiota. Recently, with the advent of genome sequencing, it has been possible to shed light on the genetic elements that are considered important for the adaptation of bifidobacteria to the human intestine. The public availability of genome sequences of natural inhabitants of the human infant intestine, *B. breve* UCC2003, *B. bifidum* PRL2010 and *B. infantis* ATCC15697, as well as human adult-specific species such as *B. adolescentis* 22L, will be used to explore the interactive genome-functionality of these intestinal microbes and their interactions with the human host using a murine model.

The main aim of this proposal is to identify specific microbe- and host-responses to colonization using the above mentioned bifidobacteria. Therefore, we propose to investigate the molecular interactions that are presumed to exist between bifidobacteria and a murine model host, as well as interactions between different residents of an artificially composed intestinal microbiota.

The generated knowledge will provide an understanding of the molecular mechanisms underlying host-microbe interactions, and genetic adaptation of bifidobacteria to the interactions that exist between various components of the human gut ecosystem.

The high complexity of the human gut microbiota and the large number of variables that affect its composition (e.g., host genotype, sex, age, diet and disease status) (Eckburg et al., 2005; Gill et al., 2006; Palmer et al., 2007) has motivated prominent researchers in the field to return to the reductionist principle by working with isogenic gnotobiotic mice, inoculated with specific bacteria. Recently, efforts in resolving host-microbe interactions using gnotobiotic models have been made by different groups utilizing members of the genus *Lactobacillus*, such as *Lactobacillus johnsonii* NCC533 (Denou et al., 2007; Denou et al., 2008; Denou et al., 2009) and *Lactobacillus plantarum* WCFS1 (Marco et al., 2009), as well as *Bifidobacterium longum* NCC2705 and other components of the gut microbiota (Sonnenburg et al., 2006).

However, little is known about the precise impact of administration of probiotic bacteria to other (resident) components of the intestinal microbiota and on the host (Ventura et al., 2007). Recent studies have highlighted how the presence of bifidobacteria in the human gut elicits an expansion in the diversity of polysaccharides targeted for degradation by *Bacteroidetes* and induces host genes involved in innate immunity (Boesten and de Vos, 2008; Sonnenburg et al., 2006). It expands its repertoire of prominently expressed glycoside hydrolases, among which enzymes involved in mannoside and xyloside degradation, and up-regulates xylose metabolism.

Furthermore, *in vivo* analyses using *B. bifidum* PRL2010 cells indicates the up-regulation of a gene set involved in host-glycan metabolism (i.e. mucus-derived glycoproteins), and suggests the existence of an intriguing microbe-host symbiosis (Turroni et al., 2010). Other genes whose expression was shown to be highly influenced during *B. bifidum* colonization of mice are represented by those specifying the production of pili and lipoteichoic acids (applicant's unpublished data), which in other gut commensal bacteria are known as key drivers of microbe-host interactions and host immune responses (Mandlik et al., 2007).

The current proposal thus aims to investigate the molecular mechanisms underlying host-microbe responses following colonization of a model murine host with *B. bifidum*, *B. breve*, *B. infantis* and *B. adolescentis* cultures, either on their own or in various combinations.

The proposed research will represent a logical extension of the investigations by the applicant regarding the cross-talk that occurs between infant-associated bifidobacterial species (*B. bifidum*, *B. breve* and *B. infantis*) as well as the molecular response following the interaction of these infant-type bifidobacterial population and bifidobacterial species typically present in the adult-type intestinal microbiota (*B. adolescentis*) (Turroni et al., 2009a). Notably, the presence of adult-type bifidobacterial species such as *B. adolescentis* in the infant gut has been associated with the development of allergy in infants (Gore et al., 2008; Ouwehand et al., 2001). In fact, the applicant has collected a large amount of data concerning the bacterial genes that are differentially expressed upon colonization of mice with different bifidobacterial species. It is worth mentioning that among such differentially transcribed genes a large set of bifidobacterial genes was identified that encode for extracellular structures like pili, capsular polysaccharides, lipoteichoic acids as well as genes encoding proteins involved in the acquisition and breakdown of polysaccharides derived from the diet or from the host, such as mucins (applicant's unpublished data). The activities of these enzymes are considered to be crucial for these bifidobacteria in order to access carbon- and energy sources that will establish commensal bacteria within the human gut and thus shape the intestinal microbiota. However, the next logical step will be to investigate the molecular impact of a bifidobacterial population on their host. Thus, as mentioned above the aim of this proposal is to understand the host's response following bifidobacterial colonization of the gut using a murine model.
The main results expected from the execution of this proposal are summarized as follows:
-Identification of the genetic determinants of the murine host that are specifically expressed upon gut colonization with typical infant bifidobacterial species (e.g., *B. breve*, *B. bifidum* and *B. infantis*) compared to those induced upon colonization with adult bifidobacterial taxa (e.g., *B. adolescentis*).
-Detection of host epithelial response upon microbe colonization. A repertoire of mouse-genes encoding cytokine-responsive proteins (e.g., T cell-produced cytokine and interferon-γ) as well as antibacterial proteins (pancreatitis-associated protein) will be assessed for up- or down-regulation in response to bacterial colonization, according to (Sonnenburg et al., 2006).
These results will thus reveal interactive relationships between host and components of its intestinal microbiota, in this case specific bifidobacterial species, and such knowledge is expected to lead to an understanding of the mechanisms of action of the perceived positive health benefits of bifidobacteria.

Research methodology

In vivo experiments using animal models will be performed to explore the genome functionality of *B. bifidum* PRL2010 (Turroni et al., 2010), *B. breve* UCC2003 (O'Connell Motherway et al., 2011), *B. infantis* ATCC15697 (Sela et al., 2008) and *B. adolescentis* L22 (applicant's unpublished data). The germ-free mouse model is considered a valid tool to study functional properties of human gut microbial communities. Germ free mice belonging to either the NMRI-KI inbred lines will be housed in plastic gnotobiotic isolators under a strict 12-hour light cycle and fed a standard autoclavated chow diet *ad libitum*. Germ-free mice will be colonized with various bifidobacteria species as mentioned above either on their own or in different combinations.

Such different combinations would provide simplified microbial communities that are present in infant. In fact, the multi-heterogeneous association would represent a very simple intestinal microbiota that could be encountered in healthy infants, whereas the multi species association of bifidobacteria that include adult-type bifidobacterial species (*B. adolescentis*) would be more representative of an infant gut microbiota detected in subjects showing allergic disorders (Gore et al., 2008; Ouwehand et al., 2001).

Following a 10-day (or longer if necessary in order to establish a stable microbiota) colonization period, animals will be sacrificed, their cecum dissected and the contents of each cecum extruded from its distal end. Bacterial densities will be determined by performing serial dilutions of cecal contents, using bacterial strains carrying plasmids each conferring a different antibiotic resistance to the particular strain/species used. The remaining cecal contents will be flash frozen in liquid nitrogen immediately after their harvest from each mouse and stored at -80°C until use. An aliquot of the frozen material (about 200 mg) will be thawed in three volumes of RNAprotect and subjected to RNA extraction following the protocol described by (Sonnenburg et al., 2006). cDNA will be prepared and labeled using the Agilent Gene Expression hybridization kit (Agilent). Host transcriptional profiling experiments will be performed using a commercially available mouse array chip. Expression profiles will be referenced to datasets obtained from germ-free controls to identify significantly up/down-regulated genes. Furthermore, such data will be validated by deployment of RNAseq, which is a cutting edge technique predominantly used for the evaluation of transcriptomes of eukaryotes.
All mouse genes that exhibit up-regulation following bifidobacterial supplementation will be divided according to the family-pathways using the GenMAPP software. In a similar manner, bifidobacterial genes which exhibit significant up- or down-regulation during bacterial transit in the murine intestine will be investigated with respect to the metabolic pathways to which they belong, and with respect to their functions.

Originality and innovative nature of the project, and relationship to the 'state of the art' of research in the field

The human intestine is home of an almost inconceivable large number of microorganisms (Arumugam et al., 2011). Thus, it seems appropriate to view ourselves as a composite of many species, and our genetic landscape is thus an amalgamation of genes embedded in our *Homo sapiens* genome plus those of our affiliated microbial partners (the microbiome). The human gut microbiota can therefore be pictured as an organ placed within a host organism. The human gut microbiome, which in total may contain >100 times the number of genes present in our genome, endows us with functional features that we did not have to evolve ourselves (Gill et al., 2006; Hooper et al., 1999). Our relationship with components of this microbiota is often described as commensal (one partner benefits and the other is apparently unaffected) as opposed to mutualism, where both partners experience increased fitness. However, use of the term commensal may simply reflect our lack of knowledge or at least agnostic attitude concerning the contributions of most components of this microbial society to our own fitness or the fitness of other community members (Savage, 2001).

The human intestine contains representatives of all three domains of life, bacteria, Archaea and eukarya. Within the bacterial group, bifidobacteria represents the first most numerous species encountered in the colon of infant humans, considerably outnumbering other groups such as *Lactobacillus* species (Favier et al., 2002; Turroni et al., 2009a; Turroni et al., 2012; Turroni et al., 2009b; Zoetendal et al., 2006b). Bifidobacteria are anaerobic, Gram-positive, irregular or branched rod-shaped bacteria that are commonly found in the gastro-intestinal tracts (GIT) of humans and most animal and insects (Ventura et al., 2007). Bifidobacteria were first isolated and described 100 years ago from human feces and were quickly associated with a healthy GIT due to their numerical dominance in breast-fed infants compared to bottle-fed infants. In the human GIT their presence has been associated with beneficial health effects, such as prevention of diarrhea in antibiotic-treated patients, amelioration of lactose intolerance, cholesterol reduction and immunomodulation (Marco et al., 2006; O'Hara and

Shanahan, 2007; Ouwehand et al., 2002). These correlations have led to the widespread use of bifidobacteria as components of health-promoting or probiotic foods. Despite the generally accepted importance of bifidobacteria as probiotic components of human GIT microflora, and their use in health-promoting foods, there is a paucity of information about the molecular background covering their probiotic features (e.g., interaction with the host and with the components of the gut microbiota) (Ventura et al., 2007). Thus, the proposed project will be very important in order to shed light into the genetics underlying bifidobacteria-host interactions and will offer crucial information to facilitate the discovery of 'probiotic' genes.

Recently, investigation of the bifidobacterial population in infants has highlighted a small number of species whose presence in the infant gut is correlated with atopic disease (allergy). The importance of bifidobacterial composition in triggering allergic disorders has been suggested by (Gore et al., 2008; Ouwehand et al., 2001), who have noticed that the presence in the infant microbiota of bifidobacterial species which typically represent an adult-type microbiota such as *B. adolescentis* is much more common in infants with atopic diseases. In this context it has also been noticed that mothers that already suffer from allergies may transfer *B. adolescentis* species to their infants more often than healthy mothers leading to an aberrant compositional development of the microbiota, which may predispose infants towards atopic diseases. Thus, the lack of specific infant- bifidobacterial species (e.g., *B. longum* subsp. *infantis*, *B. breve* and *B. bifidum*) would be taken into consideration as potential bacteria triggering allergic disorders in model systems directed to investigate the host-microbe cross talk. The use of infant-related species in our experiments (e.g., *B. breve*, *B. infantis* and *B. bifidum*) as well as a typically related adult-type bifidobacterial species will describe the molecular basis of interaction between these two ecologically distinct groups of bifidobacteria, and thus generate genetic data that will be used for future investigative studies regarding the molecular aspects triggering allergic disorders.

Bacterial genome sequencing is considered the gateway to a new era of biological investigation which will shed light on the interactive genetics underlying all microbial properties. A number of current genome projects focus on microorganisms with important roles in food, including starter cultures used in the production of fermented foods and food-grade ingredients, and organisms with probiotic potential. Recently, a novel discipline called probiogenomics has been developed that aims to understand the genetic mechanisms underlying health-promoting activities elicited by probiotic bacteria (Ventura et al., 2009a). Moreover, substantial efforts have been made to determine the genome sequence of a large number of human pathogens, due to the interest in identifying pathogenicity determinants and discovery of new gene targets for rational drug design. Each new sequenced organism reveals novel features about genome organization, gene regulation, gene content, gene transfer and the biochemical potential entrenched in the microbial world. Thousands of novel genes, previously unknown to biology, have been deposited into public databases. To date, 1140 completed genome sequences have been deposited in the NCBI data base. However, so far bifidobacterial genomics represent an under-explored field since from a total of over 38 currently recognized species of this genus only the genomes of one or more strains of *Bifidobacterium longum* species, *Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium asteroides*, *Bifidobacterium animalis* subsp. *lactis*, *Bifidobacterium adolescentis* and *Bifidobacterium dentium* are publicly available (Barrangou et al., 2009; Bottacini et al., 2012; Lee et al., 2008; O'Connell Motherway et al., 2011; Schell et al., 2002; Sela et al., 2008; Turroni et al., 2010; Ventura et al., 2009b). Nevertheless, genome analyses have provided clear indications as to how these bacteria are genetically adapted to survive in the human intestinal niche. In this proposal the scientific focus will be on a small number of

bifidobacterial genomes which have been completed in the Host lab. (*B. breve* UCC2003, *B. bifidum* PRL2010, *B. adolescentis* 22L) or that are public available (*B. infantis* ATCC15697). These genomes represent the bifidobacterial models of the infant gut microbiota (*B. infantis*, *B. bifidum* and *B. breve*) as well as a typical representative of the bifidobacterial population encountered in adult (e.g., *B. adolescentis*).

In general, bifidobacteria represent an ideal target to study the genetics of health-promoting (probiotic) bacteria because they are common inhabitants of the human intestine where they are considered to play a positive effect on the health. However, the impact of these presumed probiotic species on the composition and functioning of the human gut microbiota, the degree to which their effects are mediated through the resident microbiota versus direct signaling to the host, and the impact of the host genotype on probiotic responses remain open questions. The answers to such questions will have important implications for the design of clinical trials that aim to explore the specific and general effects of different probiotics, and to determine whether probiotic consumption should be dictated by the microbial ecology or other features of the host, including diet and genotype.
As mentioned above the human intestine is populated by a very complex microbiota, which varies between individuals, primarily at the level of species and subspecies (Eckburg et al., 2005). Moreover, within a given intestinal environment some microbial members are fully entrenched or "resident" (representing the so-called autochthonous components), whereas others act more like hitchhikers (so-called allochthonous members) that pass through together with food, water or other components of the environment (Sonnenburg et al., 2004).
All these considerations highlight the importance of creating simplified and defined model systems for studying the functional properties and operating principles of our gut microbial communities and the consequence of consuming probiotics. In this proposal we would like to shed light into the specific microbe- host responses to colonization using bacterial strains for which the genome sequences are available.

Timeliness and relevance of the project
In the last number of years significant efforts have been dedicated to develop a wide variety of probiotic bacteria to be added to new functional foods. However, a large part of the assessments on the beneficial effects of probiotic bacteria to the consumer's health are speculative and not supported by any molecular understanding of the health-beneficial effect. Thus, a clear identification of probiotic functionalities (e.g., synergic/antagonistic action with the gut microbiota) encoded by genetic entities will be necessary in order to provide a robust scientific basis for using such probiotics in functional foods, based on the so far existing ecological and microbiological knowledge, bifidobacteria represent excellent candidates for such studies.

It is without question that whole transcriptome profiling of selected bacterial species in the gut habitat and of the intestinal epithelium is crucial to shed light on the genetic basis that underscores the molecular crosstalk between the commensal/probiotic and its host. It goes without saying that the probiotic scientific field, the consumer and the relevant industries will all benefit if distinct beneficial activities are identified with scientifically proven mechanisms of action.
Another relevant target of the current proposal is to provide a gnotobiotic mouse model representing a controlled case study of how resident "probiotic" species (e.g., *B. bifidum*, *B. breve*, *B. infantis*) adapt their substrate utilization in response to the presence of another species, and it will illustrate both the generality and specificity of the relationship between host, a component of its microbiota, and intentionally introduced microbial species. The data

so far available are limited only to *B. longum* NCC2705 (Sonnenburg et al., 2006) or to other bacteria such as *Lactobacillus*. Thus, this study will highlight for the first time the cross-talk occurring between bifidobacteria and their host based on a number of different bifidobacterial species (e.g., *B. breve*, *B. bifidum*, *B. infantis* and *B. adolescentis*). Due to the fact that these species, apart from *B. adolescentis*, are commonly detected in the gut of (human) infants, the current proposal will uniquely represent a simplified infant gut model, which will highlight the microbe-host relationships occurring in this particular environment. The possibility of investigating the molecular mechanisms underlying the microbe-host interaction in the infant gut will be of crucial importance in order to analyze predisposition to disease (such as atopic diseases).

Furthermore, the investigation of the molecular responses that occur between bifidobacterial species commonly found in healthy infants (e.g., *B. breve*, *B. bifidum* and *B. infantis*) among each other or in the presence of non-typical infant bifidobacterial species, which has been linked to allergy (e.g., the biomarker *B. adolescentis*), will be crucial in order to understand the cross-talk that exists between these different ecological types of bifidobacteria (infant-type and adult-type) and the possible link to the development of allergic disorders.

Match between the fellow's profile and project

As outlined in the CV of the applicant, her areas of experience entail the genomics of bifidobacteria and the understanding of the biodiversity of the human gut. The knowledge of these subjects is indispensable in order to carry out the currently proposed project, which is aimed at analyzing microbe-microbe and microbe-host interactions. In fact, in order to dissect the molecular basis responsible for microbe-host interaction in the human gut compartment, it is crucial to have a profound knowledge about the microbial composition of the population residing in this environment. Bifidobacteria are considered as model microorganisms among the human intestinal gut microbiota and for this reason such bacteria have been chosen for this project. Notably, the applicant possesses all the necessary genomics background about this group of microorganisms represents, thus rendering her a perfect candidate to perform the proposed study.

The required skills to carry out this project entail a microbiological background on gut bacteria and in particular with bifidobacteria, and expertise in the use of transcriptomic tools (e.g., microarray as well as RNAseq experiments). As indicated in her CV, the applicant possesses all the above mentioned skills, and the current project thus represents the next logical step forward. Although she has no specific experience with mouse experiments, she will receive all necessary support for this specific task at the host institute where such expertise and its relevant infrastructure is available.

Potential for reaching a position of professional maturity

The Alimentary Pharmabiotic Centre (APC) at University College of Cork (UCC), was established relatively recently and is in an ongoing phase of vigorous development, both in terms of personnel, expertise and infrastructure.

The strong scientific record of and multidisciplinary expertise available at the APC, the highly dynamic environment of University College of Cork, together with the exponentially growing importance of comparative and functional genomics in modern biological research, renders this proposal ideal for further career development and enhanced employment prospects. In addition, the host laboratory has developed a vast network of scientific collaborations with many research institutions around the world, including laboratories placed in the original country of the applicant. These clearly will offer future job opportunities for the applicant at the end of this PostDoctoral experience. The applicant will also participate in APC-organized

workshops on scientific writing, proposal preparation and how to recognize and capture scientific results that have commercial value and how to validate and secure such intellectual property.

Potential to acquire new knowledge

As indicated in the CV, the applicant has participated in numerous international congresses, symposiums as well as workshops, including a lecture at the last edition of the Lactic Acid Bacteria Symposium (LAB10) on host-microbe interaction, which represented a clear sign of her interest in acquiring new knowledge and in keeping herself updated on scientific progress. Furthermore, the applicant has been well motivated from the very beginning of her scientific career to learn new experimental techniques and acquire novel methodologies from different areas of genetics (e.g., from other Actinobacteria members), which she later applied to the subject of her research (bifidobacteria). The current proposal, if funded, will substantially expand her knowledge and expertise and will target a very important knowledge gap that impacts on the regulatory approval and therefore commercialization potential of probiotic products.

Feasibility and credibility of the project, including work plan

The duration of the project is 12 months. The project will be divided in four main parts. The work for each part will be performed to a large degree in parallel and the indicated Milestones are therefore achieved at the end of the project (Month 12). Furthermore, since we cannot anticipate the number of interesting genes found, it will be difficult to provide an exact description and timing of the deliverables, in particular when it concerns characterization of genes that are highly expressed, differentially expressed or specifically required during the bacterial-host interaction.

The deliverables and milestones for each of the parts are listed below.

Part 1. Colonization of germ-free mice.

Deliverables

Month 3: Six- to 8 week old germ-free mice belonging to either NMRI-K1 or C57BL/6J inbred lines will be inoculated with 10^8 CFU of each bacterium used in this study.

Month 4: After 15 days colonization period (this will be monitored by selective plating of faecal contents), animals will be sacrificed and their intestine will be dissected. Intestinal contents will be treated with RNAase inhibitors and quickly frozen and stored at -80 ^oC until use.

Milestone (M1; month 4): Colonization and collection of bacterial samples.

Part 2. Preparation of murine RNA from intestinal epithelium.

Deliverables

Month 4: An aliquot of the frozen intestinal epithelia of caecum will be used for RNA extraction. RNA extraction will be carried out following the protocol described by (Zoetendal et al., 2006a) and further treated with DNase in order to eliminate any DNA contamination.

Month 5: An aliquot of 500 ng of total RNA will be subjected to reverse transcription.

Month 5: Aliquots of cDNA targets will be prepared and used for transcriptome experiments labelled with Labelling kit (Kreatech).

Milestone (M2; month 5): RNA extraction and cDNA synthesis.

Part 3. Analysis of murine transcriptomes. Transcriptome for all the bacteria that will be used for mouse-colonization will be performed using a commercial array chip (Agilent Technologies); the host laboratory has arrays, all software, hardware and expertise on array analysis) Furthermore, mouse transcriptomic investigations will be carried out using the RNAseq approach, which will consist of direct cDNA sequencing using the Ion Torrent technology.

Deliverables

Months 5-10: Transcriptional profiling experiments will be carried out following a previously described protocol (Zomer et al., 2009). Briefly, labeled cDNA will be hybridized using the Agilent Gene Expression hybridization kit as described in the Agilent Gene Expression Analysis (v4.0) manual. Following hybridization, microarrays will be washed as described in the manual and scanned using Agilent's DNA microarray scanner G2565A. The scanning

results will be converted to data files with Agilent's Feature Extraction software. DNA microarray data will be processed as previously described (Garcia de la Nava et al., 2003; van Hijum et al., 2005) up-regulated/down-regulated mouse genes will be identified.

Months 6-10: RNAseq will be performed through direct sequencing of cDNA from intestinal epithelia by using RNAseq kit from Ion Torrent. The achieved cDNA libraries will be sequenced using the 318 Ion Torrent chip and the generated reads will be analyzed through the Partek Flow software (Partek Genomics Suite^{TM}).

Milestone (M3; months 10): Bacterial transcriptional profiling hybridizations.

Part 4. Analysis of mouse transcriptomics datasets.

Deliverables

Months 10-12: All transcriptomic data sets generated in both transcriptional profiling experiments, i.e., microarray- and RNAseq-based dataset, will be analysed in order to identify genes whose expression displayed a significant change. Comparisons will be performed on specified gene chip datasets to identify genes up-regulated in the experimental group relative to the baseline group. Criteria followed to determine significant changes in the expression will be guided by the median False Discover Rate (FDR) of 50 permutations in which samples will be randomly shuffled between groups. The following criteria will be used to identify significantly up-regulated genes while maintaining an empirical FDR of less than 0.01: a) E/B > 1,2 using 90% confidence bound of fold-change; b) E-B >50; c) E+B$p \leq 0.05$.
Moreover, an ANOVA model will be used in order to determine the statistical significance of each effect for each gene. Such analysis will be carried out as previously described (Sonnenburg et al., 2006).
Further, we will assess the classification of genes whose expression was significantly changed according to the Cluster Orthologous Groups (COG). Such analysis will highlight if any of the COG families for each genome will be affected when each of the microorganisms studied will colonize the mouse gut alone or in combinations with the other strains.

Milestone (M4; month 12): Identification of the genetic basis of commensal function of bifidobacteria.

Potential of acquiring competencies

The applicant will have the possibility to acquire new competencies/skills that will significantly expand her already existing scientific background in the field of health promoting bacteria and human gut bacteria. Such novel competencies will also enable the applicant to reach higher level of maturity and independence in the field of functional genomics of gut bacteria. The development of a gnotobiotic model for studying human gut interactomics will offer the applicant the access of a methodology that in the future could be applied to a more complex bacterial populations (e.g., more bacterial species), or a different human body compartment (e.g., the oral cavity, or the stomach).

Contribution to career development or re-establishment where relevant

Currently, the field of research in probiotics is changing rapidly and novel probiotic bacteria (called new generation probiotics) are under development. Such bacteria need to be well

studied, and the molecular mechanisms underlying their capabilities to interact with the human host as well as with other components of the gut microbiota must be elucidated. Such interest in the work/competence of the applicant will be at both academic as well as industry level. The acquisition of these competences will thus offer an important opportunity for career development to the applicant.

Contribution to European excellence and European competitiveness

The current proposal represents an essential element of the Food and Health programme that is being undertaken by various European research institutes. It will generate primary data regarding the functionality of health promoting bacteria such as bifidobacteria. So far tremendous efforts have been made by many European dairy factories to develop a wide variety of probiotic or functional foods. However, large part of the assessments on the beneficial effects of probiotic bacteria like bifidobacteria to the consumer's health are largely speculative and not necessarily supported by any experimental evidence. Thus, a clear identification of probiotic functionality encoded by genetic entities will be necessary in order to provide a sound scientific basis for using such bifidobacterial probiotics.
In vivo analysis of bifidobacterial population and their interaction with the human host as well as with other components of the human intestinal microbiota will represent the logical follow-up of bacterial genomics which is currently involving many European research groups working on probiotics. The execution of this project will increase the currently limited knowledge on the molecular background sustaining the interaction between intestinal commensals and human host, which is an under-explored area as compared to other bacterial groups (e.g. pathogens). Such scientific achievements will put Europe at the forefront of research in this new area and will offer great opportunities to European Food Industry in order to develop novel effective functional foods.

Ethical Issues

The research project proposed will involve the use of a murine model based on germ free mice. Animals will be used since they represent a preclinical model for the study of microbe-host (human) interaction. The estimated number of animals required by the proposed project will be around 70 mice. However, this number may increase according to the combination of different bacterial strains that will need to be used during the project.
All the experiments involving animals will be performed in compliance of the "3Rs" policy of Refinement, Reduction and Replacement endorsed by the European Union (99/167/EC: Council Decision of 25/1/99). This proposal will perform studies in the mouse model adhering to national regulations on animal experimentation (implementing 86/609/CEE Directive). Procedures involving animals and their care will be conducted in conformity with the institutional guidelines that are in compliance with international laws and policies (EEC Council Directive 86/609, OJ L 358, 1, Dec. 12, 1987; Guide for Care and Use of Laboratory Animals, U.S. National Research Council, 1996). The studies on animals will be carried out under strict containment, in a well organized biosafety class II animal facility, equipped with a cupboard with appropriate ventilation, pressure, and filters to limit animal and environmental contamination and by qualified personnel to minimize any possible discomfort to the animals.

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