Proceedings of the 2nd International Conference on Biological Engineering and Medical Science (ICBioMed 2022), Part II
DOI: 10.54254/2753-8818/4/20220644

Application and Research Progress of Recombinant Lactic Acid Bacteria by Genetic Engineering

Zhenghan Ding[1,†], Jiayi Huang[2,†], Yueyin Zhang[3,4,†]

[1] College of pharmacy, Fujian University of Traditional Chinese Medicine, Fuzhou, China
[2] School of Life Science and Technology, Central South University of Forestry and Technology, Changsha, China.
[3] School of Life Sciences, Sun Yat-sen University, Guangzhou, China

[4]zhangyy299@mail2.sysu.edu.cn
[†]These authors contribute equally.

Abstract. Lactic acid bacteria, as a kind of food-grade microorganism, is widely used in food industry, medical care and other fields. The special function of lactic acid bacteria has been increasingly valued and needed, and the traditional strain screening method is complex, long cycle and low cost, which hinders the development and progress of lactic acid industry. At this time, the rapid development of modern genetic engineering has brought new opportunities for the medical application of LAB. By developing new functional strains of LAB, genes with specific functions can be introduced into the genome of LAB, so that LAB can meet the requirements of designers and users. First of all, this article summarizes the characteristics of lactic acid bacteria and discusses the dominant types of lactic acid bacteria in transgenic bacteria. Then, six medical applications of LAB in vaccine delivery vector, virus prevention and control, allergy treatment, cervical cancer, inflammation, lactose intolerance after genetic engineering were reviewed. Finally, the genetically motified LAB is used in the diagnosis and treatment of diseases, such as immune tolerance, vector development, immune mechanism and other issues were reviewed, and the future application of LAB in the field of genetic engineering and cancer treatment was discussed.

Keywords: genetic, lactic acid bacteria, engineering.

1. Introduction

With the rapid development of genetic engineering technology, through the introduction of new genes to achieve their expression in engineered bacteria, to achieve the purpose of changing their metabolic function or expressing some functional proteins, such as antimicrobial peptides. The use of genetic engineering techniques to edit, improve existing strains or breed and develop new strains is a hot topic in the field of research. With the development of food technology and changes in hygiene requirements, the application of lactic acid bacteria as a genetically engineered host bacteria has attracted widespread attention from researchers. Lactic acid bacteria are a general term for one of the gram-positive bacteria that can allow carbohydrates to ferment to produce lactic acid, including lactobacillus, lactococcus, bifidobacteria, azococcus, and at least 23 genera. Lactic acid bacteria are

© 2025 The Authors. This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (https://creativecommons.org/licenses/by/4.0/).

DOI: 10.54254/2753-8818/4/20220644

widely used in the food fermentation, pharmaceutical production, health care drugs and feed additive industries because they are considered safe microorganisms.

The problems existing in genetically modified LAB are mainly the following points: the problems of marker genes, the selection of host bacteria, immune tolerance, vector development, and immune mechanisms.

This review focused on the current status of the application of genetically modified LAB for different diseases, including vaccines, allergy treatment, inflammation, virus prevention and control, cervical cancer and lactose intolerance.

2. Application of recombinant lab

2.1. The delivery vehicle for the vaccine

The live vector vaccine of LAB is the vector bacterium of LAB, and the pathogenic genes of viruses, bacteria or parasites are reconstituted by genetic engineering technology, so as to construct the recombinant LAB that can express exogenous antigen or carry exogenous DNA vaccine. These recombinant bacteria can deliver antigens to the mucosal system of the host through oral or nasal drops, and then stimulate the immune host to produce immune responses at the mucosal level [1]. They can stimulate humoral, cellular and mucosal immune responses simultaneously, and the immune process is consistent with pathogen infection. Can prevent pathogen infection at the mucosal level [2]. Recombinant LAB not only has such an effect to disease prevention, but also have a certain treatment and inhibition of virus infection [3]. In addition, the production cost of LAB live vector vaccine is low, the preparation method is simple, does not require DNA vaccine or peptide vaccine preparation process purification, easy to standardize; Good safety, no side effects, no risk such as virulence return of live attenuated vaccine; Easy to use, no need to cooperate with adjuvants, save a lot of labor, material resources, especially suitable for large-scale breeding farm animal herd immunity. Therefore, LAB have important research significance and pathogenic antigens are used to play a role in transmission and small molecule active peptides.

The main advantage of LAB live vector vaccine is to stimulate the mucosal immune system of the body to produce secreted antibody sIgA, sIgA is enriched at the mucosal level of the immune host, and timely eliminate pathogens invading through the mucosal system. When the vaccine is delivered to the gastrointestinal mucosa by oral or nasal delivery, it is ingested by microfolded cells in the rich area of mucosal epithelial lymphoid follicles and transmitted to the underlying macrophages and dendritic cells, and then promotes the differentiation of T lymphocytes and B lymphocytes, resulting in specific humoral and cellular immune responses. LgA accounts for 80% of immunoglobulin in mucosal tissues, and sIgA is the main mucosal immune barrier against infection [4]. The proliferation of T lymphocytes promotes the increase of cytokine levels and enhances the activity of natural killer cells, thereby providing immune protection at the cellular level [5].

At present, there are two main forms of vaccine antigen delivery using LAB as the carrier: Directly using LAB as the carrier to express the target antigen; Eukaryotic expression plasmids carrying recombinant target antigen genes using LAB as vectors. The former LAB vaccine can directly deliver antigen to the host mucosal system and generate mucosal immune response. However, recombinant lactobacillus vaccines carrying eukaryotic expression plasmids are invasive. After being phagocytic, the eukaryotic expression plasmids are lysed and released, and the released eukaryotic expression plasmids can synthesize target antigens using the protein synthesis system of host cells, and then induce the body to produce corresponding immune responses [6]. To ensure that recombinant LAB vaccines carrying eukaryotic expression plasmids can be recognized and phagocytosed by host cells, the surface of LAB has host receptor-binding protein. When Listeria internalized egg white A gene (inlA) is integrated on the chromosome of LAB, the internalized protein A of epidamide can be displayed on the cell wall. Furthermore, the recombinant bacteria were promoted to enter the gastrointestinal mucosal epithelial cells [7]. The advantages of DNA vaccines delivered by LAB are LAB themselves do not need to express the target antigen, the preparation cost is low; Eukaryotic

DOI: 10.54254/2753-8818/4/20220644

expression plasmids can exist in host cells for a long time after being phagocytosed and released, and exert immunogenicity for a long time. After antigen expression in host cells, it can complete protein modification and folding by the host's own modification system, which has stronger immunity against phytophthora [8]. The expression of exogenous antigens by LAB mainly has three forms: intracellular, secreted and anchored. The fusion of the gene encoding Nuc or LEISSTCDA between the signal peptide and the target protein gene can significantly enhance the expression and secretion of foreign proteins [9]. Different recombinant LAB with the same expression form have different immune efficacy, and most studies believe that anchoring recombinant bacteria can induce a higher level of antibodies and provide a higher level of challenge protection than other forms of recombinant bacteria [10]; However, Dieye et al. (2003) and Perez et al. (2005) reported that secretory recombinant lactobacilli could deliver antigens more efficiently. The difference of immune effect is not only related to the expression mode of LAB, but also may be related to the amount of antigen carried by LAB. The higher the expression of antigen, the earlier the host can stimulate immune response [11].

2.2. Allergy treatment

Allergen-specific immunotherapy is currently an effective treatment for allergic diseases, but it requires repeated administration of allergen extracts over a period of 3 to 5 years and often causes adverse events. Many probiotic strains have shown beneficial effects in the treatment of allergic diseases, and recombinant LAB expressing relevant allergens have emerged successively for the production or delivery of allergens or allergen-derived peptides to the mucosal surface to induce tolerance.

By Western blot analysis, Der p2 was confirmed to be expressed at different sites in recombinant Lactobacillus. Animal experiments showed that all three different recombinant Lactobacilli induced immune tolerance by reducing cellular immune responses in allergic mice, modulating Ig E/Ig G2a expression, and inducing mesenteric lymphatic vessel expression. The three recombinant LAB suppressed allergic reactions by decreasing cellular immune responses, regulating Ig E/Ig G2a expression, and inducing the production of Tregs in mesenteric lymph nodes in allergic mice [12]. The in vivo immune effects of the three different recombinant LAB were similar, indicating that the expression site of Der p2 in recombinant LAB had no significant effect on the in vivo immune effects of recombinant LAB.

In a study by Kayo et al, recombinant Lactobacillus expressing the Japanese cedar pollen allergen Cryj1 was orally administered to a mouse model of cedar pollen fever, and the results showed that oral administration of the Lactobacillus vaccine carrying Cryj1 improved nasal symptoms and the production of allergen-specific IgE responses in a mouse model of cedar pollen fever [13].

2.3. Inflammatory bowel disease treatment

Crohn's disease varies with the site of the gut where inflammation occurs and the severity of the inflammatory response. The development of inflammation may be closely related to the body's immune response to intestinal microbes. Treatment of inflammatory bowel disease requires long-term administration of anti-inflammatory and immunosuppressive drugs to control its flare-ups. Interleukin 10 (IL-10) is one of the most important anti-inflammatory factors in the intestine, and direct treatment of IBD by oral IL-10 is very difficult.IL-10 is highly degradable in the intestine and this treatment can also cause some side effects.Carmen et al. investigated the anti-inflammatory effect of IL-10-producing Lactococcus lactis fermented cow's milk. The results showed that the use of IL-10-containing Lactococcus fermented bovine milk can be used for the prevention and control of IBD and that fermented bovine milk can be used as a delivery vehicle for therapeutic proteins [14]. Qiu et al. investigated the therapeutic effects of Lactobacillus casei expressing IL-10 using a murine model of induced enteritis. Expression of IL-10 recombinant Lactobacillus casei CECT 5276 could treat rats with colitis by inhibiting the release of inflammatory factors. Using this approach reduced the probability of enteritis in mice by 50% and is expected to be used in the treatment of human IBD [15].

DOI: 10.54254/2753-8818/4/20220644

UC is a nonspecific aggressive inflammatory disease of the colon that causes patients to develop features such as mucosal inflammation, erosions, and ulcers.The degree of UC disease is divided into exacerbation and remission phases. Treatment includes induction of remission and maintenance of remission with anti-inflammatory molecules (i.e., 5-AsA compounds); treatment can be administered with systemic and topical corticosteroids, immunosuppressive drugs such as 6-mercaptopurine and antibodies to tumor necrosis factor-alpha. However, these treatments come with certain side effects, which means that a large proportion of patients do not tolerate the available treatments [16]. Probiotic therapies can improve treatment by combining with probiotics (a non-digestible oligosaccharide that is absorbed in the upper intestine). This combination is known as symbiosis. In a double-blind randomized controlled trial, Furrie et al. [17] demonstrated that administration of symbiosis (B. Longum + Synergy 1) to patients with active UC for one month improved the full clinical picture of chronic inflammation. As a result, the pro-inflammatory cytokines tumor necrosis factor-α and interleukin 1-α were significantly reduced after treatment.

The effectiveness of probiotics in the treatment of IBD currently requires more detailed mechanistic studies to determine their potential beneficial effects. On top of this, preliminary studies on cellular and animal models are needed to explore the potential mechanisms of probiotic action. Finally, specific clinical trials are needed to determine the effects of fermented dairy products on disease development and maintenance.

2.4. Influenza virus prevention and control

Avian influenza is an avian infectious disease caused by influenza A virus. Humoral immunity generated by conventional vaccines fails to produce a cross-protective response, so targeting conserved internal antigenic proteins is a candidate antigen for vaccine construction. The nucleoprotein (NP) and matrix protein (M1) of avian influenza viruses are the most representative and attractive internally conserved proteins.

Rui-Ling Chen constructed recombinant LAB that correctly expressed the HA protein of H9N2 avian influenza virus and induced high levels of mucosal immune response after immunization of mice, induced proliferation of CD3+CD4+ and CD3+CD8+ T cells in spleen, PP nodes and intestinal lymph nodes, increased alveolar SIgA and serum-specific immunoglobulin levels, and promoted the expression of various cytokines [18]. The adjuvant properties of CHIL-2 were also shown by combining chicken interleukin 2 (ChIL-2)-expressing LAB with avian influenza hemagglutinin (H5). Lactic acid may be a promising antigenic carrier in avian influenza vaccines.

2.5. Cervical Cancer Treatment

Human papillomavirus (HPV) is the etiologic agent of cervical cancer (CxCa), the most common sexually transmitted pathogen in the world. The human papillomavirus type 16 (HPV-16) E7 oncoprotein, a component protein of CxCa, is a good candidate antigen for the development of new therapeutic CxCa vaccines.Li et al. constructed lactococci carrying human papillomavirus E7 protein and IL-12 DNA, and found that in mice injected with an E7-expressing tumor cell line (TC-1), the experimental group Kawana et al. conducted an 8-week observational phase 1/2a clinical trial with recombinant Lactobacillus mutans secreting the mutant HPV-16 E7 protein by oral administration, and showed that oral administration of Lactobacillus mutans secreting the HPV-16 E7 protein was effective in triggering an antigen-specific immune response. They showed that oral administration of recombinant HPV-16 E7-secreting bacteria increased serum HPV-16 E7-specific antibody production, thereby inducing protective humoral immunity, and that this mode of immunization was safe.

2.6. Lactose Intolerance

Under normal physiological conditions, lactose is hydrolyzed into glucose and galactose in the small intestine by lactase located at the brush-like edge of the epithelial cells of the mucosa of the small intestine, and can eventually be absorbed by the body.

DOI: 10.54254/2753-8818/4/20220644

However, when the human body lacks lactase or its activity is reduced, lactose will not be hydrolyzed and absorbed, but directly to the lower part of the small intestine and colon, and finally under the fermentation of colon bacteria, lactose is fermented into short-chain fatty acids such as acetic acid, propionic acid, butyric acid and other short-chain fatty acids and methane, H2, CO2 and other gases, thereby increasing intestinal osmotic pressure, causing gastrointestinal dysfunction, so that the body has diarrhea, abdominal pain, bloating and other symptoms, clinically called lactose intolerance.

The common method of treating lactose intolerance by LAB is that the human body obtains the β galactosidase that is lacking by ingesting LAB fermentation products independently, which can delay the rate of gastric emptying, slow down the intestinal transit time, and ultimately improve the intestinal microbiome balance.

Construct the LAB that secrete β-galactosidase, let it colonize in the human intestine, and then improve the intestinal microecological environment, accelerate intestinal peristalsis, enhance the body's immunity, reduce the risk of carcinogenesis, reduce blood lipids and cholesterol, so as not only to fully express the characteristics of the probiotic, but also to continue to secrete β-galactosidase and release into the gastrointestinal tract, through the digestion of lactose to promote the absorption of the body, fundamentally solve the lack of lactase in the human body.

3. Conclusion

With the development of genetic engineering technology, LAB expression system has been realized in several fields. It has been applied in several fields such as microecological preparations, vaccines against viral infections, and vaccines for the prevention of microbial infections. Lactobacillus as genetically engineered receptor bacteria can survive for a long time in vivo and continuously express exogenous proteins, which can produce a long-lasting immune response in a single inoculation; expressed proteins do not need to be purified, eliminating the complex process of protein post-processing, thus reducing costs and suitable for mass production. However, there are still some challenges for Lactobacillus as an engineered bacterium, such as the tolerance problem of Lactobacillus colonization in animal intestine; the expression amount of exogenous genes expressed by Lactobacillus is not ideal; the vectors and marker genes in the expression system may also have safety risks; and the need to develop suitable mucosal delivery systems, including ideal antigen delivery systems and specific adjuvants. This is not only a problem for Lactobacillus as a genetically engineered bacterium, but also a problem for genetically engineered bacteria as a system. It is expected that in the near future, more research and clinical trials promote recombinant lactobacilli as a new therapeutic approach to prevent and treat various human diseases. In the future, there is still a need to develop various medical functional proteins using recombinant LAB, apply them in the corresponding disease models, and conduct various animal immunoprotective tests to verify their safety, efficacy and usefulness.

References

[1] L. G. Bermúdez-Humarán, P. Kharrt, J. M. Chatel, et al. Lacto-cocci and Lactobacilli as mucosal delivery vector for the rapeuticproteins and DNA vaccines [J]. Mcirobial Cell Factories, 2011, 10(1): S4.

[2] H. Moeini, R. A. Rahim, A. R. Omar, et al. Lactobacillus acidophilus as a live vehicle for oral immunization against chicken a nemiavirus [J]. Microbiol Biotechnol,2011,90:77~88.

[3] G. BermúdezHumaránL N.G. Cortes-Perez, et al. A Novel mucosal vaccine based on live Lactococci expressing E7 antigen and IL-12 induces systemic and mucosal immune responses and protects mice against human papillomavirus type16 induced tumors [J]. Immunol,2005,175(11):7297~7302.

[4] G, BermúdezHumaránL. Lactococcusl actisas a live vector for Mucosal delivery of the rapeutic proteins[J]. Human Vaccines,2009,5(4):264~267.

DOI: 10.54254/2753-8818/4/20220644

[5] H.W. Chen. Research progress on the gene expression system of animal mucosal immunization vaccine: LAB [J]. China Animal Husbandry and Veterinary Medicine,2010,37(10):194~197.
[6] V. D. Guimaraes, et al. Internalin expressing Lactococcus lactisis able to invade small intestine of Guinea pigs and deliver DNA into mammalian epithelial cells [J]. MicrobesInfect, 2005,7(5):836~844.
[7] L. A. Ribeiro, et al. Production and targeting of the Brucella abortus antigen L7/L12 in Lactococcus lactis: A first step towards food-grade live vaccines against Brucellosis[J]. Appl Environ Microbiol,2002,68(2):910~916.
[8] G. L. Bermúdez-HumaránL et al. Intranasal immunization with recombinant Lactococcus lactis secreting murine interleukin-12 enhance santigen-specific Th1 cyto-kine production [J]. InfectImmunity,2003,71(4):1887~1896.
[9] H. Poo et al. Oral administration of human Papillomavirus type 16 E7 displayed on Lactobacillus casei induces E7-specific antitumor effects in C57/BL6 mice[J]. Int J Cancer,2006,119(7):1702~1709.
[10] L. Pathangey, et al. Effect of expression Level on immune responses to recombinant oral Salmonella enterica serovar Typhimurium vaccines[J].Vaccine,2009,27(20): 2707~2711.
[11] C. Q. AI. Study of Der p2-expressing LAB on the prevention of house dust mite allergy [D]. Southern Yangtze University.2015(in Chinese)
[12] K. Ohkouchi et al. Prophylactic effect of Lactobacillus oral vaccine expressing a Japanese cedar pollen allergen [J]. J Biosci Bioeng, 2012,113(4):536-541.
[13] S. del Carmen et al. Evaluation of the anti-inflammatory effect of milk fermented by a strain of IL-10-producing Lactococcus lactis using a murine model of Crohn's disease. J Mol Microb Biotech, 2011, 21(3-4): 138-146.
[14] Z. B. Qiu et al. Effect of recombinant Lactobacillus casei expressing interleukin-10 in dextran sulfate sodium-induced colitis mice. Journal of Digestive Diseases, 2013, 14(2): 76-83.
[15] P. Marteau et al., “Ineffectiveness of Lactobacillus johnsonii LA1 for prophylaxis of postoperative recurrence in Crohn’s disease: a randomised, double blind, placebo controlled GETAID trial,” Gut, vol. 55, no. 6, pp. 842–847, 2006.
[16] E. Furrie, S. Macfarlane, A. Kennedy et al., “Synbiotic therapy (Bifidobacterium longum/Synergy 1) initiates resolution of inflammation in patients with active ulcerative colitis: a randomised controlled pilot trial,” Gut, vol. 54, no. 2, pp. 242–249, 2005
[17] R. L. Chen. Construction of dendritic cell-targeted recombinant Lactobacillus against H9N2 avian influenza virus and evaluation of immune efficiency [D]. Chang chun: Jilin University, 2016. (in Chinese)
[18] K. Szatraj, A. K. Szczepankowska et al., (2014) Expression of avian influenza haemagglutinin (H5) and chicken interleukin 2 (chIL-2) under control of the ptcB promoter in Lactococcus lactis. Acta Biochim Pol 61(3):609–614.