

Recombinant Probiotics: Future Perspectives in Disease Treatment

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Editorial

In recent years, considerable progress has been made towards understanding the role of the microbiota in health and diseases. The microbiota significantly contributes to immune function, digestion, metabolism, gut development and physiology. More scientific data show that the new approaches for the treatment of some diseases can be found by manipulation of the host microbiota. Modulation of the microbiota is becoming an acceptable effective strategy for prophylaxis and treatment of infectious and inflammatory diseases, metabolic disorders, psychotic conditions and cancer. Probiotics are important tools for correction of microbiota changes and maintaining health status of the host. Efficacy of probiotic treatment has been confirmed for acute and antibiotic-associated diarrhea [1], inflammatory bowel disease [2,3], allergic conditions [4], cancer [5], arthritis [6].

Many probiotic bacteria with different spectrum of activity and mechanisms of action are on the market now. However, there are a lot of human health problems needed to be addressed. Modern methods of genetic engineering open the new possibilities for creation of probiotics strains with the desired characteristics. Such probiotics can be designed to improve and to strengthen their existing properties or to influence the critical steps in the pathogenesis of diseases.

One of the most serious threats for healthcare and public safety worldwide is the emergence of multi-resistant strains of pathogens. This problem raises the question as to the discovery of novel therapeutics for the treatment of these infections. Antibacterial activity of probiotic bacteria are well documented in the scientific literature [7-9]. Probiotic bacteria can also significantly influence the host immune resistance by induction of antimicrobial peptides expression in the organism [10]. But probiotic properties vary considerably in microbial strains [11,12], so construction of new strains that express different antimicrobials and keep probiotic benefits could be a dual strategy for treatment of multi-resistant pathogens. This approach was used for engineering of *Lactococcus lactis* to produce and secrete heterologous antimicrobial peptides with activity against Gram-negative pathogenic *Escherichia coli* and *Salmonella* [13]. *Lactobacillus lactis* and *Escherichia coli* were used for construction of recombinant strains with activity against Gram-positive bacteria, including methicillin resistant *Staphylococcus aureus* [14,15]. Novel antimicrobial strategy was proposed by Saedi et al. [16]. Authors engineered commensal *Escherichia coli* strain capable of sensing and killing a pathogenic *Pseudomonas aeruginosa* strain through the production and release of antimicrobial peptide pyocin [16].

Combined efficacy of probiotic against pathogenic bacteria and viruses has been achieved by transformation of antagonistically active *Bacillus subtilis* strain with plasmid DNA, coding human alpha-2 interferon [17]. Antiviral activity of *B. subtilis* recombinant strain against influenza virus, herpes virus, and equine encephalomyelitis

virus was shown in vitro and in experimental animal infections [18]. Efficacy of animal protection against 10 LD50 doses of influenza virus by oral administration of a recombinant strain was 70%, against 100 LD50 doses was 50% [19].

The use of effective vaccines presents a valuable approach in the protection against pathogens. But the currently available vaccines are expensive to produce, require multiple doses, cause side effects in some individuals and are not always effective [20,21]. Additionally, current methods of vaccination target the systemic immune system and elicit insufficient mucosal immune response [22]. Effective mucosal response can be achieved when the vaccine is delivered directly onto mucosal sites (oral, nasal, rectal, vaginal). Live attenuated vaccine vectors such as *Samonella*, *Bordetella*, and *Listeria* have been successfully used as mucosal delivery systems of heterologous antigens in animal models [23,24]. These bacteria are highly immunogenic, but the possibility of reversion to virulence is a significant safety concern [25,26]. Recombinant probiotics offer major advantages as live vaccine vectors. They are safe, possess adjuvant properties, and have additional probiotic effects. Probiotic bacteria can survive transit through the intestinal tract and deliver recombinant vaccines in situ. Lactic Acid Bacteria (LAB) have been intensively used for construction of effective mucosal delivery vehicles. Different efficient expression systems were developed for LAB, which enabled successful expression of various antigens derived from pathogens in lactobacilli. One of the first recombinant LAB expressing the Tetanus Toxin Fragment C (TTFC) antigen was protective after intranasal administration, against lethal challenge with tetanus toxin in mice [27]. Efficacy of engineered LAB as vaccine delivery vehicle was demonstrated against different bacterial pathogens: *Streptococcus pneumoniae* [28]; *Yersinia pseudotuberculosis* [29]; *Bacillus anthracis* [30], *Listeria monocytogenes* [31]. Interestingly, that some LAB recombinant vaccine afforded better protection against pathogens than vaccination with purified antigen [28] or traditionally used injected vaccine [25]. Antiviral vaccine based on LAB also showed high efficacy in animal studies. Oral administration of recombinant *Lactococcus lactis*, expressed avian influenza H5N1 hemagglutinin completely protected the mice against lethal challenges with H5N1 virus [32]. Vaccine strain *Lactobacillus jensenii* expressing the HIV-1 entry inhibitor cyanovirin-N demonstrated a 63% reduction of HIV infection in macaques after repeated vaginal challenges [33]. Authors concluded that this approach can be applied to reduce transmission of HIV in women.

Bacillus bacteria attract attention of scientists as a perspective vehicle for vaccine delivery [34]. Effective surface display systems based on Bacillus spores were proposed for construction of recombinant vaccine strains [35]. Bacillus spores have many advantages for this purpose: a safety record of the use as probiotics and starter cultures; a low level of anti-spore response in mammalian host; a high stability in the organism, as well as during production and

storage [34-38]. *B. subtilis* spores and cells engineered to express TTFC were effective in inducing robust and consistent systemic immunity in mice and piglets after intranasal or sublingual immunization [39]. This vaccine stored as lyophilized powder maintained protective immunogenicity for 12 months at 45°C. High level of protection against *B. anthracis* was achieved by intraperitoneal immunization of mice with recombinant *B. subtilis* strain expressing *B. anthracis* protective antigen [40]. Oral immunization of hamsters with *B. subtilis* spores with the displayed cell binding domain of *Clostridium difficile* toxin A protected animals from challenge with a toxigenic *C. difficile* strain [41]. *B. subtilis* strains were used for expression of UreA antigen of *Helicobacter acinonychis* [42], UreB antigen of *Helicobacter pylori* [43], heat-labile toxin of *Escherichia coli* [44]. *B. subtilis* vaccine strain designed to express murine rotavirus VP6 was effective in protection of mice against rotavirus challenge [45]. Efficacy of protection against enterovirus 71 infection was shown after oral and intranasal immunization of mice with *B. subtilis* strain expressing viral structural protein VP1 [46]. Recombinant *B. subtilis* strain expressing antigens from Foot-And-Mouth Disease Virus (FMDV) type Asia 1 protected guinea pigs against the viral challenge [47]. The spore display approach was applied also for successful expression of different proteins: green fluorescent protein GFPuv [48]; β -glucuronidase [49]; proinsulin [50]; human serum albumin [51].

Bacillus strains can be also engineered for extracellular production of recombinant proteins because these bacteria have an intrinsic characteristic to secrete different proteins. *B. brevis* strain was transformed with the plasmid, carrying a synthetic gene of a single chain human insulin precursor [52]. Biological activity of the recombinant product, obtained from the culture supernatant was similar to human insulin. Efficient production of proinsulin into the culture medium was demonstrated by recombinant *B. subtilis* strain [53].

Probiotic bacteria were successfully engineered for targeted delivery of cytokines at airway mucosa or mucosa of the colon. Specific anti-TTFC antibody responses in mice immunized intranasally with *L. lactis*, producing TTFC intracellularly and secreting functional murine IL-2 or IL-6, were up to 15-fold higher in comparison with the control animals [54]. Murin colitis were effectively treated by oral administration of *L. lactis* secreting IL-10 [55]. Mucosal delivery of IL-10 and Glutamic Acid Decarboxylase (GAD65) by genetically modified *L. lactis* prevented development of diabetes in NOD mice [56].

Despite promising efficacy of the recombinant bacteria demonstrated in vitro and in animal studies, only two strains were tested in humans: *L. lactis* producing IL-10 [57] and *B. subtilis* secreting human interferon alpha-2 [58]. Both trials showed safety of the recombinant bacteria for patients without any side-effects. *B. subtilis* recombinant strain was further examined in clinical trials for the treatment of hepatitis and meningoencephalitis [59,60]. The results of clinical trials confirmed safety of a recombinant strain and its efficacy. *B. subtilis* recombinant strain was approved for medical use in Ukraine.

Recombinant probiotics can be successfully tailored as antimicrobials and gene therapy vectors, for delivery of vaccines and other therapeutics. For future progress with genetically modified probiotics, establishing the criteria for assessment of environmental safety and tracing the fate of recombinant DNA *in vitro* and *in vivo* are of great importance. However, these modified microorganisms have a

great potential to address novel approaches to prevention and treatment of different human and animal pathological conditions.

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