Probiotics in the Prevention of Urogenital Tract Infections. Mechanisms Involved

M. E. Fátima Nader-Macías*

CERELA-CONICET. Chacabuco 145. 4000. Tucumán. Argentina

Abstract: Urogenital tract infections affect a very high number of women worldwide, producing many clinical situations that imply increasing costs to the health systems, and a consequent morbility and mortality. The Urinary Tract Infections are more common in pre pubers or postmenopausal women, while the Genital Infections are more related to Sexually Active Women. Many of the applied therapeutics imply the use of antibiotics or other drugs that produce adverse effects not only in the urogenital tract, but at general level. This tract is also affected by other external or internal factors that have effect on the equilibrium of the urogenital microbiota, increasing the incidence of infections. During the last years the preventive measures are being applied around the world. In the urogenital tract, they are considerably important by the relationship with pregnancy development, newborn status and mother complications. The application of probiotics for many clinical situations, and for the restoration of the urogenital microbiota and prevention of infections, is more and more frequent. Probiotics are defined as live microorganisms administered in high numbers to the host to produce a physiological effect. The mechanisms involved in the probiotic effect include the production of antagonistic substances, the competitive exclusion phenomenon, the competition for nutrients, the colonization ability, the biofilm formation and/or the stimulation of the immune system. Some of these mechanisms have been showed by "*in vitro*" assays, but not yet in many "*in vivo*" experiments. The diversity of strains claimed as probiotics is higher every time, without the publication of clinical trails supporting their beneficial effect. These aspects and other related with the rationale of probiotic application in the urogenital tract are discussed in the present review

1. PROBIOTIC DEFINITION

The concept of the application of *Lactobacillus* species for the prevention and treatment of infections is not new. The consumption of fermented milks has a long history from ancient times, as a way to increase the storage of food for consumption. Elie Metchnikoff, a Russian scientist published in 1906 a paper on the beneficial effects produced by the consumption of milk fermented with lactobacillus to control the putrefactive intestinal microflora [1]. Even though the fermented products has been considered beneficial from the nutricional point of view, during the last two decades there has been a renewed interest in the use of probiotics (also called biotherapeutic agents) for human and animal use.

The term "probiotic" was derived from the Greeks, meaning "for life". Probiotics are live micro-organisms, which when administered in adequate amounts confer a health benefit on the host [2, 3]. The concept of probiotic application in other tracts has a long story, and numerous studies support their use, but not many in the urogenital tract [4]. The main objective of the probiotic application is the restoration of the ecological equilibrium of the indigenous microbiota to participate in the host´s physiology and thus to protect the host from infections. There has been an exclussion in the number of publications on the area, directed by the consumer´s requirements and the free press reviewed recently in scientific meetings sponsored by the World Health Organization (WHO) and Food and Agricultural Organization (FAO) [2, 5]. These organizations have stated that there is adequate scientific evidence to indicate that there is potential for probiotic foods to provide health benefits and that specific strains are safe for human use [2, 5].

Some probiotic products have been used therapeutically for the modulation of the immune system, to decrease the cholesterol level, to treat rheumatoid artritis, to prevent cancer, to improve lactose intolerance [3, 4, 5, 6, 7], lately to prevent *Helicobacter pylori* infections [8]. But there is only clinical evidence of their use for the treatment of traveler´s diarrhea, rotavirus diarrhea and antibiotic-associated diarrhea [9, 10, 11, 12, 13]. There are a lower number of evidences in the prevention of atopic dermatitis [14] constipation, candidiasis or UTI or even in some inflammatory syndromes of the gastrointestinal tract, as Crohn´s disease or pouchitis [15, 16]. Lately, they have been suggested as adjuvants in some syndromes associated with the nervous system as autism, or hepatic encephalopathy [17, 18, 19]. In some papers published on the last years, and also in the conclusion of the scientific meetings on the subject, as the International Scientific Association for Probiotics and Prebiotics (ISAPP) [2, 5, 20] there is a constant request for well planned clinical and animal studies to support the beneficial effects of the microorganisms, either preventives or therapeutics to be proclaimed as a probiotic strain.

The number and diversity of microorganisms suggested as probiotics is higher every time. Many of them are supported by the food producer companies that are trying to encourage studies to demonstrate the claimed effect [2, 5, 20]. But only a few of them have shown the clinical effects,

*Address correspondence to this author at the CERELA-CONICET. Chacabuco 145. 4000. Tucumán. Argentina; Tel: 54-381-4311720; Fax: 4005600; E-mail: fnader@cerela.org.ar

1573-4048/06 $50.00+.00

or the mechanisms involved in animal models. There are some products in the pharmacological market containing lactobacilli. Many of them do not have any reference to support the properties or characteristics of the bacteria included, even beneficial or detrimental. Some of them are misslabeled, do not contain the reported microorganisms [21] and are not supported by clinical or animal studies showing their probiotic or beneficial effect. This is one of the main reason by which the use of probiotic bacteria needs a scientific background to support their application and frequent use [2, 5].

2. INDIGENOUS MICROBIOTA OF THE UROGENITAL FEMALE TRACT

The vaginal microflora was first described by Doderleïn in 1892 [22]. Beijerink, in 1901 [23] demonstrated that it was mostly composed by Lactobacilli. But it is only since few years that its composition as a mixed flora has started to be known, thanks to the application of molecular biology tools. The vagina is colonized by up to 50 species of organisms during a lifetime, being lactobacilli the predominant genus, which can exist at 10^7 cells/ml of vaginal fluid [24, 25]. Many of the species cannot be detected by traditional culture methods, but by the application of PCR-based techniques [26, 27, 28, 29, 30]. These lactobacilli are essentials to a balanced vaginal ecosystem [31, 32, 33] and necessary for the defence of the mucosa to protect the host from infectious diseases, including some that are sexually transmitted or those that affect the urinary tract.

During the pregnancy, the fetus is sterile [34]. After born, the neonate urinary tract is also sterile, and with the exception of the distal urethra that acquires a limited microbiota derived from the perineum, the sterility is maintained during the health status. After born, the sterile vagina of the neonates acquires the microbiota of the mother's vagina, intestine and skin, together with those from the environment having a similar composition up to 2-3 months of life [31]. After three weeks of life, the metabolism and secretion of estrogens derived from the mother stop. The vaginal epithelium is exfoliated and the epithelial glycogen contents decreases. These changes are associated with an increased vaginal pH from 4.5 to 7.0 that selects a microbiota less resistant to acids: _*-hemolytic Streptococcus (viridans group), Escherichia coli, Klebsiella, coagulase negative Staphylococcus, Corynebacterium spp* [24, 25].

During the puberty, the level of estrogens and the content of epithelial glycogen increase, and the vaginal pH decreases again to 4.0-4.5, selecting then a glycogen-fermented microbiota, acid tolerant, predominantly of the genus Lactobacillus [36]. The redox potential (Eh) is also increased, providing an environment less favorable to the growth of strict anaerobes. Lately, some new species have been isolated, as for example *Atopobium vaginalis*, or some strains of the genus *Lactobacillus* 26, 29, [37]. The described ecosystems have ecological niches associated that are later susceptible to certain modifications depending of the menstrual cycle and women age [24]. The endocervix and vagina are considered unique ecological niches with a distinctive microbiota, because the epithelium, the pH and the other conditions are different to the mucosa of other tracts. The cervical os and lower canal can show a microbiota similar to the distal vagina. The upper cervical canal is normally sterile, or shows only a very low number of microorganisms [38]. The cervical microbiota is less characterized than those from vagina, because the variable anatomy of the cervix is modified with the contraception and pregnancy. The majority of the microorganisms isolated from these environmments are Prokaryota (Bacteria), but also some Eukaryota members are present as Fungi or Parasites, and subcellular microorganisms as Viruses. The application of molecular biology techniques will direct to the evidence of the presence of a very diverse indigenous microbiota, mainly the "non cultivable" microorganisms, enriching thus the overview of the described microbiota until these days [26, 27, 28, 29, 30].

3. FREQUENT UROGENITAL PATHOLOGIES AND THEIR CONSEQUENCES

In normal conditions, the urogenital tract has unique properties that counteract infection [39]. The normal urogenital environment considered as an ecological niche can be disrupted by many internal factors (physiological and hormonal status [40], advancing age, sexual activity, pregnancy, primary immune suppression, etc) or external (antibiotics [41, 42], hormones, foreign bodies - intrauterine devices and diaphragms [43], immunosuppressive drugs, spermicidal agents [44], vaginal douching, tight pants, multiple sex partners [45, 46], etc) which affect directly the ecological balance of the tract producing disequilibria of the microorganisms. The infectious situations related to the female genital tract are more and more frequent by their direct relationship to the increase of sexual transmitted diseases (STD), which include those produced by bacteria, virus, fungi and parasites. In these situations, the protective effect of the normal microbiota is not acting, because some of the indigenous microorganisms are depleted, some are increased -as in the Candidiasis or Bacterial Vaginosis (BV) cases- favoring thus the production of some pathologies, or the income of new pathogenic microorganisms. Is not clear yet if the depletion of lactobacilli, the main population of bacteria present in a healthy vaginal tract, is produced by some bacteriophages that kill the bacteria (probably from sexual transmission) allowing thus an overgrowth of the other bacterial genus representative of BV [47, 48]. Once the ecological equilibrium is disrupted, the physiological status of the tract is not normal, the low pH is increased, the secretions are altered, and the inflammatory/immune response is targeted [49]. The incidence of urinary tract infection, bacterial vaginosis and yeast vaginitis, is estimated to affect one billion women each year, being the rate for urinary tract infection alone 0.5 cases per person per year [37, 50]. Many therapies are being applied to attack the pathogenic microorganisms, and to avoid the concomitant sintomatology, mainly antibiotics. But, the concept of restoring the Lactobacillus content of the vaginal microbiota as a barrier to prevent infection, called restoration therapy for preventive purposes was conceived in the early 70s, and is only applied lately, but not very frequently [51, 52, 53].

All the pathologies that affect the urogenital tract have a significant importance, not only for the women health, but primarily for the pregnant women and the potential affection

to the fetus or to the newborn [49]. Then, this becomes a main problem in terms of health prevention [54].

The pathologies that affect the urogenital tract produced by microorganisms are summarized according to the area of the body they affect, as follows:

3A.Vaginitis or Vulvovaginitis and Cervicitis

produced by different etiological agents.

-**Bacterial Vaginosis** (BV). Mc Lean [55] defines BV as a non-inflammatory disease characterized by a depletion of vaginal lactobacilli and an overgrowth of a vaginal mixed flora composed by aerobic, anaerobic and microaerophiles in high numbers including *Gardnerella vaginalis*, *Mycoplasma hominis*, *Peptostreptococcu*s species, *Bacteroides* and other anaerobic Gram-negative rods [56].

-**Vulvovaginal candidiasis:** produced by species of the genus Cándida (*C. albicans in* 67 to 95%), the majority of them are part of the indigenous microflora, and increase when an external or internal factor disequilibrate the ecosystem [57].

-**Trichomoniasis** is produced by a eukaryotic parasite, *Trichomonas vaginalis*, usually sexually transmitted, mainly with multiple sex-partner.

-**Chlamydia Cervicitis.** *Chlamydia trachomatis* is an intracellular obligated parasite that affects only human beings.

-**Gonococal Cervicovaginitis**. The responsible microorganism is *Neisseria gonorrhoeae*, a Gram negative coccus, infecting cilindric and transitional epithelial cells of the urogenital tract.

-**Herpes Cervicitis and vulvovaginitis.** This is a sexual transmitted infection, being the etiological agent the type I and II (HSV I y HSV II) Herpes Virus, ADN single chain viruses, with an envelope of glucoprotein chain.

-**Human Papilloma Virus (HPV)** presents a high prevalence and frequency as STD in women with all of the clinical manifestations, because some lesions are produced in the posterior vagina or cervix. The prevalence of some particular serotypes (for example 16 and 18) is related more frequently with the genetic modifications in the vaginal cells that can derive in cervical cancer [58, 59].

-**Chemical or non-specific Vaginitis:** include all the pathologies that can be produced by any substance potentially itching (spermicides agents, excipients or vehicles of medical compounds, vaginal douches, powders douches, local antimicrobial agents).

-**Human Immunodeficiency Viruses:** eventhough the incidence of the different clinical situations and syndromes produced by these viruses have increased in the last years, there are not definitive reports on the role of lactobacilli on the prevention of this type of infections. There are some papers published on the interacción of H_20_2-producer lactic acid bacteria and viruses on cell cultures or "*in vitro*" experimental models [60]. But there is not yet an available animal experimental model to study the protective effect of lactobacilli against these viruses.

3B. Urinary Tract Infections (UTI)

The UTI are included into the most common bacterial infections, being one of the main problems worldwide in the women, both ambulatory and hospitalized, producing a very high morbility. They are present in a very wide range, from asymptomatic bacteriuria to kidney infection and failure. They are very related to the higher costs derived from health care systems in the whole world [61, 62]. The lower UTI, non-complicated symptomatic, are a problem in pre and post-menopausal women, with a risk percentage higher that 20%. Around 60% of women have a UTI once in their lives, being reported 10 millions of symptomatic UTI and also of asymptomatic bacteriuria in the United States [63]. The sexual activity is the most significant risk for the UTI, and one third part of women older that 50 years present urogenital symptoms related with the estrogenic deficiency. The advanced age is associated with the declination of many physiological processes that include those related with the urogenital tract [64]. The Enterobacteria is one of the groups of pathogens responsible of UTI, including *Escherichia coli*, *Klebsiella* and *Proteus.* The infection is generally ascendant, produced by pathogens that are members of the urethral and vaginal microflora, and sometimes as a contamination from the rectum and perianal area [50, 65, 66]. The cited patterns are modified when referred to the nosocomial infections, percentages that are increased in the pre-pubers girls and in post-menopausal women, where there is a clear evidence of the hormonal status effect. A high proportion of post-menopausal women present a modification of the vaginal pH and thus of the microbiota by the effect of estrogens, which allow an easier incoming of the pathogenic microorganisms. In this particular age group, the frequency of recurrences is also increased, because 1/3 of them produce permanent recurrences, more common in 25 to 29 years old women, or older that 55 years old [25, 32]. The recurrences are a common indication of the long-term antimicrobial prophyl-axis treatments that produces the emergent antimicrobial resistance of the pathogenic microorganisms [67].

4 THERAPEUTIC MEASURES AND CONVEN-TIONAL TREATMENTS APPLIED

4a. Antibiotic Therapies

The UTI are treated effectively with antibiotic therapy, even though this treatment produces disequilibrium of the indigenous microflora. In USA there are 11 million women receiving antimicrobials for the UTI infections, which implies around $1.6 Billion dollars [63, 66]. Even though the antimicrobial agents are very effectives to get the clinic resolution of the bacterial infections, the drug resistance is being increased worldwide, as for example in *E. coli* was reported a resistance of 18% to Trimetoprime-Sulfametoxazol in USA and Canada [66], while in Spain is increased to 30% for quinolones. This is one of the main situations that show the requirement of the development of valid alternatives for the management of these therapies.

Also, the transmission of extra chromosomal elements has been described as the responsible of the resistance transference to antibiotics [67], which add another problem to the situation.

The STD are treated with antimicrobial, antimicotic, antiparasitic or antiviral agents, according to the etiological agent responsible of the infection. The therapy costs for infectious diseases involved many millions dollars world-wide, with the consequent adverse effect. These types of infections are increasing their importance by the high morbility and mortality that can cause in the newborn [67, 68].

4b. Application of "Homemade" or Non-Conventional Preventive Therapies

The application of a series of "homemade" or tradition-transferred therapies has been observed specifically in the urogenital tract, as the application of vinegar (acetic acid). Also, medicines acquired over the pharmacy counter, [69] or consumption of foods or nutraceutics with probiotic properties demonstrated in the digestive tract, as for example, yogurt [70, 71]. One of the most frequent product employed, transmitted from generation to generation, is the application of vinegar in the vaginal douched, or the inclusion of garlic powder in the foods. The local inoculation of yogurt or the intravaginal application of garlic has been suggested for the treatment of candidiasis, in some internet pages [72].

Other foods, as for example the cranberry juice, has been considered historically as inhibitory of the UTI. The effect of cranberries on urine [73] showed that the excretion of hippuric acid from the berries helped the urine to remain acidic, which could explain why they can be used to treat and prevent infections. Habash *et al.* [74] have reported that some of the components, together with ascorbic acid, inhibit the adhesión of uropathogens to silicone tubes. Later, the proanthocyanidines, which are condensed tannins, have been identified as a characteristic molecular structure responsible of the bioactivity of the juice [73, 75]. The comparison between sweet cranberry juices, unsweetened raisins [75], or even with *L. rhamnosus* GG [76] to prevent UTI has been performed, without definitive results. The scientific evidence indicates that there is not enough controlled clinical studies to determine the effectivity of the cranberry juice on the prevention of UTI, suggesting the type of clinic assays that should be performed to assign a preventive or curative effect proclaimed for this type of medicines [77]. There is a general consensus of further research to demonstrate the beneficial effect and also the dose-response and pharmacokinetics studies of the active compounds.

Referred to the vaginal tract, there are also some papers published on the treatment of BV in pregnant women by the local administration of yogurt [78], and in non-pregnant [79], but without definitive conclusions. Some other authors report the use of different alternative medicines, as single peroxide vaginal douching [80, 81] or lactic acid [82] combined with metronidazole, or the tea tree oil and garlic [83]. But these types of alternatives or "at the counter" medicines for the treatment of vaginal symptoms increase the costs of health cares without producing any benefit, as discussed by Nyrjesy [84] who indicates the amount of money that patients with vaginal symptoms waste when buying these products. Again, there is evidence of the lack of alternative preventive or therapeutic medicines for the urogenital tract infections at the present.

5. BENEFITS OF PROBIOTIC BACTERIA ON THE UROGENITAL TRACT. MECHANISMS INVOLVED AND APPLICATIONS

One of the main objectives of the probiotic administration is the maintenance of the ecological equilibrium of this particular complex niche composed by epithelial cells in constant desquamation, layers of mucoproteins, other gland secretions, secreted A immunoglobulin (IgA) and a complete array of diverse microorganisms. In this way, the probiotic bacteria can participate or promote the formation of a biofilm, produce antagonistic substances, stimulate the immune system and protect in this way to the infectious agents.

The mechanisms by which the probiotic bacteria can exert their role on the urogenital microbiota, and thus the desired effect in the urogenital tract has been suggested and showed in some "*in vitro*" experimental models, not being demonstrated yet if all of them are playing a determinant role in the tract, or if they are the result, combination or addition of the suggested effects. Some of them are directly related to the characteristics of the bacterial surface, other to the metabolic ways or enzymes present, but others depend on the interaction of microorganisms with other cells, mainly eukaryotic cells of the host. Then, the actual mechanism of action of probiotics in the urogenital tract is multifactorial.

For understanding purposes, the suggested mechanisms can be divided according to the main effect produced, even though we do not yet which of them are really acting in "*in vivo*" situations.

5a. Adhesion to Epithelial Cells, Colonization Capabilities

The bacterial adherence to the mucosal surfaces is considered a fundamental prerequisite for the pathogenic colonization and subsequent infection. Many studies suggest that the different composition of the mucosal surfaces affect bacterial adhesion. The indigenous and pathogenic bacteria are able to adhere to the epithelial host cells [85]. This adhesion to the vaginal mucosa is an essential factor for the colonization of the microbiota, being assumed that this first union is a critical parameter for the colonization of the mucosal surfaces by microorganisms. Two mechanisms are involved in the adhesion process of micro-organisms [85] a non specific adhesion based on physico chemicals properties which originates different interaction between bacteria and mucosa, and specific adhesion involving external structures from bacteria (adhesins) to specific receptor on the epithelium. The contribution of these adhesins to the pathogens colonization in the host has been deeply studied [43, 52]. However, their role in the colonization of microorganisms that are part of the normal, indigenous microbiota is not yet understood, neither the mechanisms involved into the adhesion between epithelial cells and vaginal lactobacillus, which are the structures involved in the adhesión of the indigenous microorganisms? A wide variety of surface adhesins [87, 88, 89] that can be proteins, lipoteichoic acids, and polysaccharides are involved. The hydrophobicity of the cell surface can be used as a predictor of the adherence [90].The receptor site for adhesion is located on the epithelial cells surface (glycolipids) or on the

mucus [91]. But there are not definitive results through "*in vivo*" experimental models to demonstrate the definitive relationship between the external structures of the indigenous microbiota and their adhesion to mucosa and colonization capability

There are evidences of the type of structures present on the surface of lactobacilli related to their adhesion capabilities. Lactobacilli assayed through transmission electronic microscopy have shown to possess some structures similar to flagella (one or two) located at polar or subpolar position appearing when microorganisms are grown either in liquid or solid media [92]. The fimbria production was maximal when lactobacilli were grown in solid media and anaerobic environment, and the fimbriation increases significantly the "*in vitro*" adhesión of lactobacilli to epithelial cell. On the other side, the S-layer described in some members of the indigenous microbiota has been assigned as one of the proteic nature structure responsible of the adhesion of microorganisms to mucus or epithelial cells [93, 94]. Lactobacilli also adhere to a variety of epithelial cell by "*in vitro*" assays [95]. A bacterial component, proteic in nature, mediated the adhesion of *L. acidophilus* [96], *L. fermentum* [95, 97] or *Lactobacillus salivarius* [89, 90] to epithelial cells, while other components as lipoteichoic acid can mediate the adhesion in *L. johnsonii* [98]. Synergies between lactobacillus strains is also participating in their adhesion capacity to the mucosa, as for example *L. casei* enhanced the adhesion of *L. gasseri in vitro* on intestinal epithelial cells [99].

The indigenous microbiota can adhere to the cell surface, to the mucus layer or to the other components of the mucosal epithelium. The extracellular matrix of the epithelium is composed by many secreted proteins, mainly collagen, fibronectin, laminin and proteoglycanes located on the surfaces of the endothelial or epithelial cells, which have been shown to mediate the bacterial adherence to host cells. Some strains are able to adhere to collagen, one of the proteic components of the extracellular matrix, mainly present in the connective tissue [100, 101]. Fibronectin is present on the extracellular matrix and in the body fluid as vaginal fluid. It modulates interactions between extracellular matrix and cells by the formation of integrins, but also plays a role in the adhesion of microflora to mucosa [102]. Fibronectin enhances the establishment of normal microbiota, and some lactobacilli isolated from vagina from healthy women are able to adhere specifically to fibronectin. This adhesion is stronger when pH decreases to 4 corresponding to vaginal conditions [103], and the union to fibronectin is related with the adherence to epithelial cells [104]. These results support the hypothesis that the indigenous microflora is able to bond to the Extracellular Matrix Proteins which could contribute to the initial establishment of the ecological niche and then to the competitive exclusion of pathogenic microorganisms playing thus an important role in the decrease of BV [103]. The involvement of other structures, as the S-layer of *L. crispatus* [105] to collagen, or *L. fermentum* biosurfactants to collagen-binding proteins that inhibit pathogen adhesion has been stated as responsible of the adhesion of microorganisms [101, 105].

The mucous layer covering the epithelial cells of the mucous membrane could be also the initial step for the contact between the bacterial cell and the host. The adhesion of microorganisms, viable or inactivated, to mucous glycoproteins extracted from faeces of infants and adults, and glycoproteins from the small intestine isolated from ileostomic effluent [106, 107, 108] has been demonstrated, or either from the vaginal mucus [89, 90].

It can be concluded that further research is needed to demonstrate the definitive relationship between the adherence capability of different probiotic strains and the colonization to the host. It has been suggested that some cell-signaling molecules are produced by *Lactobacillus*, but they were not demonstrated at the present [50].

5b. Biofilm Formation

Once the bacteria are adhered to the mucus layer or epithelial cell surface, they participate in the formation of a protective biofilm, which has properties different to the planktonic bacteria. This biofilm formation was first described for microorganisms of the oral cavity [109] being later indicated in the urogenital tract [110].The colonization of microorganisms is then promoted, which means the maintenance of a constant population. The aggregation or co aggregation properties will help to the maintenance of stable populations, and also promote the bacterial colonization, helping that constant numbers of bacteria are present. In this way, they can participate in the competence for the specific adherence sites or by steric blockage (competitive exclusion), or either by using the available nutrients, thus avoiding the income, growth or multiplication of the pathogenic microorganisms [111, 112]. When incorporated into the biofilm, they have a different sensibility to antimicrobial substances, characteristics that must be tested in the probiotic strains before their clinical use [109].

5c. Production of Biosurfactants

Biosurfactants are detergent molecules produced by microorganisms [113]. As they interfere on surfaces tension, several physiological roles are attributed to biosurfactants [114], for example the participation of biosurfactant-producing bacteria in the adhesion as a competitive barrier to pathogens [115]. The production of biosurfactants has been described in the genus *Lactobacillus*, a particular surlactine in *L. acidophilus* and *L. fermentum* strains [116]. Surlactine molecule inhibits the adhesion of *E. faecalis*, *E. coli*, *C. albicans* and almost all the pathogens responsible for urogenital infections. Proteins included into the surfactants molecules produced by *L. fermentum* RC14 could have a signalization function, because they are secreted and disgregated at low pH. They are also able to spread in the cells acting then in the pathogen inhibition [97]. There are also some evidences of the signaling proteins in lactobacilli, including a peptidic pheromone of 19 residues secreted, co-transcript with genes that codify histidine kinase and a response regulator that induce the bacteriocin production [50]. The gene of the surface S-layer of *L. brevis* has been described together to the expression signals [117]. The information available at the moment suggests that the production of biosurfactants can play an important role in the

biofilm formation by probiotic bacteria, and then in the exclusion of pathogens in the urogenital tract.

5d. Aggregation and Co-Aggregation

Aggregation is the interaction between two microorganisms of the same strain, while co-aggregation is the interaction between two microorganisms of different strains or species. Both are considered fundamental in several ecological systems [118]. In the vaginal environment, the first process can promote the biofilm formation or the colonization of beneficial microorganisms, as suggested for lactobacilli in the gastrointestinal tract [119]. The coaggregation can happen between lactobacillus and pathogens, enabling the pathogens to bind to the vaginal mucosa [120]. Both phenomena are specific to certain strains, being described the aggregation in micro-organisms isolated from different ecological niches, such as the oral cavity [121], the human [87, 120] or bovine [122] urogenital tract, the gastrointestinal tract of chickens [123], pigs [124] and humans [125]. The aggregation was also demonstrated in strains isolated from the vaginal environments [111, 112], and their coaggregation with some members of the indigenous microbiota as *C.albicans, E. coli and G. vaginalis* [126]. This characteristic is genetically determined, because the genes responsible of the synthesis of an auto-aggregating factor of 32 kDa of *L. gasseri* 4B2 [126] are expressed more remarkably in the exponential growth phase rather than in the stationary phase, and a specific factor promoting aggregation (FPA) has been detected in certain vaginal lactobacillus strains, as in *L. gasseri* or *L. coryniformis* co aggregating *E. coli* and *Campylobacter jejuni*. [127].This characteristis is transmissible between microorganisms, because the aggregation-promoting factor of *L. gasseri* 4B2 was able to mediate a high frequency of conjugation between *Lactobacillus* strains that could allow the acquisition of new phenotypic characteristics [128] and have an essential function in the maintenance of the cellular shape [118, 128]. Many factors, included the environmental conditions, the cellular functions and activities influenced by regulator systems operating under high-cell density conditions, as the quorum sensing signals, are included between those that affect both, aggregation and co aggregation [129, 130].

The autoaggregating characteristic determined through "*in vitro*" assays, has also been assayed in experimental models of the gastrointestinal tract, as the aggregation of *L. crispatus* and its relationship with the adhesion and colonization in cultured cells [119]. In a mice animal model, the protection against Dextran Sodium sulfate induced colitis of an auto aggregating *L. crispatus* (in a dose-dependent way), strain adherent to human mucus, was demonstrated, while the non-aggregating mutant does not produce such effect [131]. But there is not yet any evidence of the relationship between these characteristics and the protection against infections in the urogenital tract.

5e. Production of Antagonistic Substances

The probiotic bacteria must exhibit some beneficial properties derived from their metabolic capabilities, which are directly related with their genetic composition, and with the enzymatic pathways that drive their behavior. Some of the antagonistic substances produced by the members of the genus Lactobacillus that have significance in the urogenital tract are the following:

-Organic acids: glycogen is an important source of carbon in the vaginal environment. It is secreted at the surface of the epithelium and its level varies with the hormonal cycle. Lactic acid bacteria produce the fermentation of glycogen or glucose to predominantly lactic [35, 36]. This lactic acid production has been historically one of the main characteristic of the lactic acid and related microorganisms (*Lactobacilli, Bifidobacterium. Streptococci*), being inhibitory to a wide spectrum of pathogenic microorganisms. Lactobacilli have been related to the maintenance of the low pH of the vaginal tract, (pH 4.5 or lower), pH that is modified when the ecological balance is disrupted by the increase in numbers of other microorganisms, as in the case of Bacterial Vaginosis. There was some type of discussion on the responsible of the isomeric lactic acid present in the vaginal fluid, either D, L, or a mixture of them [35, 36, 132].

The probiotic bacteria must be able to produce very high levels of lactic acid, inhibiting then the survival of pathogenic microorganisms [132, 133]. There are other short-chain organic acids, such as acetic, propionic or formic acid which are produced by heterolactic microorganisms, also inhibitory to pathogens.

-Hydrogen peroxide: this is one of the most desirables metabolic properties of the vaginal probiotic bacteria, because many researches have shown that the lactobacilli present in the healthy vaginal tract are those able to produce H_20_2, while in a unbalanced or other pathological situations, the lactobacilli present are non-producing H_20_2 [31, 55, 134]. *Lactobacillus* producing hydrogen peroxyde (H_2O_2) plays an important role in the vaginal flora balance. 96% of healthy women present these lactobacilli in their flora (*L.crispatus and L.jensenii*) as these lactobacilli can be isolated in only 3.5 % of women having bacterial vaginosis [134]. This characteristic is not related with any bacterial specie, genus or metabolic group. It is genetically determined, present only in some particular strains, because this metabolite is not produced by all the vaginal isolated strains [134, 136, 137]. H_2O_2 and its metabolites ($OH^{\cdot}$, O_2^{-}) are toxic to the cells by their action on nucleic acid, proteins, leading to cellular death. The H_20_2 can react with mieloperoxidase to form reactive molecules toxic to human immunodeficiency Virus (HIV) [138, 139]. The H_2O_2 produced by *Lactobacillus i*nhibits the growth of *G. vaginalis*, *E. coli* and *S. aureus*. It is also likely that *Atopobium vaginae*, anaerobic bacteria involved in BV is sensitive to H_2O_2. Furthermore, it has been shown that a high concentration of lactobacillus producing H_2O_2 with peroxydase and halogenure present in the vaginal fluid inhibits HIV *in vitro* [140]. This finding relates to the higher virus load found in women with *Mycoplasma hominis* induced vaginosis that is inversely correlated in women displaying normal microflora [140]. Redondo Lopez *et al.* [31] reported how is possible the inhibition of pathogenic microorganisns by adding mieloperoxidase and one halure in an "*in vitro*" experimental model. The addition of other biological mediators, as the mieloperoxidase enzyme (MPO) present in neutrophyles and monocytes, is

able to react with the hemme and clorure to destroy mycroorganisms after phagocytosis. It has been suggested that the MPO instillation in vagina could increase the vaginal defenses against STD because the vaginal microbiota is characterized by H_2O_2 producing lactobacilli, but the studies performed in adult macaque model evidenced that the endogenous basal MPO [141] is variable and does not reduce the number of H_2O_2 producer bacteria, neither produce vaginal or cervical colposcopic changes

Even though this is one of the most desirable properties of the vaginal probiotics derived from epidemiological studies, it has not been yet demonstrated the presence of H_2O_2 in *in vivo* models or patients, probably because the available methods do not allow the detection of the small amounts of peroxide produced. In the urogenital tract all the ions and metabolic products needed to this pathway to work are present, and then is one of the bacterial traits with higher possibility to be suitable and actives at the tract [25, 32].

-Bacteriocin production. Bacteriocins are proteins synthetized by bacteria that present narrow spectrum microbicidal properties [142]. Molecules assimilated to bacteriocins ("bacteriocin-like") are a little bit different from bacteriocins as they present a large spectrum of microbicidal activities. Bacteriocins bind to specific receptor on target cells and form pores into the cytoplasmic membranes, disrupting the cell membrane transport. The production of bacteriocins is other of the suggested probiotic mechanisms acting at the urogenital level. Our research group have isolated one *L. salivarius* strain, able to produce a bacteriocin-like substance active against a wide variety of urogenital pathogens, tested by "*in vitro*" assays, as *Gardnerella vaginalis*, *Enterococus faecalis*, *Neisseria gonorrea* [143]. We have demonstrated also the optimal conditions for growth and bacteriocin production [144, 145]. Some lactobacilli isolated from vaginal sphere have shown an inhibitor activity on most of pathogens encountered during vaginal infection [146]. Even though this trait is a very desirable characteristic applied mainly in the area of food production to avoid the pathogen growth in the elaboration and storage stages, it was not demonstrated yet to be active in "*in vivo*" situations.

5f. Competition of Nutrients

This concept is related in some way with those microorganisms able to form and survive into the biofilm, because the nutrients available in these types of films are different from those available for free or planktonic cells [121]. Into this particular ecosystem, the competence for the available nutrients in the medium provides a selective advantage to those microorganisms that can use them in a first moment, and selectively, to their own growth and survival. This type of effect has been described and demonstrated in experimental models resembling the intestinal tract, allowing the growth and survival of some groups of microorganisms, and limiting or inhibit the other [147, 148], but not in the urogenital tract.

5g. Enzymes Production

Lactobacilli can inhibit the growth and proliferation of pathogenic bacteria by metabolizing the arginine in citrulline, thus depriving the medium from arginine, which is the required molecule to produce polyamines by pathogens. This metabolic pathway is performed by the Arginine desaminase enzyme described in the genus lactobacillus. The main effect of these enzymes has the principle of application in the BV, characterized by the replacement of lactobacilli by *Gardnerella vaginalis* and other anaerobic bacteria responsible of the full odor. This odor is due to the production of polyamines and trimethylamines [149]. The polyamines (spermine, spermidine, putrescine and cadaverine) are responsible for the negative effect on the host, by promoting the exfoliation of the epithelial cells from the vagina leading to the mucosa rupture [150]. A study including several women treated with *L. brevis,* which has a strong arginine-desaminase activity has shown decrease of the vaginal polyamines concentration associated to the return of the vaginal microflora [151], suggesting thus the role that the probiotic bacteria could have in the prevention of this type of pathology.

5h. Immune System Stimulation

Another mechanisms involved in the probiotic action is directly related to the capability to stimulate the local immune system, and then to protect from infections. The immune system represents an important component of the reproductive tract [152], influencing many of the biological functions of the host. The mucosal epithelium of the reproductive tract provides the specialized organ with the first barrier of defense and is equipped to respond to pathogens, whilst sustaining commensal microorganisms.

The urogenital mucosal surface together with other mucosal surfaces is a wide area of interaction of antigens with the immune system. Mucosal surfaces are places for antigenic exclusion, antigenic sampling and immune regulation [153]. In these mucosal surfaces interact those microorganisms that are part of the indigenous microbiota and also all the antigens that enter the host through the tracts. The vaginal immune system is part of the common mucosal immune system, developed and connected with the gastrointestinal, respiratory and glandular systems [154]. In this context, even though there are not present immune organs or immune system organized in lymphoid folicles, or similar structures, the vaginal tract is populated with cells that are part of this immune system. Different subsets of lymphocytes: T, B, NK (Natural Killer), K (Killer), fibroblasts, Antigen-Presenting Cells (APC), Macrophages and dendritic cells, are present. Cytoquines or lymphoquines interacting between them and with the different cells in a very complex network are also present, not very deeply studied until now in the vaginal tract, presenting important differences with other mucosal membranes [155].

The reproductive tract is an inductive site for the immune response. Ogra and Ogra [156] demonstrated that antigen placed in the uterus and vagina of women resulted in specific antibodies in uterine and cervicovaginal secretions. The T vaginal cells are phenotipically different to the peripherical T cells, because TCD4+ cells from vagina and those derived systematically can be differentiated at their protein and molecular levels, supporting the concept of immunological independence or mucosal compartamentalization [157].

In addition to the cells of the immune system, the mucosal epithelium of the female reproductive tract may actively participate in the control of pathogens, but the precise mechanism is poorly understood. The uroepithelial cells are part of a mucosal network [158]. The bacterial infections stimulate the cytokines production in vaginal mucosa. The cytokine response of the epithelial cells as a consequence of their interaction with bacteria has been examined in various models [159], where it has been demonstrated that the resident bacteria can activate the primary response of cytokines at mucosal sites, stimulating the epithelial and non-epithelial cells and induce the migration of circulating cells to the mucosal site of infection. The combination of the primary and secondary responses from the local and recluted cells establish a "local environment of cytokines". The type of cytokines present on the mucosal determines which are the cells to be activated [157], being also modified by the pregnant state [160, 161, 162, 163].

The role of the Toll-like receptors in the reproductive tract, as key components of the immune innate system which recognize conserved sequences on the surface of microorganisms and trigger effector cell functions has been recently reviewed [164]. The expression of these Toll-like receptors in the epithelial cells of female reproductive tissues has been demonstrated, which suggests their role in the innate immune response in the tract. Some of the structures that stimulate the immune system are located on the external surface of the probiotic bacteria, and some others come from the interaction of the bacteria with the immune cells that leads to the production of immune mediators.

Some other cells has been evidenced in the murine vaginal tract, as Langerhams cells –with Antigen Presenting function previously demonstrated in the epidermis [165]- in the stratified epithelium of murine vagina and cervix. The ultrastructural details of the process showed that these APC are involved in the phagocitosis of death cells. They phagocitate epithelial apoptotic cells and contribute thus to the epithelial vaginal change during the estral cycle. The antigen presentation by vaginal cells as well as their influence on the B and T cell proliferation is regulated also by estradiol [163].

Some of the evidences of the effect of certain lactobacillus strains on the immune system of the vaginal tract are the following: *L. gasseri* secretes a molecule called gasserokine that activate CD_{18} on macrophages that trigger them to migrate. It is well know that migration of macrophages to the infection site is a necessary step for the elimination of the pathogen [166]. *L. gasseri* also induced the production of interferon-alpha by murine macrophages in 3 to 6 hours [167]. This property has also been described for *L. acidophilus* [168]. Our research group has demonstrated the different strains of lactobacilli stimulates at variable levels the IgA and IgM producing cells on the mucosal vaginal of mice intravaginally inoculated with different strains [169]. This mucosal immune system is under the hormonal influence [160, 161, 162, 163] and the APC are also under this effect [163].

Again, the majority of the scientific evidences of the effect of lactic acid bacteria on the immune system are related to the gastrointestinal tract mainly related to the consumption of foods fermented with these microorganisms, or to the bacteria alone at different doses and duration of the treatment. The relationship between the vaginal mucosal system and other mucosal sites is being studied lately, mainly the vagina as a target organ to deliver vaccines for other mucosal sites. The effectivity of the intranasal immunization referred to the urogenital protection, or the intestinal tract as a delivery system for urogenital probiotics is also being studied [170].

6. TECHNOLOGICAL PROPERTIES

When trying to design a pharmaceutical product, another set of characteristics of the probiotic product or strains must be considered, which will be briefly cited in the present review:

6a. General Characteristics

Origin, Safety, Identification of the strains. These characteristics are related to the origin of the strain, the organ or mucosa in which will be applied, and also the safety characteristics. The probiotic strains must be included into the GRAS (Generally Regarded as Safe) microorganisms, characteristic that comprises the genera *Lactobacillus*, *Pediococci*, *Bifidobacteria* and some *Streptococcus* strains. Historically data indicates that they are safe for human use, based on their occurrence as normal commensals of the human microbiota, and their safe use in different foods and products worldwide. The FAO and WHO guidelines recommended that probiotic strains be characterized at a series of tests [2, 5].

Based in the host specificity of the indigenous microflora described by Savage [171], the strains must be isolated from the same host where will be applied, and also from the same ecological niche to favor the survival of the inoculated microorganisms. Another important consideration is to identify and characterize the strains to the genus and species level with internationally accepted methods, as suggested by FAO and WHO guidelines [2, 5], and also to have a clear strain designation to track the publications on the probiotic benefits.

6b. Resistance to Enviromental Conditions

The probiotic strains when administered to the host, must resist the conditions of the tract where will be inoculated, and to the therapies frecuently applied. Referred to the urogenital tract, the strains must resist the low pH of the vaginal tract, and the enzymes present normally in the tract [36, 37, 42]. They must survive to the antibiotics, hormones therapies, spermicides and other substances regularly applied in the tract, to be able to maintain the level of protective microorganisms. Another important test to be performed is their resistance to bacteriophages, based on the "Sexual Transmitted Diseases Theory" of this extrachromosal elements, or "Bacterial phaginosis Theory" supported by the Tao´s group results, who showed the lysis os lactobacilli by phages transmitted sexually, present in seminal and vaginal fluids [47, 48]. All of the characteristic tested through "*in vitro*" assays, should be later studied in an animal experimental model, or through clinical studies to prove their

survival and maintenance in the tract to obtain the proclaimed effect on the host [2, 5].

6c. Resistance to Technological Conditions

An important condition to be assayed is if the probiotic microorganisms can be scaled up to obtain enough biomass and number of viable microorganisms to be included into the final product. This study can be performed through he application of mathematical or statistical models, which help in the prediction of the behavior of the microorganisms [172]. Another group of characteristics to be tested is if the microorganisms can survive to the excipients and storage at different conditions [173, 174, 175], based in the fact that the administered microorganisms must remain viable and in enough numbers to colonize the epithelia, or to produce the desire probiotic effects [76].

The detailed production of tablets or capsules for the delivery of lactobacilli in the vaginal tract is not available in the published literature, because most of them are protected by patent processes or under the "confidential clausules" of the manufacturers or pharmaceutical companies [177, 178, 179]. There is a tendency to include bioadhesive molecules without the production of adverse effects on the host. Maggi *et al.*. included 10 different lactobacillus strains in singles or doubles vaginal tablets. The double tablet was characterized by different properties in each one of the layers: one with effervescents characteristics to allow a fast and complete distribution of one active ingredient on the complete vaginal surface, while the second layer has a sustained composition able to release the microorganisms for a longer period of time [180, 181]. The intravaginal dosage based in bioadhesive polymers (polycarbophil, hydroxipropil methyl cellulose and sodium SALT of hyaluronic acid) included in pessaries made of semisynthetic solid triglycerides was also reported [182].

The live microorganisms can be freeze dried, or spray dried, and later included into the designed product, where a combination of different type of substances can be tested, from sugars, cryoprotectant, antioxidants, vitamins, or bacteriocin or antibiotic substances [173, 175].

6d. Maintenance of the Probiotical Characteristics

Once lyophilized, or incorporated into the excipients/ pharmaceutical product, the microorganisms must maintain the probiotic characteristics by which was selected, as for example the production of lactic acid [133], bacteriocin [143], hydrogen peroxide [135,136], or the properties of adhesion or autoaggregation [112, 173].

7. *IN VIVO* STUDIES IN EXPERIMENTAL MODELS. ANIMAL MODELS

Some of the mechanisms described previously have been demonstrated in experiments performed "*in vitro*" [183]. But this characterization is insufficient to define a probiotic organism. The expression of the probiotic properties "*in vivo*" and the safety characteristics are needed to predict their behavior in the human body. To determine which are the mechanisms acting "*in vivo*", a mice experimental model to study the colonization capability of microorganisms was set up at the laboratory [184]. The indigenous lactobacilli were able to colonize in a more effective way the urinary tract when included in agarose beads [185]. The protective effect against uropathogenic *E. coli*, and *Klebsiella pneumoniae* when lactobacilli were intraurethrally inoculated was also demonstrated [186]. The effect of estrogens and antibiotics was more effective in the protection against the pathogen [187, 188] when mice were previously inoculated with lactobacilli. The administration of lactobacilli either in the urinary or vaginal tract does not produce any adverse effect on the mice, nor structural or ultrastructural modifications [189]. Recently, the protective effect of lactobacillus against *Proteus mirabilis* ascending urinary tract infection in the same experimental model was published [190].

Which are the mechanisms involved in the protective effect? There is an increase of the level of anti-pathogen antibodies in sera and in urine when lactobacillus was intraurethrally administered [186] and an increase in the number of IgA and Ig M producing cells at the vaginal mucosal level when intravaginally inoculated. The protection was obtained not only with viable microorganisms, and in a lower amount with cells elaborated with heat-killed or sonnicated lactobacillus (non published results), indicating a combination of steric blockage and competitive exclusion.

All of these results indicate that even thoug there are many suggested mechanisms derived from "*in vitro*" experiments, when studyng an animal experimental model, there is a combination or addition of many of them: stimulation of the immune system, colonization capability, competitive exclusion and steric blockage. We still have to demonstrate if the aggregation and production of antagonistic substances are mechanisms that are functioning in "*in vivo*" situations, and which are the other mechanisms that could be involved in the probiotic effect.

8. ASSAYS IN HUMAN BEINGS

Probiotic products containing Lactobacilli have been proposed for the treatment of bacterial infections as was cited previously. However, commercially available products sold as dietary supplements do not always contain the Lactobacillus species advertised on the label and do not always contain H_20_2-producing lactobacilli [4], or microorganisms with other beneficial properties [21]. This fact has originates some discussions lately.

The number of studies performed in patients for recolonization of the urogenital tract is very low. Some of them were included into Phase 2 Clinical studies that assess the efficacy of a product against a placebo. There is not yet a Phase 3 Clinical study at the vaginal tract, which regularly would asses the effectivity of a product in comparison with a standard therapy. Some of the human studies published in the urogenital area are the following:

Oral administration o*f L. rhamnosus* GR1 and *L fermentum* RC 14 once or twice daily for 28 days has been correlated with healthy vaginal flora [191, 192]. Also, the local administration of capsules prevents from Urinary tract infections [193]. *L. rhamnosus* GR-1 reduces the incidence of UTI in women from six episodes per year to 1.6 per patient. These results are supported by the duplication of the number of lactobacilli in vagina referred to the beginning of the treatment, being administered capsules containing 10^7 CFU intravaginally to reconstitute the vaginal flora [194].

Evidence from a 64-patient randomized, placebo controlled trial indicates that daily oral intake of 10^9 to 10^{10} *L. rhamnosus* GR-1 and *L. fermentum* RC-14 leads to transfer of the organisms from the rectum to the vagina, as well as a depletion of coliforms and yeast in the vagina [195]. These strains were able to recolonize the vaginal tract of the consumer women. The authors applied genetic methodology to identify the administered microorganisms, and determined that GR-1 is present in the tract after 14 days following intravaginal administration, while *L. rhamnosus* GG disappeared five days after administration [195]. This strain causes temporary infantile colonization after the oral administration to their pregnant mother [196].This group of Canadian researchers have shown that the cited strains are able to colonize the vagina following vaginal suppository use [193] and reduce the risk of urinary tract infections, yeast vaginitis and bacterial vaginosis [192]. Recent studies have shown that daily ingestion of capsules containing *L. rhamnosus* GR-1 and *L. fermentum* RC-14 by 19 women with BV microbiota, resulted in a normal microbiota in 81% of cases, compared with 50% in women given placebo [191]. Hillier *et al.* reported the results of a pilot study where the intravaginal administration of three days gelatine capsules containing *L. crispatus* CTV-05, a H_20_2 producer strain, was detected in seven of nine women while there were not many microorganisms recovered in a Macaca model [197]. Results published on the web site indicate that this strain given intravaginally after metranidazole treatment for BV resulted in a clinical cure at 30 days in subjects colonized by lactobacilli (70%) compared to noncolonized (47%) patients receiving placebo [198].

The prevention of BV is significantly important in women at risk of some infections, as for example HIV, because the women population with BV shows an increased risk of HIV, as evidenced by some publications [29, 60, 199, 200, 201]. There is not still evidence of what could be the mechanisms by which lactobacilli could reduce the risk of HIV infection.

Referred to the prevention of urinary tract infection Reid el at, administered once weekly a vaginal suppository containing 10^8 *L. rhamnosus* GR1 and *L. fermentum* B-54 for one year. They compared the rate of UTI occurrence with that in the previous year in 25 women [194]. There was a significant reduction in UTI during lactobacillus use (from 6 to 1.6 episodes per year)

In 1995 Parent *et al.* [202] reported on the treatment of BV by using Lactobacilli and low doses of estriol. The product is called Gynoflor, containing 50 mf of lyophilized *L. acidophilus* (H_20_2 producer) and 0.03 mg estriol. This strain recolonizes vagina and increases the number of lactobacillus in vaginal swab of patients. Recently, another paper reported the effectivity of this product in turkey women [203] for the restoration of the vaginal flora after treatment of bacterial infections.

There is other product in the European market, Fermalac, containing *L. rhamnosus* and other strains (Rosell, Montreal Canada) which is also being studied in human population to determine the effectivity [204], but there have not peer-revised studies proving the eradication of BV.There are some other publications on the administration of lactobacilli in the vaginal tract, without definitive conclusions on their effectivity [205, 206, 207, 208].

9. FUTURE DIRECTIONS.

9a. Lactobacillus as Vaccine Vectors

The more recent applications of lactic acid bacteria are in the area of vaccine vectors, in which there were significant advances, mainly in the gastrointestinal tract [170, 209]. Even though the urogenital is not the optimal tract to produce an "in situ" stimulation of the immune system, some studies performed in experimental animal models showed the effectivity of the rectal or intranasal immunization to obtain antibodies in the vaginal secretion. Based mainly on the GRAS nature of LAB, and the advances in the area of molecular biology for the insertion of genes or groups of genes, codifying for antigens to be expressed in the cellular surface, maintained inserted at the cellular wall, or expressed intracellular to be released after the lysis of the micro-organisms. This is a potential area of wide application for vaccines that will act against the high spectrum of STD [210].

9.b. Anti Tumoral Effect of *Lactobacillus* in the Urogenital Tract

The firsts papers on the capability of lactobacilli to inhibit tumors in the urogenital tract were published very early in the last century reporting the antitumoral effect of lactobacillus instilled locally on the bladder tumours [211]. There is a more recent paper related to this subject that shows the chemopreventive effect of the oral administration of 10^8 CFU of *L. rhamnosus* GG orally during seven days on the growth of a bladder culture line implanted subcutaneously in mice. This paper showed that the consumption of the microorganisms increased the number of T /CD3, CD4 and CD8a lymphocytes and NK cells from spleen, suggesting a modulation of the immune system in the control of the tumour growth. Brandau *et al.* [213] reported the antitumoral effect of intravesical instillation of heat-killed cells of *Lactobacillus casei* strain shirota on the murine bladder tumor. More studies must be performed to really assign the anti-tumoral effect of lactobacillus in the urogenital tract.

9c. Effect on Group B Streptococcus Infections

Neonatal group B streptococcal infections are one of the main health problems because of the high mortality and morbility rates in certain countries. Some approaches include the perinatal antibiotic therapy or vaccination with bacterial polysaccharides. The inhibition produced by *Lactobacillus* on *group B streptococci*, is opening a new possibility of the use of this genus in the prevention of such neonatal infections [214].

9d. Production of Peptides, Vitamins, Antioxidants, Complementation with Prebioticos

There are others metabolites produced by lactic acid bacteria, that could be studied for their beneficial characteristics, as prebiotics [215], vitamins [216], antioxidants [217], or even terpenoids-based substances for the tissue regeneration, that are being applied in some other tracts or mucosaes. Recently, the comparison between cranberry juice

and unsweetened raisins for the prevention of UTI has been published, what indicates the importance that the consumption on foods is beginning to have in the prevention of infections, or either to maintain a health status, with high life quality parameters [75]. Further studied should be performed to assign the correct beneficial effect of such substances in animals and later in human trials.

9e. Looking at the Future. Perspectives

Even thoug the GRAS nature of functional foods and probiotics allows their administration to the human or animal hosts, and considering that the regulatory issues sometimes are very general, some specific considerations must be taken referred to the unknown aspects or those that needs further research, as for example:

a. What is the effect of the long-term administration of probiotics, either on the tract under study or on the other areas of the host?

b. Do they produce some adverse or collateral effect, or structural modification in the target organs?

c. Can they really be applied in a preventive way? How effective are they? More clinical assays, double blind, randomized studies are needed for the evaluation of this characteristic.

d. Which are the clinic groups that can use them?

e. Do they have a therapeutic effect on groups of patients with certain pathologies, or on defined clinical cases? More clinical studies and population studies are needed.

f. Which is the effect on the high quality life parameters? Is worth to test them?

g. These are some of the questions that remain without defined answers, encouraging the scientists working on the area to go deeper on these subjects.

CONCLUSIONS

The present status of probiotics in clinical practices and the evidence of their effects still remain to be fairly proved. They are not a main clinical practice in many countries of the world. The analysis of the probiotic strains shows that very few are currently available at the market, either pharmaceutical or food related. Many strains do not fullfile the FAO and WHO criteria for probiotics, even though there is a lot of non-traditional, complementary or alternative medicines within the probiotic products containing lactobacilli, bifidobacteria and streptococci are included.

Several factors are leading to physicians to examine probiotics and alternative medicines to standard pharmaceutical drugs. These include the increasing levels of multidrug resistance of pathogenic microorganisms, the demands of consumer for natural products, and the instauration of preventive politics around the world.

A lot of researchers are working in the probiotic area, mainly in the gastrointestinal tract, trying to demonstrate the efficacy of the administration of probiotic products, and to understand which are the mechanisms underlying such beneficial effect on the host. But, there is not so much information available at the urogenital tract, where there is still a lot more to demonstrate.

The emergence of scientific and clinical evidence showing the efficacy and effectiveness of some specific probiotic strains, together with the FAO and WHO guidelines, will help to the availability of reliable and clinically proven probiotic during the next years.

ACKNOWLEDGEMENTS

The experimental results reported in the present review were supported by grants from CONICET (National Council of Scientific and Technological Researchs) from Argentina and from Becas Carrillo Oñativia (Ministery of Public Health) from Argentina.

The described results derived from the PhD thesis of Clara Silva de Ruiz, Virgina Ocaña and Silvina Juarez Tomas, that were developed at the National University of Tucumán.

Some CRL vaginal *Lactobacillus* strains were licensed to Anidral, an Italian company from the Mofin-Alce group.

REFERENCES

[1] Metcnikoff E. The prolongation of life. Optimistic studies. Heinemann, London, 1906; 151-83.

[2] Food and Agriculture Organization of the United Nations and World Health Organization. FAO/WHO. Guidelines for the evaluation of probiotics in Foods. Evaluation of health and nutritional properties of powder milk and live lactic acid bacteria. FAO of the United Nations and WHO Expert Consultation Report. 2001. *www.fao.org/es/ESN/Probio/probio.htm* (online)

[3] Reid G, Sanders ME, Gaskins R, *et al*. New Scientific paradigms for probiotics and prebiotics. J of Clin Gastroent 2003; 37: 108-18.

[4] Hughes VL, Hillier SL. Microbiological characteristics of Lactobacillus products used for colonization of the vagina. Obstet Gynecol 1990; 75: 244-8.

[5] Food and Agriculture Organization of the United Nations and World Health Organization. Regulatory and clinical aspects of dairy probiotics. FAO/WHO Expert Consultation Repor 2002. (online)

[6] Dunne C, O´Mahony L, Murphy L, *et al*. *In vitro* selection criteria for probiotic bacteria of human origin: correlation with *in vivo* finding. Am J Clin Nutr Sup 2001; 386S-392S.

[7] de Roos NM, Katan MB. Effects of probiotic bacteria on diarrhea, lipid metabolism and carcinogenesis: a review of papers published between 1988 and 1998. Am J Clin Nutr 2000; 71: 405-11.

[8] Felley CPI, Cortéis-Theulaz JL, Rivero P, *et al*. Favourable effect of an acidified milk (LC-1) on *Helycobacter pyloris* gastritis in man. Eur J Gastroenterol Hepatol 2001; 13: 15-9.

[9] D-Souza AL, Rajkumar C, Cooke J, Bulpitt CJ. Probiotics in prevention of antibiotic associated diarrhea: metaanalysis. BMJ 2002; 324: 1361.

[10] Szajewska H, Mrukowics JZ. Probiotics in the treatment and prevention of acute infectious diarrea in infants and children: a systematic review of published randomized, double blind, placebo controlled trials. J Pedriatr Gastroentl Nutr 2001; 33: Sup2: S17-25.

[11] Sartor RB. Probiotic therapy of intestinal inflammation and infections. Curr Opin Gastroenterol 2005; 21: 44-50.

[12] Sullivan A, Nord CE. Probiotics and gastrointestinal diseases. J Intern Med 2005; 257: 78-92.

[13] Cremonini F, Di Caro S, Nista EC, *et al*. Meta-analysis: the effect of probiotic administration on antibiotic-associated diarrhoea. Aliment Pharmacol Ther 2002; 16: 1461-7.

[14] Weston S, Halbert AR, Richmond P, Prescott SL. Effect of probiotics on atopic dermatitis: a randomised controlled trial. Arch Dis Child 2005; 29.

[15] de Vrese M, Schrezenmeir J. Probiotics and non-iintestinal infectious conditions. Br J Nutr 2002; 88: S59-66.

[16] Gill HS, Guarner F. Probiotics and humal health: a clinical perspective. Postgrad Med J 2004; 80: 516-26.

[17] Bongaerts G, Severijnen R, Timmerman H. Effects of antibiotics, prebiotics and probiotics in treatment for hepatic encephalopathy. Med Hypotheses 2005; 64: 64-8.
[18] Boca M, Vyskocil M, Mikulecky M, *et al*. Complex therapy of chronic hepatic encephalopathy completed with probiotic: comparison of two studies. Cas Lek Cesk 2004; 143: 324-8.
[19] Logan AC, Katzman M. Major depressive disorder: probiotics may be an adjuvant therapy. Med Hypotheses 2005; 64: 533-8.
[20] Reid G. The importance of guidelines in the development and application of probiotics. Curr Pharm Des 2005; 11: 11-6.
[21] Huff BA. Caveat emptor "Probiotics" might not be what they seem. Can Pam Physic 2004; 50:583-7.
[22] Döderlein A. Das Scheidensekret und seine Bedeutung für Puerperalfieber. Zbl Bakt 1892.; 11: 699.
[23] Beijerinch MW. Sur les ferments lactiques de l'industrie. Archives of Neetherland Science 1901; 6: 212-43.
[24] Marrie TJ, Hardging G, Ronald AR. Anaerobic and aerobic flora in healthy females. J Clin Microb 1976; 8: 62-72.
[25] Larsen B. Vaginal Flora in Health and Disease. Clin Obstet Gynecol 1993; 36: 107-21.
[26] Pavlova S, Kilic A, Kilic S, *et al*. Genetic diversity of vaginal lactobacilli from women in different countries based on 16S rRNA gene sequences. J Applied Microbiol 2002; 92: 451-9.
[27] Hyman RW, Fukushima M, Diamond L, Kumm J, Giudice L, Davis R. Microbes on the human vaginal epithelium PNAS 2005; 102: 7952-7.
[28] Antonio MAD, Hillier SF. DNA Fingerprinting of *Lactobacillus crispatus* strain CTV-05 by Repetitive Elements Sequence-Based PCR Analysis in a Pilot Study of Vaginal Colonization. J Clin Microb 2003; 41: 1881-7.
[29] Burton JP, Reid G. Evaluation of the bacterial vaginal flora of 20 postmenopausal women by direct (Nugent score) and molecular (polymerase chain reaction and denaturating gradient gel electrophoresis) techniques. J Infec Dis 2002; 186: 1770-80.
[30] Zariffard MR, Saifuddin M, Sha BE, Spear GT. Detection of bacterial vaginosis related organisms by real-time PCR for *Lactobacilli, Gardnerella vaginalis and Mycoplasma hominis.* FEMS Immunol Med Microbiol 2002; 13: 277-81.
[31] Redondo-Lopez V, Cook R, Sobel J. Emerging role of lactobacilli in the control and maintenance of the vaginal bacterial microflora. Rev Infect Dis 1990; 12: 856-72.
[32] Overman, BA. The vagina as an ecologic system. J Nurse-Midwifery 1993; 38: 146-51.
[33] Lahlack N, Ageron E, Zampati E, Michel O, Grimont G. Composition of the *L acidophilus* complex isolated from vaginal flora. Microbiologica 1996; 19.
[34] Reid G. When microbes meets human. CID 2004; 39: 827-30.
[35] Boskey ER, Cone RA, Whaley KJ, Moench TR. Origins of vaginal acidity: high D/L lactate ratio is consistent with bacteria being the primary source. Hum Reprod 2001; 16 (9): 1809-913.
[36] Boskey ER, Telsch KM, Whaley KJ, Moench TR, Cone RA. Acid production by vaginal flora *in vitro* is consistent with the rate and extent of vaginal acidification. Infec Immun 1999; 67: 5170-5.
[37] Reid G, Jass J, Sebulsky Tm, Mc Cormick JK. Potential uses of probiotics in clinical practice. Clin Microb Rev 2003; 16: 658-72.
[38] Sparks JW. Human intrauterine growth and nutrient accretion. Semin Perinatol 1984; 8: 74-93.
[39] Rajan N, Cao Q, Anderson BE, *et al*. Roles of glycoproteins and oligosaccharides found in human vaginal fluid in bacterial adherence. Inf Immun 1999; 67: 5027-32.
[40] Cauci S, Driussi S, De Santo D, *et al*. Prevalence of bacterial vaginosis and vaginal flora changes in peri- and postmenopausal women. J Clin Microbiol 2002; 40: 2147-52.
[41] Bannatyne R, Smith A. Recurrent bacterial vaginosis and metronidazole resistance in *Gardnerella vaginalis*. Sex Trans Infect 1998; 74: 455-66.
[42] Hillier S, Holloway M, Rabe L. Antimicrobial agent susceptibility of *Lactobacillus crispatus, L jensenii, L gasseri* and other Lactobacillus species. 39th Interscience Conference on Antimicrobial Agents and Chemotherapy. ASM's Annual Meeting on Infectious Diseases. American Society for Microbiology, Washington 1999.
[43] Hooton T, Fihn S, Johnson C, Roberts P, Stamm W. Association between bacterial vaginosis and acute cystitis in women using diaphragms. Arch Intern Med 1989; 149: 1932-6.
[44] Mc Groarty J, Tomeczek L, Pond D, Reid G, Bruce A. Hydrogen peroxide production by Lactobacillus species: correlation with susceptibility to the spermicidal compound nonoxynol-9. J Infect Dis 1992; 165: 1142-4.
[45] Ness R, Hillier S, Richter H *et al.* Douching in relation to bacterial vaginosis, lactobacilli and facultative bacteria in the vagina. Obstet Gynecol 2002; 100: 765-72.
[46] Chiaffarino F, Parazzini F, DeBesi P, Lavezzari M. Risk factors for bacterial vaginosis. Eur J of Obst Gynec Reprod Biol 2004; 117: 222-6.
[47] Blackwell, AL. Vaginal Bacterial Phaginosis?. Sex Trans Infect 1999; 75: 352-3.
[48] Pavlova SI, Kilic AO, Mou SM, Tao L. Bacteriophage infection in human vaginal lactobacilli: an *in vitro* study. Infect Dis Ob Gyn1997; 5: 36-44.
[49] Andrews WW, Goldenberg RL, Hauth JC. Preterm-labor: Emerging role of genital tract infections. Inf Ag Dis 1995; 4: 196-211.
[50] Reid G, Burton J, Devillard E. The rationale for pobiotics in female urogenital tract. Med Gen Med 2004; 6: 49-56
[51] Reid G, Bruce AW. Urogenital infections in women; can probiotics help?. Postgrad Med J 2003; 79: 428-34.
[52] Stapleton A. Novel approaches to prevention of urinary tract infections. Infect Dis Clin North Am 2003; 17: 457-571.
[53] Morelli G, Papaleo E, Ferrari A. Lactobacilli for prevention of urogenital infections: a review. Eur Rev Med Pharmacol Sci 2004; 8: 87-95.
[54] Hay PE, Lamont RF, Taylor-Robinson D, Morgan DJ, Ison CA, Pearson J. Abnormal bacterial colonization of the genital tract and subsequent preterm delivery and late miscarriage. Br Med J 1994; 308: 295-298.
[55] McLean NW, Rosenstein IJ. Characterisation and selection of a *Lactobacillus* species to re-colonise the vagina of women with recurrent bacterial vaginosis. J Med Microbiol 2000; 49: 543-52.
[56] Hill GB. The microbiology of bacterial vaginosis. Am J Obstet Gynecol 1993; 169: 450-56.
[57] Odds FC. Candidiasis of the genitalia. Odds, FC. Candida and candidiasis: A review and bibliography. 2nd ed, Bailliére Tindall. London 1988; 124.
[58] Castle PE, Giuliano AR. Genital tract infections, cervical inflammation and antioxidant nutrients-assessing their roles as human papillomavirus cofactors. J Natl Cancer Inst Monogr 2003; 31: 29-34.
[59] Karlsson R, Jonsson M, Edlung K, *et al*. Lifetime number of partners as the only independent risk factor for human Papilloma Virus Infection: a population-based study. Sex Trans Dis 1994; 120-7.
[60] Klebanoff S, Coombs W. Viricidal Effect of Lactobacillus acidophilus on Human Immunodeficiency Virus-Type I: their role in Heterosexual transmission. J Exp Med 1991; 174: 289-92.
[61] Reid G, Bruce A, Fraser N, Heinemann C, Owen J, Henning B. Oral probiotics can resolve urogenital infections. FEMS Immunol Med Microbiol 2001; 30: 49-52.
[62] Reid G. Probiotic agents to protect the urogenital tract against infection. Am. J Clin Nutr 2001; 73(suppl):437-43.
[63] Hooton TM. Recurrent urinary tract infections in women. Int J Antimicrob Agents 2001; 17: 259-68.
[64] Raz R, Stamm W. A controlled trial of intravaginal estriol in postmenopausal women with recurrent urinary tract infections. N Eng J Med 1993; 329: 753-6.
[65] Gupta K, Stapleton AE, Hooton TM, Roberts PL, Fennell CL, Stamm WE. Inverse association of H_2O_2- producing lactobacilli and vaginal Escherichia coli colonization in women with recurrent urinary tract infections. J Infect Dis 1998; 178: 446-50.
[66] Kontiokari T, Nuutinen M, Uhari M. Dietary factors affecting susceptibility to urinary tract infections. Pediatr Nephrol 2004; 19: 378-83.
[67] Nelson RR. Intrinsically vancomycin-resistant Gram positive-organisms: clinical relevance and implications for infection control. J Hospital Infec 1999; 42: 275-82.
[68] Martius J, Krohn M, Hillier S, *et al.* Relationships of vaginal Lactobacillus species, cervical *Chlamydia trachomatis*, and bacterial vaginosis to preterm birth. Obstet Gynecol 1988; 71: 89-95.
[69] Ferris DG, Dekle C, Litaker MS. Women's use of over the counter antifungal medications for gynaecological symptoms. J Fam Pract 1996; 42: 595-600.

[70] Shalev E, Battino S, Weiner E, Colodner R, Keness Y. Ingestion of yogurt containing *Lactobacillus acidophilus* compared with pasteurized yogurt as prophylaxis of recurrent candidal vaginitis and bacterial vaginosis. Arch Farm Med 1996; 5: 593-6.

[71] Hilton E, Isenberg H, Alperstein P, France K, Borenstein M. Ingestion of yogurt containing Lactobacillus acidophilus as prophylaxis for Candidal vaginitis. Annals Internal Med 1992; 116: 353-7.

[72] (www.ciudad futura.com)

[73] Hutchinsons J. Do cranberries help prevent urinary tract infections? Nurs Times.2005; 101 38-40.

[74] Habash MB, Van der Mei HC, Busscher HJ, Reid G. The effect of water, ascorbic acid and cranberry derived supplementation on human urine and uropathogens adhesion to silicone rubber. Can J Microbiol 1999; 45: 691-4.

[75] Greenberg JA, Newmann SJ, Howell AB. Consumption of sweetener dried cranberrier versus unsweetened raisins for inhibition of uropathogenic *Escherichia coli* adhesion in human urine: a pilot study. J Altern Complement Med. 2005;11:875-8

[76] Howell AB. Cranberry proanthocyanidins and the maintenance of urinary tract health. Crit Rev Food Sci Nut 2002; 42: 273-8.

[77] Jepson RG, Mihaljevic L, Craig J. Cranberries for treating urinary tract infections. Coch Database Sys Rev 2000; 2: CD001322y.

[78] Neri A. Sabah G, Samra Z. Bacterial vaginosis in pregnancy treated with yogurt. Acta Obstetricia et Gynecologica Scandinavica 1993; 72: 17-9.

[79] Chimura T, Funayama T, Murayama K, Numazaki M. Ecological treatment of bacterial vaginosis. Jpn J Antibiot 1995; 48: 432-6.

[80] Chaithongwongwatthana S, Limpongsanurak S, Shitthi-Amorn C. Single peroxide vaginal douching versus single dose oral metronidazole for the treatment of bacterial vaginosis: a randomized controlled trial. J Med Assoc Thai 2003; 86: S379-4.

[81] Cardone A, Zarcone R, Borrelli A, Di Cunzolo A, Russo A, Tartaglia E. Utilisation of hydrogen peroxide in the treatment of recurrent bacterial vaginosis. Minerva Gunecoil 2003; 55: 483-92.

[82] Andreeva P, Slavchec B, Kovachev S, Nacheva A, Vacheca R. Treatmnent of bacterial vaginosis with high dosage metronidazol and lactic acid. Akish Ginekol 2002; 41: 36-9.

[83] Van Kessel K, Assefi N, Marrazzo J, Eckert L. Common Complementary and alternative therapies for yeast vaginitis and bacterial vaginosis: a systematic review. Obstet Gynecol Surv 2003; 58: 351-8.

[84] Nyrjesy P, Weitz MV, Grody MH, Lorber B. Over-the counter and alternative medicines in the treatment of chronic vaginal symptoms. Obstet. Gynecol 1997; 90: 50-3.

[85] Vesterlund S, Palttla J, Karp M, Ouwehand AC. Measurements of bacterial adhesion *–in vitro* evaluation of different methods. J Microbiol Methods 2005; 60: 225-33.

[86] Andreu A, Stapleton A, Fennell C, Hillier S and Stamm W. Hemagglutination, adherence, and surface properties of vaginal *Lactobacillus* sp. J Infect Dis 1995; 171(5): 1237-43.

[87] Boris S, Suarez J, Barbes J. Characterization of the aggregation promoting factor from *Lactobacillus gasseri*, a vaginal isolate. J Appl Microbiol 1997; 83(4): 413-20.

[88] Boris S, Suarez J, Vazques F, Barbes C. Adherence of Human vaginal Lactobacilli to vaginal epithelial cells and interaction with uropathogens. Infect Immun 1998; 66(5):1985-9.

[89] Ocaña V, Nader-Maciás ME. Adhesion of Lactobacillus Vaginal Strains with Probiotic Properties to Vaginal Epithelial Cells. Biocell 2001; 25(3): 327-9545.

[90] Ocaña VS, Bru E, Ruiz Holgado A, Nader-Macías ME. Surface characteristics of lactobacilli isolated from human vagina. J Gen Appl Microbiol 1999 ; 45: 203-12.

[91] Bourlioux P. Ecologie microbienne du tractus digestif humain. De Roissart H and Luquet F. Les bactéries lactiques II. Uriage, Lorica 1994; 369-81.

[92] Mc Groarty JA. Cell surface appendages of lactobacilli. FEMS Microbiol Lett 1994; 15: 415-09.

[93] Lortal S, van Heijenoort J, Gruber K, Sleytr UB. S-layer of *L helveticus* 12046: Isolation, chemical characterization and re-formation after extraction with lithium chloride. J Gen Micr 1993; 138: 611-8.

[94] Frece J, Kos B, Svetec IK, Zgaga Z, Mrsa V, Suskovic J. Importance of S-layer proteins in probiotic activity of Lactobacillus acidophilus M92. J Appl Microbiol 2005; 98: 285-92.

[95] Henricksson A, Szewzyk R, Conway PL. Characteristics of the adhesive determinants of L fermentum 104. App Envir Micro 1991; 57: 499-502.

[96] Greene JD, Klaenhammer TR. Factors involved in adherence of lactobacilli to human Caco-2 cells. App Environ Microbiol 1994; 60: 4487-94.

[97] Heinemann C, van Hylckama JET, Janssen D, Busscher HJ, van der Mai HC, Reid G. Purification and Characterization of a surface-binding protein from *Lactobacillus fermentum* RC-14 that inhibits adhesion of *Enterocuccus faecalis* 1131. FEMS Microbiol Letters 2000; 190: 177-80.

[98] Granato D, Perotti F, Masserey I, *et al*. Cell surface-associated lipoteichoic acid acts as an adhesion factor for attachment of *L johnsonii* La1 to human enterocyte-like Caco-2 cells. App Environ Microbiol 1999; 65: 1071-7.

[99] Azuma Y, Sato M. Lactobacillus casei NY1301 increases the adhesion of *Lactobacillus gasseri* NY0509 to human intestinal Caco-2 cells 2001; 2326-9.

[100] Aleljung P, Shem W, Rozalska B, Hellman U, Ljungh A, Wadstrom T. Purification of collagen-binding proteins of Lactobacillus reuterii NCIB 11951. Curr Microb 1994; 28: 231-6.

[101] Styriak I, Demeckova V, Nemcova R. Collagen (Cn-I) binding by gut lactobacilli. Berl Munch Tierarztl Woschenschr 1999; 112: 301-4.

[102] Poulouin L, Gallet O, Imhoff J. La fibronectine: propriétés et fonctions de la molecule native et de ses fragments. Médicine-Sciences 1997; 13: 657-61.

[103] Nagy E, Froman G, Mardh P. Fibronectin binding of lactobacillus species isolated from women with or without bacterial vaginosis. J Med Mic 1992; 37: 38-42.

[104] Kapczynski DR, Meinersmann RJ, Lee MD. Adherence of Lactobacillus to intestinal 407 cells in culture correlates with fibronectin binding. Curr Microbiol 2000; 41: 136-41.

[105] Toba T, R. Virkola, B. Westerlund *et al* A collagen binding S-layer protein in *L crispatus*. App Envir Micro 61 1995; 2467-71.

[106] Ouwehand AC, Salminen S, Tolkko S, Roberts P, Ovaska J, Salminen E. Resected human colonic tissue: new model for characterizing adhesion of lactic acid bacteria. Clin Diagn Lab Immunol 2002; 9: 184-6.

[107] Ouwehand AC, Tuomola EM, Lee YK, Salminen S. Microbial interaction to intestinal mucosal models. Methods Enzymol 2001; 337: 200-12.

[108] Ouwehand AC, Tolkko S, Kulmala J, Salminen S, Salminen E. Adhesion of inactivates probiotic strains to intestinal mucus. Let App Microbiol 2000; 31: 82-6.

[109] Rickard AH, Gilberts P, High N, Kolenbrader P, Handley P. Bacterial coaggregation: an integral process in the development of multi-species biofims. Trends Microbiol 2003; 11: 94-100.

[110] Velreads MM, van de Belt B, Busscher HJ, Reid G, van der Mei HC. Inhibition of uropathogenic biofilm growth on silicone rubber in human urine by lactobacilli- a teleologic approach. World J Urol 2000, 18: 422-6.

[111] Ocaña VS, Nader-Macias ME. Vaginal Lactobacilli: self and co-aggregating ability. Br J Biomedical Sci 2002; 59: 183-90.

[112] Juarez Tomas MS, Wiese B, Nader-Macias ME. Effects of culture conditions on the growth and autoaggregation ability of *vaginal Lactobacillus johsonii* CRL 1294. J Appl Microbiol 2005.

[113] Velraeds M, Van Der Mei H, Reid G, Busscher H. Inhibition of initial adhesion of uropathogenic *Enterococcus faecalis* by biosurfactants from *Lactobacillus* isolates. Appl Environm Microbiol 1996; 62(6):1958-63.

[114] Velraeds M, Van De Belt-Gritter B, Van Der Mei H, Reid G, Busscher H. Interference in initial adhesion of uropathogenic bacteria and yeasts to silicone rubber by a *Lactobacillus acidophilus biosurfactant*. J Med Microbiol 1998; 47(12): 1081-5.

[115] Velraeds M, Van Der Mei H, Reid G, Busscher H. Inhibition of initial adhesion of uropathogenic *Enterococcus faecalis* to solid substrata by an adsorbed biosurfactant layer from *Lactobacillus acidophilus*. Urology 1997; 49(5): 790-4.

[116] Reid G, Heinemann C, Velraeds M, vander Mai HC, Busscher HJ. Biosurfactant produced by *Lactobacillus.* Meth Enzymol 1999; 310: 426-33.

[117] Djordjevic GM, Tchieu JH, Saier MH. Genes involved in control of galactose uptake in *Lactobacillus brevis* and reconstitution of the regulatory system *in Bacillus subtilis*. J Bacteria 2001; 183: 3224-36.

[118] Jankovic I, Ventura M, Meylan V, Rouvet M, Elli M, Zink R. Contribution of aggregation-promoting factor to maintenance of cell shape in Lactobacillus gasseri 4B2. J Bacteriol 2003;185: 3288-96.

[119] Cesena C, Morelli L, Alander M, Siljander T, Tuomola E, Salminen S, Mattila-Sandholm T, Vilpponen-Salmela T, von Wright A. *Lactobacillis crispatus* and its nonaggregating mutant in human colonization trials. J Dairy Sci 2001; 84: 1001-10.

[120] Kmet V, Lucchini F. Aggregation-promoting factor in human vaginal Lactobacillus strains. FEMS Immunol Med Microbiol 1997; 19: 111-4.

[121] Kolenbrander PE. Oral Microbial Communities: Biofilms, interactions, and genetic systems. Ann Rev of Microbiol 2000; 54: 413-37.

[122] Bujnakova D, Kmet V. Aggregation of animal lactobacilli with O157 enterohemorrhagic *Escherichia coli.* J Veterin Med 2002; 49: 152-4.

[123] Vandevoorde L, Christiaens H, Verstraete W. Prevalence of coaggregation among chicken lactobacilli. J Applied Bacteriol 1992; 72: 214-9.

[124] Roos S, Lindgren S, Jonsson H. Autoaggregation of *Lactobacillus reuteri* is mediated by a putative DEAD-box helicase. Mol Microbiol 1999; 32: 427-36.

[125] Kos B, Suskovic J, Vukovic S, Simpraga M, Frece J, Matosic S. Adhesion and aggregation ability of probiotic strain *Lactobacillus acidophilus* M92. J Appl Microbiol 2003; 94: 981-7.

[126] Ventura M, Jankovic I, Walker DC, Pridmore RD, Zink R. Identification and characterization of novel surface proteins in *Lactobacillus johnsonii* and *Lactobacillus gasseri.* Appl Environm Microbiol 2002; 68: 6172-81.

[127] Schachtsiek M, Hammes W, Hertel C. Characterization *of Lactobacillus coryniformis* DSM 20001T surface proteine Cpf mediating coaggregation with and aggregation among pathogens. Appl Environ Microbiol 2004; 70(12): 7078-85.

[128] Reniero R, Cocconcelli P, Bottazzi V, Morelli L. High frequency of conjugation in Lactobacillus mediated by an aggregation-promoting factor. J Gen Microbiol 1992; 138: 763-8.

[129] Kjelleberg S, Molin S. Is there a role for quorum sensing signals in bacterial biofilms? Curr Opin Microbiol 2002; 5: 254-8.

[130] McNeill K, Hamilton IR. Acid response of biofilm cells of *Streptococcus mutans*. FEMS Microbiol Let 2003; 221: 25-30.

[131] Castagliulo I, Galeazzi F, Ferrari S, *et al*. Beneficial effect of auto-aggregating *Lactobacillus crispatus* on experimentally induced colitis in mice. FEMS Immun Med Microbiol 2005; 43: 197-204.

[132] Berrebi A, Ayoubi JM. Le déséquilibre de la flore vaginale. Genesis: Gynecologie obstrétique – Endocrinologie 1999; 44: 1-4.

[133] Juárez Tomás MS, Ocaña V, Wiese B, Nader-Macías ME. Growth and lactic acid production by vaginal *Lactobacillus acidophilus* CRL 1259. Inhibition of uropathogenic *Escherichia coli.* J Med Microbiol 2003; 52: 1117-24.

[134] Escenbach D, Davick P, Williams S, Klebanoff S, Young-Smith C, Holmes KK. Prevalence of hydrogen-peroxide -producing Lactobacillus species in normal women and women with bacterial vaginosis. J Clin Microb 1989; 27: 251-6.

[135] Ocaña V, Ruiz Holgado A, Nader-Macias ME. Selection of vaginal-H_2O_2 generating Lactobacillus species for probiotic use. Curr Microbiol 1999; 38: 279.

[136] Ocaña VS, Bru E, Ruiz Holgado AP, Nader-Macías ME. Growth inhibition of *Staphylococcus aureus* by H2O2- producing *Lactobacillus paracasei subsp paracasei* isolated from the human vagina. FEMS Immunol Med Microbiol 1999; 23: 87-92.

[137] Juárez Tomás MS, Bru E, Nader-Macías ME. Comparison of the growth and hydrogen peroxide production by vaginal probiotic lactobacilli under different culture conditions. Am J Obstet Gynecol 2003; 188: 35-44.

[138] Klebanoff SJ, Hillier SL, Eschenbach DA, Waltersdorph AM. Control of the microbial flora of the vagina by H2O2 -generating lactobacilli. J Inf Dis 1991; 164: 94-100.

[139] Al Harthi L, Roebuck KA, Olinger GG, *et al*. Bacterial vaginosis-associated microflora isolated from the female genital tract activates HIV-I expression. J Acq Imm Defic Syndr Human Retrovirol 1999; 21:194-202.

[140] Sha B, Zarrifard M, Wang Q, *et al.* Female genital-tract HIV load correlates inversely with Lactobacillus species but positively with bacterial vaginosis and *Mycoplasma hominis*. J Infect Dis 2005; 191: 25-32.

[141] Lichtenwalner AB, Patton DL, Klebanoff SJ, Headley CM, Hillier SL. Vaginal mieloperoxidase and flora in the pig-tailed macaque. J Med Primatol 2000; 29: 36-41.

[142] Jack R.W, Tagg J.R, Ray B. Bacteriocins of Gram-positive bacteria. Microb Rev 1995; 59:171-200.

[143] Ocaña V, Ruiz Holgado A, Nader-Macías ME. Characterization of a bacteriocin-like substance produced by a vaginal *Lactobacillus salivarius* strain. Applied Environ Microbiol 1999; 65: 5631.

[144] Juárez Tomás MS, Bru E, Wiese B, de Ruiz Holgado AAP, Nader-Macías ME. Influence of pH, temperature and culture media on the growth and bacteriocin production of vaginal *Lactobacillus salivarius* CRL 1328. J Appl Microbiol 2002; 93: 714-24.

[145] Juárez Tomás MS, Otero MC, Ocaña VS, Nader-Macías ME. Production of antimicrobial substances in lactic acid bacteria I. Determination of hydrogen peroxide. Spencer JFT, Ragout de Spencer AL, eds. Methods in Molecular Biology. Public Health Microbiology: Methods Protocols. Vol. 268. Totowa, NJ, Humana Press Inc; 2004: 337-46.

[146] Rousseau V, Lepargneur J, Roques C, Remaud-Simeon M, Paul F. Prebiotic effects of oligosaccharides on selected vaginal lactobacilli and pathogenic microorganisms. Anaerobe 2005.

[147] Freter R, Brickner H, Botney M, Cleven D, Aranki A. Mechanisms that control bacterial populations in continuous-flow culture models of mouse large intestinal flora. Infect Immun 1983; 39: 676-85.

[148] Freter R, Nader-Macías M.E. Probiotics prospects of use in opportunistic infections. Fuller R. (ed.), Old Herborn, University Seminar Monograph 1995; 256.

[149] Hillier SL, Krhonh MA, Rabe LK, Klebanoff S, Eschenbach D. The normal vaginal flora, H_20_2-producing lactobacilli and bacterial vaginosis in pregnant women. Clin Inf Diseas1993; 16: S273-81.

[150] Lepargneur JP, Rousseau V. Rôle protecteur de la flore de Doderleïn. J Gynecol Obstet Biol Reprod. 2002; 31: 485-94.

[151] Famularo G, Pieluigi M, Coccia R, Mastroiacovo P, DeSimone C. Microecology, bacterial vaginosis and probiotics: perspectives for bacteriotherapy. Med Hypoth 2001; 56: 421-30.

[152] Witkin SS. Immunology of the vagina. Clin Obst Gynecol 1993; 36: 122-28.

[153] Kutteh WH. Mucosal Immunity in the Human Female Reproductive Tract. Mucosal Immunol 1999; 90: 1423-34.

[154] Kutteh WH, Mestecky J. Secretory immunity in the female reproductive tract. J Am J Reprod Immunol 1994; 31: 40-6.

[155] Kutteh WH, Edwards RP, Menge AC, Mestecky J. Immunity in female reproductive tract secretions. In: "Local Immunity in Reproductive Tract Tissues". Oxford University Press, New Delhi, India. 1993; 229-43.

[156] Ogra PL, Ogra SS. Local antibody response to polio vaccine in the human female genital tract. J Immunol 1973; 110: 1307-11.

[157] Kutteh WH, Moldoveanu Z, Mestecky J. Mucosal immunity in the female reproductive tract: correlation of immunoglobulins, cytokines, and reproductive hormones in human cervical mucus around the time of ovulation. AIDS Res Hum Retroviruses 1998; 14: S51-5.

[158] Hedges SR, Agace WW, Svanborg C. Epithelial cytokine responses and mucosal cytokine networks. Trends in Microbiol 1999; 3: 266-70.

[159] Wallace TD, Bradley S, Buckley ND, Green-Johnson JM. Interactions of lactic acid bacteria with human intestinal epithelial cells: effects on cytokine production. J Food Prot 2003; 66: 466-72.

[160] Kutteh WH, Franklin RD. Quantification of immunoglobulins and cytokines in human cervical mucus during each trimester of pregnancy. Am J Obstet Gynecol 2001; 184: 865-72.

[161] Sakai M, Ishiyama A, Tabata M, *et al.* Relationship between Cervical Mucus Interleukin-8 Concentration and Vaginal Bacterial in Pregnancy. Am J Reprod Immunol 2004; 52: 106-12.

[162] Mestecky J, Fultz N. Mucosal Immune System of the Human Genital Tract. J of Infec Dis 1999; 179: Suppl 3. S470 –4.

[163] Wira C, Rossoll R, Kaushic C. Antigen-Presenting Cells in the female reproductive tract: influence of estradiol on antigen presentation by vaginal cells. Endocrinol 2000; 141: 2877-85.

[164] Abrahams VM. Toll-like receptors in the cycling female reproductive tract and during pregnancy. Curr Women´s Health Rev 2005; 1: 35-42.

[165] Parr MB, Kepple L, Parr EL. Langerhans cells phagpcytose vaginal epithelial cells undergoing apoptosis during the murine estrous cycle. Biol Reprod 1991; 45; 252-60.

[166] Kitazawa H, Ino T, Kawai Y, Itoh T, Saito T. A novel immunostimulating aspect of *Lactobacillus gasseri*: induction of "Gasserokine" as chemoattractants for macrophages. Int J Food Microbiol 2002; 77: 29-38.

[167] Kitazawa H, Tomioka Y, Matsumura K, *et al*. Expression of mRNA encoding IFN alpha in macrophages stimulated with *Lactobacillus gasseri*. FEMS Microbiol Lett 1994; 120: 315-21.

[168] Kitazawa H, Matsumara K, Itoh T , Yamaguchi T. Interferon induction in murine peritoneal macrophage by stimulation with Lactobacillus acidophilus. Microbiol Immunol 1992; 36: 311-15.

[169] Vintini E, Ocaña V, Nader-Macias ME. Effect of lactobacilli administration in the vaginal tract of mice: evaluation of side effects and local immune response by local administration of selected strains. Methods Mol Biol 2004; 268: 401-10.

[170] Morelli L, Zonenschain D, Del Piano M, Cognein P. Utilization of the intestinal tract as delivery system for urogenital probiotics. J Clin Gastroenterol 2004; 38: S107-110.

[171] Savage DC, Kotarski SF. Models for study the specificity by which lactobacilli adhere to murine gastric epithelium. Inf Immun 1979; 26: 966-73.

[172] Juárez Tomás MS, Bru de Labanda E, de Ruiz Holgado AP, Nader-Macías ME. Estimation of vaginal probiotic lactobacilli growth parameters with the application of the Gompertz model. Can J Microbiol 2002; 48: 82-92.

[173] Juárez Tomás MS, Ocaña V and Nader-Macías ME Viability of vaginal probiotic lactobacilli during refrigerated and frozen storage. Anaerobe 2004; 10: 1-5.

[174] Zárate G, Nader-Macias ME. Viability and biological properties of probiotic vaginal lactobacilli after lyophilization and refrigerated storage into gelatin capsules. (2005) In press.

[175] Zárate G, Juarez Tomas MS, Nader-Macias ME. Effect of some pharmaceutical excipients on the survival of probiotic vaginal lactobacilli. Can J Microbiol 2005: 51: 483-89.

[176] Reid G, Beuerman D, Heinemann C, Bruce AW. Probiotic Lactobacillus dose required to restore and maintain a normal vaginal flora. FEMS Immunol Med Microbiol; 2001: 32: 37-41.

[177] Chrisope G, (Boulder, CO). Vaginal lactobacillus medicant. US PATENT & TRADEMARK OFFICE (United States Patent), GyneLogix, Inc; 2000: 093,394- 6-21.

[178] Larsen B. (Huntington, WV). Vaginal pharmaceutical compositions. US PATENT & TRADEMARK OFFICE (United States Patent), Marshall University Research Corporation. (Huntion, WV) 1999; (5)958: 461-7-34.

[179] Reid G, (London, CA), Bruce A W. (Toronto, CA). Lactobacillus and skim milk compositions and methods for preventing microbial urogenital infections. US PATENT & TRADEMARK OFFICE (U. S. Patent), Research Corporation Technologies, Inc. (Tucson, AZ). 1997; (5)643: 830-1-1.

[180] Maggi L, Mastromarino P, Macchia S, *et al*. Technological and biological evaluation of tablets containing different strains of lactobacilli for vaginal administration. Eur J Pharm Biopharm. 2000: 50: 389-395.

[181] Mastromarino P, Brigidi P, Macchia S, *et al.* Characterization and selection of vaginal Lactobacillus strains for the preparation of vaginal tablets. J Appl Microbiol 2002; 93: 884-893.

[182] Geschel GC, Mafei P, Lombardi Borgia C, Ronchi C, Rossi S. Development of a mucoadhesive dosage form for vaginal administration. Drug Develop Industrial Pharmacy 2001; 27: 547-57.

[183] Saxelin M, Tynkkybeb S, Mattila Sandholm T, de Vos WM. Probiotics and other functional microbes: from market to mechanisms. Curr Opin Biotechnol 2005; 16: 204-211.

[184] Nader-Macias ME, Lopez-Bocanera ME, Silva-Ruiz C, Pesce-Ruiz Holgado A. Isolation of lactobacilli from the urogenital tract of mice. Elaboration of beads for their inoculation. Microbiologie-Aliments-Nutrition 1992; 10:43-7.

[185] Silva-Ruiz C, Nader-Macias ME, Lopez-Bocanera ME, Pesce-Ruiz Holgado A. *Lactobacillus fermentum* administered in suspensions and in agarose beads to mice: a comparative study. Microbiologie-Aliments-Nutrition1993; 11:391-97.

[186] Nader-Macias ME, Silva-Ruiz C, Lopez-Bocanera ME, Pesce-Ruiz Holgado A. Behaviour of lactobacilli on prevention and therapeutic effects on urinary tract infections (UTI) in mice. Anaerobe 1996; 2; 85-93.

[187] Silva-Ruiz C, Lopez-Bocanera ME, Nader-Macias ME, Pesce-Ruiz Holgado A. Effect of lactobacilli and antibiotics on *E. coli* urinary infections in mice. Biol Pharm Bull 1996;19: 88-93.

[188] Silva de Ruiz C, Rey R, Nader-Macias ME. Experimental administration of estradiol on the colonization of *Lactobacillus fermentum* and *Escherichia coli* in the urogenital tract of mice. Biol Pharm Bull 2001; 24: 127-34.

[189] Silva de Ruiz C, Rey R, Nader-Macias ME. Structural and ultrastructural studies of the urinary tract of mice inoculated with *Lactobacillus fermentum*. Br J Urol 2003; 91: 878-82.

[190] Fraga M, Scavone P, Zunino P. Preventive and therapeutic administration of an indigenous Lactobacillus sp strain against Proteus mirabilis ascending urinary tract infection in a mouse model. Antonie van Leeuwenhook 2005; 88: 24-34.

[191] Gardiner G, Heinemann C, Beuerman D, Bruce AW, Reid G. Persistence of *Lactobacillus fermentum* RC-14 and *L. rhamnosus* GR-1, but not *L. rhamnosus* GG in the human vagina as demonstrated by randomly amplified polymorphic DNA (RAPD). Clin Diagn Lab Immunol 2002; 9:92-96.

[192] 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.

[193] Reid G, A. Bruce and M. Taylor. Influence of three days antimicrobial therapy and Lactobacilli vaginal suppositories on recurrence of Urinary Tract Infections. Clin Therap 1992; 14: 11-16.

[194] Reid G, Beuerman D,Heinemann C, Bruce AW. Probiotic Lactobacillus dose required to restore and maintain normal vaginal flora. FEMS Immunol Med Microb 2001; 32: 37-41.

[195] 195. Reid G, Charbonneau D, Erb J, *et al*. Oral use of *Lactobacillus rhamnosus* GR-1 and *L. fermentum* RC-14 significantly alters vaginal flora: randomized, placebo-controlled trial in 64 healthy women. FEMS Immunol Med Microbiol 2003; 35: 131-134,120:51-56.

[196] Schultz M, Gotti C, Young RJ, Iwen P, Vanderhoof JA. Administration of oral probiotic bacteria to pregnant women causes temporary infantile colonization. J Pediatr Gastroenterol Nut 2004; 38: 293-297.

[197] Patton DL, Cosgrove Sweeney YT, Antonio MA, Rabe LKm Hillier SL. *Lactobacillus crispatus* capsules: single use safety study in the Macaca nemestrina model. Sex Transm Dis 2003; 30: 568-570.

[198] The Medicines Company and NIH Start Clinical Trial of CTV-05, a Natural Probiotic for Bacterial Vaginosis and Urogenital Diseases

[199] Martin HL, Richardson BA, Nyange PM, *et al.* Vaginal lactobacilli, microbial flora, and risk of human immunodeficiency virus type 1 and sexually transmitted disease acquisition. J Infect Dis 1999; 180: 1863.

[200] Schwebke JR. Role of vaginal flora as barrier to HIV acquisition. Curr Infect Dis Reports 2001; 3: 152-5.

[201] Sewankambo N, Gray RH, Wawer MJ, *et al*. HIV-1 infection associated with abnormal vaginal flora morphology and bacterial vaginosis. Lancet 1997; 350: 546-50.

[202] Parent J, Bossens M, Bayot D, *et al.* Therapy of bacterial vaginosis using exogenously applied lactobacillus acidophilus and a low dose of estriol. Drug Res 1996: 46; 68-73.

[203] Ozkinay E, Terek MC, Yayci M, Kaiser R, Grob P, Tuncay G. The effectiveness of live lactobacilli in combination with low dose oestriol (Gynoflor) to restore the vaginal flora after treatment of vaginal infections. BJOG 2005; 112: 234-40.

[204] Chlebecek J, Reich I. Fermalac Vaginal (Rougier Inc.) in the prevention of colpitis pregnancy. Cesk Gynekol 1993: 58; 237-8.

[205] Kaivolas S, A Klvijarvi, *et al*. Lactobacillus-vagitorio toisturan vulvovaginitis hordossa. Organon tiedoke 1985; 17: 29-32.

[206] Devillard E, Burton JP, Hammond JA, Lam D, Reid G. Novel insight into the vaginal microflora in postmenopausal women under hormone replacement therapy as analyzed by PCR-denaturing gradient gel electrophoresis. Eur J Obstet Gynecol Reprod Biol 2004; 117: 76-81.

[207] Baerheim S, E Larsen, A Di Granes. Vaginal application of lactobacilli in the prophylaxis of recurrent lower urinary tract infections in women. Scand J Prim Health Care 1994; 12: 239-42.

[208] Reid G, Anukam K, James VI, *et al*. Oral Probiotic for maternal and newborn health. J Clin Gastroenterol 2005; 39: 353-4.

[209] Cross ML. Immune signaling by orally-delivery probiotic bacteria: effects on common mucosal immnoresponses and protection at

distal mucosal sites. Int J Immunopathol Pharmacol 2004; 17: 127-34.

[210] Rush C, Hafner LM, Timms P. Lactobacilli: vehicles for antigen delivery to the female urogenital tract. Advances in Mucosal Immunity. J Mestecky *et al* (ed). Plenum Press, NY 1995.

[211] Lim BK, Mahendran R, Lee YK, Bay BH. Chemopreventive effect of *Lactobacillus rhamnosus* on growth of a subcutaneously implanted bladder cancer cell line in the mouse. Jpn J Cancer Res 2002; 93: 36-41.

[212] Brandau S, Bohle A. Antitumor effects of the intravesical instillation of heat killed cells of the *Lactobacillus casei* strain shirota on the murine orthotopic bladder tumos MBT2. J Urol 2002; 168: 199-200.

[213] Acikgoz ZC, Gambersade S, Gocer S, Ceylan P. Inhibitory effect of vaginal lactobacilli on group B Streptococci. Mikrobiyol Bull 2005; 39: 17-23.

[214] Bengmark S, Ortiz de Urbina JJ. Symbiotics: a new strategy in critically ill patient treatment. Nutr Hops 2005; 20: 147-56.

[215] Petersen EE, Magnani P. Efficacy and safety of Vitamin C tablets in the treatment of no specific vaginitis: a randomised, double blind, placebo-controlled study. Eur J Obstet Gynecol Reprod Biol 2004; 10: 70-5.

[216] Ouwehand AC, Batsman A, Salminen S. Probiotics for the skin; a new area of potential application? Let App Microbiol 2003; 36: 327-31.

[217] Vattem DA, Ghaedian R, Shetty K. Enhancing health benefits of berries through phenolic antioxidant enrichment: focus on cranberry. Asia Pac J Clin Nut 2005; 14: 120-30.