Lactobacillus Mucosal Vaccine Vectors: Immune Responses against Bacterial and Viral Antigens

Jonathan S. LeCureux,a Gregg A. Deanb

aDepartment of Natural and Applied Sciences, Evangel University, Springfield, Missouri, USA
bDepartment of Microbiology, Immunology and Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colorado, USA

ABSTRACT Lactic acid bacteria (LAB) have been utilized since the 1990s for therapeutic heterologous gene expression. The ability of LAB to elicit an immune response against expressed foreign antigens has led to their exploration as potential mucosal vaccine candidates. LAB vaccine vectors offer many attractive advantages: simple, noninvasive administration (usually oral or intranasal), the acceptance and stability of genetic modifications, relatively low cost, and the highest level of safety possible. Experimentation using LAB of the genus Lactobacillus has become popular in recent years due to their ability to elicit strong systemic and mucosal immune responses. This article reviews Lactobacillus vaccine constructs, including Lactobacillus species, antigen expression, model organisms, and in vivo immune responses, with a primary focus on viral and bacterial antigens.

KEYWORDS Lactobacillus, mucosal immunity, mucosal vaccines

Lactic acid bacteria (LAB), alongside other food-based platforms, have been utilized since the 1990s for therapeutic heterologous gene expression (1). The ability of LAB to elicit an immune response against expressed foreign antigens has led to their use as potential candidates as mucosal vaccine vectors. As vaccine vectors, they offer several attractive advantages: simple, noninvasive administration (usually oral or intranasal), the acceptance and maintenance of genetic modifications, low cost, and high safety levels. LAB tend to elicit minimal immune responses against themselves, instead inducing high levels of systemic and mucosal antibodies against the expressed foreign antigen following uptake via the mucosal immune system (2).

LAB for use as vaccine vectors generally include Streptococcus gordonii, Lactococcus lactis, or multiple Lactobacillus species. S. gordonii has generally fallen out of use, with a few exceptions (3). L. lactis and Lactobacillus spp. have continued to grow in use, with the number of publications continuing to increase. Several excellent reviews of L. lactis vaccines have been published (4–6), as well as articles describing how to generate these recombinant bacteria (7). Because of the large number of recent articles detailing lactobacilli as vaccine vectors, this review focuses on those publications and on the resulting immune responses generated in vivo.

Briefly, this review is divided into sections corresponding to the pathogen/disease of interest (virus, bacterium). Pathogen species or families that have been investigated in multiple studies (i.e., human immunodeficiency virus [HIV], Escherichia coli) are then highlighted, focusing on the immune responses resulting from Lactobacillus vaccination. This review covers only research involving Lactobacillus strains with heterologous gene expression. Studies conducted with unmodified Lactobacillus used either as an adjuvant or for intrinsic antibacterial or antiviral properties are excluded (8, 9). The text of this review focuses on in vivo immune responses and on selected in vitro studies with...
a significant immune component, with Table 1 highlighting viral antigens and Table 2 highlighting bacterial antigens.

**VIRUSES**

**Human immunodeficiency virus.** Human immunodeficiency virus type 1 (HIV-1) has been relegated to the status of being a treatable chronic disease, and yet infection rates are unacceptably high (10). An effective HIV vaccine is still elusive via traditional methods, with statistical significance limitations plaguing the only modestly successful clinical trial (11). Utilizing lactobacilli as mucosal vaccine vectors can provide an enhanced immune response at the typical mucosal sites of infection. Several studies have looked at lactobacilli expressing HIV antigens, thus targeting the virus at the most common site of infection, namely, the mucosa. Our laboratory has shown that expressing additional secreted molecules as adjuvants (interleukin 1β [IL-1β], *Salmonella* flagellin C) can significantly improve the mucosal (IgA) and systemic (serum IgG) immune responses against HIV proteins (MPER, Gag) in orally dosed mice (12, 13). Kuczkowska et al. have shown in vitro evidence of T cell recruitment using an *L. plantarum* strain expressing a fusion protein of CCL3/HIV Gag (14). No challenge studies in monkeys or humans have been performed to determine the efficacy of the immune response.

An alternative preventative measure against HIV is the use of prophylactic topical microbicides, which can be effective in high-risk groups (15). By incorporating microbicide expression into lactobacilli, mucosal sites can be colonized and continuously protected, reducing cost and the need for strict adherence. In two separate studies, Lagenaur et al. utilized a vagina-associated *L. jensenii* strain secreting cyanovirin-N, a promising microbicide with high affinity for HIV envelope glycoproteins. This application was safe in rhesus macaques and afforded protection against simian-human immunodeficiency virus (SHIV) challenge (16–18). That group also used lactobacilli for secretion of broadly neutralizing antibody fragments to protect the vaginal mucosa, though the work was still performed in vitro (19). Human trials are under way.

**Human papillomavirus.** The association between human papillomavirus (HPV) and various cancers, particularly cervical cancer, is well known (20). Because of this association, HPV proteins are usually expressed on the surface cervical cancer cells. This allows an immune response that not only targets potentially infectious virus but can also destroy infected, cancerous cells. There are currently two FDA-approved vaccines against the most common strains of HPV (vaccines Gardasil and Cervarix). Both generate protective immune responses via spontaneous virus-like particle (VLP) formation of the HPV L1 capsid protein (21). While these vaccines provide excellent protection and represent potential cancer therapies, the cost can prove prohibitive even in the United States (22). Only one research group has utilized *Lactobacillus* to generate VLPs using the L1 protein, resulting in serum IgG expression following subcutaneous injection in BALB/c mice (23). All other research groups have utilized surface expression of HPV proteins, either minor capsid protein L2 or the early oncoproteins E6 and E7, which are directly responsible for unregulated cellular replication (24). In an extensive set of early experiments, Poo et al. utilized an E7-expressing *L. casei* strain, observing serum IgG along with intestinal and vaginal IgA in orally immunized C57BL/6 mice. They also observed E7-specific gamma interferon (IFN-γ)-secreting cells in the vagina and spleen, as well as a therapeutic reduction in tumor size and increased animal survival following TC-1 tumor cell challenge (25). A similar study using E6 had similar results (26). Poo et al. later targeted the L2 protein in BALB/c mice, observing serum IgG, mucosal IgG and IgA, and cross-neutralization with related viruses (27). Using *L. casei* administered to C57BL/6, Adachi et al. observed increased levels of E7-specific T cells in the gut, as well as granzyme-B production. Mucosal lymphocytes were found to be capable of TC-1 cell lysis, a result which was also repeated by another research group (28, 29). Interestingly, oral administration improved the response in comparison to the results seen with subcutaneous or intramuscular administration (28). Another research group utilized *L. plantarum* expressing E7, with similar antibody and antitumor results, though they
| Pathogen | Lactobacillus species | Antigen(s) expressed | Expression | Result(s) | Intended host(s) | Reference |
|----------|-----------------------|----------------------|------------|-----------|-----------------|-----------|
| CAV      | L. acidophilus        | VP1                  | Surface    | Serum Ab, T cell response | Poultry     | 93          |
| CSFV     | L. plantarum         | E2                   | Surface    | Serum IgG, mucosal IgA, T cell response | Swine      | 62          |
| CSFV     | L. casei             | CTL 290              | Secreted   | Serum IgG, mucosal IgA, T cell response, challenge |            | 60          |
| CSFV     | L. casei             | CTL 290              | Unknown    | Serum IgG, T cell response, challenge | Swine      | 61          |
| CSFV     | L. plantarum         | ORF81                | Surface    | IgM, challenge | Fish        | 59          |
| FMDV     | L. casei, L. plantarum | VP1                 | Intracellular | Serum Ab, mucosal IgA | Human     | 95          |
| FMDV     | L. acidophilus        | VP1                  | Secreted   | Serum IgG, T cell response, challenge | Animal     | 66          |
| GPV      | L. plantarum         | VP2                  | Unknown    | Mucosal sIgA, TNF-α, IFN-γ, T cell response | Poultry     | 96          |
| HDV      | L. casei, L. plantarum | HDVag             | Intracellular | Serum Ab, mucosal IgA | Human     | 95          |
| HIV      | L. jensenii          | scFv m9, dAb m36, m36.4 | Secreted   | Stability            | Human      | 19          |
| HIV      | L. acidophilus        | Gag                  | Surface    | In vitro T cell line chemotaxis | Human      | 14          |
| HIV      | L. plantarum         | Gag                  | Surface    | Mucosal IgA            | Human      | 12          |
| HIV      | L. casei             | CV-N                | Secreted   | Safety, toxicity       | Human      | 17          |
| HIV      | L. acidophilus        | Gag                  | Surface    | Mucosal IgA            | Human      | 12          |
| HIV      | L. casei             | CV-N                | Secreted   | Challenge              | Human      | 16          |
| HIV      | L. fermentum         | Gp41                 | Surface    | Stability               | Human      | 97          |
| HIV      | L. jensenii          | CV-N                | Secreted   | Safety, toxicity       | Human      | 18          |
| HPV      | L. casei             | E7                   | Unknown    | T cell response         | Human      | 98          |
| HPV      | L. casei             | L2                   | Surface    | Serum IgG, mucosal IgA, mucosal IgA, challenge | Human     | 27          |
| HPV      | L. casei             | E7                   | Surface    | CTL response, challenge | Human      | 29          |
| HPV      | L. casei             | E6                   | Surface    | Serum IgG, mucosal IgA, challenge, cross-neutralization | Human     | 26          |
| HPV      | L. plantarum         | E7                   | Surface    | Serum IgG, challenge   | Human      | 30          |
| HPV      | L. casei             | E7                   | Surface    | Serum IgG, mucosal IgA, challenge | Human      | 25          |
| HPV      | L. casei             | E7                   | Surface    | T cell response         | Human      | 28          |
| HPV      | L. casei             | E7                   | Surface    | Unknown                | Human      | 99          |
| HPV      | L. casei             | L1, VLP             | Intracellular | Serum IgG | Human      | 23          |
| HPV      | L. plantarum         | E7                   | Surface    | Stability               | Human      | 100         |
| HPV      | L. casei             | E7                   | Unknown    | Increased cervical lymphocytes, decreased pathology | Human     | 31          |
| IBDV     | L. casei             | VP2                  | Unknown    | Serum IgG, mucosal IgA, challenge survival | Poultry     | 101         |
| IBV      | L. salivarius        | EpiC                 | Surface    | Stability, toxicity    | Poultry     | 102         |
| IBV      | L. salivarius        | EpiC                 | Surface    | Stability               | Poultry     | 102         |
| Influenza virus | L. casei | E7 | Surface | Serum IgG, mucosal IgA, challenge | Poultry | 35          |
| Influenza virus | L. casei | E6 | Surface | Serum IgG, mucosal IgA, challenge, cross-neutralization | Human | 35          |
| Influenza virus | L. delbruecki | HA | Unknown | Serum IgG, mucosal IgA, challenge | Poultry | 104         |
| Influenza virus | L. casei | E7 | Surface | Serum IgG, mucosal IgA, challenge, cross-neutralization | Human | 35          |
| Influenza virus | L. plantarum | NP | Unknown | Serum IgG, mucosal IgA, challenge, cross-neutralization | Poultry | 35          |
| Influenza virus | L. casei | M2e | Unknown | Serum IgG, mucosal IgA, challenge, cross-neutralization | Human | 105         |
| Influenza virus | L. acidophilus, L. delbruecki | HA | Unknown | Serum IgG, mucosal IgA | Human | 34          |
| Influenza virus | L. plantarum | HA | Unknown | Serum IgG, mucosal IgA, challenge | Poultry | 33          |
| Influenza virus | L. plantarum | NP, M1 | Unknown | Serum IgG, mucosal IgA, challenge, cross-neutralization | Poultry | 32          |
| Influenza virus | L. plantarum | HA | Unknown | Serum IgG, mucosal IgA, challenge, cross-neutralization | Poultry | 32          |
| Influenza virus | L. plantarum | NP, M1 | Unknown | Serum IgG, mucosal IgA, challenge, cross-neutralization | Poultry | 107         |
| Influenza virus | L. casei | NS1 | Surface | Stability              | Human | 108         |
| IPNV     | L. casei             | VP2, VP3             | Surface, secreted | Serum IgM, challenge protection | Fish       | 58          |
| IPNV     | L. casei             | VP2                  | Surface, secreted | Serum IgM, challenge | Fish       | 57          |
| NDV      | L. plantarum         | HN                   | Unknown    | Serum IgA, mucosal IgA, T cell response, challenge | Poultry | 65          |
| Norwalk virus | L. casei | VP60 | Intracellular | Stability              | Human | 109         |

(Continued on next page)
checked only for antibodies in the serum and not in the mucosa (30). Because of the observed therapeutic effect seen in several studies, a human trial using cervical cancer (cervical intraepithelial neoplasia grade 3 [CIN3]) patients was conducted and demonstrated the presence of E7-specific lymphocytes in cervical tissues but not in blood, with the majority of patient tumor pathologies being downgraded (31). Taken together, the data show great promise and potential for the development of anti-HPV Lactobacillus vaccines to meet an important public health need.

**Influenza virus.** The unpredictability of the availability of future influenza virus strains, as well as supply problems stemming from slow growth methods (egg and cell based), means that anti-influenza Lactobacillus vaccines could fill a need, particularly for treatment of infections by highly pathogenic strains such as H5N1. Shi et al. showed that oral administration of an *L. plantarum* strain expressing H9N2 hemagglutinin (HA) induced fecal IgA, bronchiolar IgA, and serum IgG. B cell levels in secondary lymphoid organs were increased, and CD8<sup>+</sup> T cell proliferation and IFN-γ secretion were greatly improved relative to the levels seen with a typical influenza vaccine. Most importantly, vaccinated mice survived lethal challenge (32). These results were seen again in assays using dendritic cell-targeting peptide (DC-pep) adjuvant, which showed improved immune responses and challenge survival in chickens (33). Similar antibody and T cell results were observed in targeting H5N1 hemagglutinin (HA<sub>1</sub>) in BALB/c mice (34) and chickens (35). Other influenza virus proteins have also been targeted. Chowdhury et al. granted BALB/c mice protection (via oral or intranasal administration) from multiple lethal challenge strains and showed that inclusion of cholera toxin subunit A1 (CTA1) significantly improved antibody levels and protection (36). A follow-up study showed that antibody levels and IFN-γ secretion and proliferation, as well as protection against lethal challenge, lasted 7 months postvaccination (37).

**Coronavirus.** Until the recent outbreaks of severe acute respiratory syndrome (SARS) (2003) and Middle East respiratory syndrome (MERS) (2014/2015), coronavirus (CoV) morbidity and mortality were generally worse for domesticated animals rather than for humans, particularly within porcine and poultry farms. Coronavirus usually

---

**TABLE 1** (Continued)

| Pathogen | Lactobacillus species | Antigen(s) expressed | Expression | Result(s) | Intended host(s) | Reference |
|----------|-----------------------|----------------------|------------|-----------|-----------------|-----------|
| PEDV     | *L. casei*            | COE                  | Surface    | Serum IgG, mucosal IgA, T cell response, neutralization | Swine     | 45         |
| PEDV     | *L. casei*            | S1, N                | Surface, secreted | Serum IgG, mucosal IgA | Swine | 44 |
| PEDV     | *L. casei*            | N                    | Surface    | Serum IgG, mucosal IgA | Swine | 110     |
| PEDV     | *L. casei*            | N                    | Surface    | Serum IgG, mucosal IgA | Swine | 46     |
| Porcine RV | *L. casei*      | VP4                  | Surface    | Serum IgG, mucosal IgA, neutralization | Swine | 53     |
| Porcine RV | *L. acidophilus* | VP7                  | Unknown    | Mucosal IgA, challenge | Swine | 111     |
| Porcine RV | *L. casei*      | VP4                  | Unknown    | Serum IgG, mucosal sIgA, neut. Ab | Swine | 112     |
| PPV      | *L. casei*            | VP2                  | Secreted   | Serum IgG, mucosal IgA, T cell response, challenge | Swine | 60     |
| PPV      | *L. casei*            | VP2                  | Secreted   | Serum IgG, mucosal IgA | Swine | 64     |
| PPV      | *L. casei*            | VP2                  | Secreted   | Serum IgG, mucosal IgA | Swine | 94     |
| PPV      | *L. casei*            | VP2                  | Secreted   | Serum IgG, mucosal IgA | Swine | 63     |
| RV       | *L. paracasei*        | ARP1                 | Surface    | Challenge | Human | 54     |
| RV       | *L. rhamnosus*        | IgGb, IgGd           | Surface    | Challenge | Human | 55     |
| SVCV     | *L. plantarum*        | GP                   | Surface    | IgM, challenge | Fish | 59    |
| TGEV     | *L. casei*            | D                    | Surface    | Serum IgG, mucosal IgA, T cell response, challenge | Swine | 47     |
| TGEV     | *L. casei*            | MDP                  | Surface    | Serum IgG, mucosal IgA, T cell response, neutralization | Swine | 41     |
| TGEV     | *L. pentosus*         | 6D                   | Surface, secreted | Serum IgG, mucosal IgA | Swine | 40     |
| TGEV     | *L. casei*            | S                    | Secreted   | Serum IgG, mucosal IgA | Swine | 39     |

CAV, chicken anemia virus; CyHV-3, cyprinid herpesvirus 3; FMDV, foot-and-mouth disease virus; GPV, goose parvovirus; HDV, hepatitis D virus; IBDV, infectious bursal disease virus; IBV, infectious bronchitis virus; NDV, Newcastle disease virus; Porcine RV, porcine rotavirus; PPV, porcine parvovirus; SVCV, spring viremia of carp virus; Ab, antibody; neut. Ab, neutralizing antibody; sIgA, secretory immunoglobulin G; scFv, single chain variable fragment.
| Pathogen | Lactobacillus species | Antigen(s) expressed | Expression | Result(s) | Intended host(s) | Reference |
|----------|----------------------|----------------------|------------|-----------|------------------|-----------|
| Bacillus anthracis | L. gasseri | PA | Unknown | Serum IgG, mucosal IgA, T cell response | Human, animal | 71 |
| Bacillus anthracis | L. gasseri | PA | Unknown | Neutr. Ab, T cell response, challenge | Human | 70 |
| Bacillus anthracis | L. acidophilus | PA | Surface | Neutr. Ab, mucosal IgA, challenge | Human | 69 |
| Bacillus anthracis | L. casei | PA | Surface, intracell., secreted | Serum IgG | Human | 68 |
| Bacillus anthracis | L. acidophilus | PA | Surface | Stability | Human | 114 |
| Borrelia burgdorferi | L. plantarum | OspA | Surface | Serum IgG, mucosal IgA | Human | 83 |
| Borrelia burgdorferi | L. plantarum | OspA | Unknown | Serum IgG, mucosal IgA, challenge | Human | 82 |
| Bordetella pertussis | L. casei | FHA | Intracell. | Serum IgG | Human | 115 |
| Clostridium botulinum | L. acidophilus | BoNT/A-Hc | Surface | Serum IgG, serum IgA, intestinal IgA, IFN-γ, challenge | Human, animal | 86 |
| Clostridium perfringens | L. casei | α-, β1-, β2-, ε-toxoids | Unknown | Serum IgG, fecal IgA, nasal IgA, IFN-γ/IL-4, T cell response, challenge | Human, animal | 116 |
| Clostridium perfringens | L. casei | β-Toxoid | Surface, intracell. | Serum IgG, serum IgA, intestinal IgA, IFN-γ, challenge | Human, animal | 117 |
| Clostridium perfringens | L. casei | α-Toxoid | Surface | Serum IgG, mucosal IgA, challenge | Human, animal | 118 |
| Chlamydia psittaci | L. fermentum | TTF | Surface | Stability | Animal | 97 |
| Clostridium tetani | L. casei | TTF | Surface, intracell., secreted | Serum IgG | Human | 119 |
| Clostridium tetani | L. plantarum | TTF | Intrad. | Serum IgG, mucosal IgA | Human | 120 |
| Clostridium tetani | L. plantarum | TTF | Intrad. | Serum IgG | Human | 121 |
| Clostridium tetani | L. johnsonii | TTF | Surface | Serum IgG, mucosal IgA | Human | 122 |
| Clostridium tetani | L. plantarum | TTF | Intracell., secreted, surface | Serum IgG, mucosal IgA, challenge | Human | 123 |
| Clostridium tetani | L. plantarum | TTF | Intracell. | Serum IgG, mucosal IgA, T cell response, challenge | Human | 124 |
| Clostridium tetani | L. plantarum, L. casei | TTF | Intracell., surface | Serum IgG, mucosal IgA, T cell response | Human | 125 |
| Chlamydia trachomatis | L. plantarum, L. fermentum | VD4 | Surface | Stability | Human | 126 |
| Chlamydia trachomatis | L. plantarum | Hirep2 | Surface | Serum IgG, serum IgA, mucosal IgA, IFN-γ | Human | 90 |
| Escherichia coli (EHEC O157:H7) | L. acidophilus | EspA, Tir | Secreted | Serum IgG, mucosal sIgA, IL-10, challenge | Human | 127 |
| Escherichia coli (EPEC) | L. casei | β-Intimin | Unknown | Serum IgG, mucosal IgM, challenge | Human | 77 |
| Escherichia coli (ETEC) | L. casei | K88 | Unknown | Serum IgG, mucosal sIgA, challenge | Human | 128 |
| Escherichia coli (ETEC) | L. casei | FaeG | Secreted | Stability | Human | 129 |
| Escherichia coli (ETEC) | L. casei | FP | Secreted | Stability | Human | 129 |
| Escherichia coli (ETEC) | L. casei | F1 | Surface | Serum IgG, mucosal IgA, challenge | Human | 75 |
| Escherichia coli (ETEC) | L. casei | K88, K99 | Surface | Serum IgG, mucosal IgA, T cell response | Human, ruminants, human | 74 |
| Escherichia coli (ETEC) | L. casei | K99 | Surface | Serum IgG, mucosal IgA | Human, ruminants, human | 73 |
| Escherichia coli (ETEC) | L. casei | F41 | Surface | Serum IgG, mucosal IgA, T cell response | Human, ruminants, human | 72 |
| Escherichia coli (ETEC) | L. reuteri | ST, LT(8) | Secreted | Serum IgG, mucosal IgA, challenge protection | Human, ruminants, human | 76 |
| Escherichia coli (ETEC) | L. acidophilus | K99 | Surface | In vitro inhibition of pathogen adhesion | Human, ruminants, human | 130 |

(Continued on next page)
infect via the gastrointestinal tract in livestock and the respiratory tract in birds and humans, causing devastating economic losses and dangerous morbidities in the young, old, and immunocompromised (38). The first coronavirus addressed using lactobacilli was transmissible gastroenteritis coronavirus (TGEV), which affects swine, particularly piglets. Several spike protein epitopes have been targeted (S, 6D), resulting in induction of serum IgG and mucosal IgA in mice (39, 40). More recently, the muramyl dipeptide (MDP) protein was targeted, utilizing tuftsin as an adjuvant, and the results showed improved antibody and T cell responses in BALB/c mice (41). The only human coronavirus addressed was SARS-CoV, with induction of serum IgG and mucosal IgA against spike proteins (SA, SB) observed in C57BL/6 mice (42). Porcine epidemic diarrhea virus (PEDV) is another coronavirus that primarily affects piglets, resulting in large economic losses (43). In a thorough set of experiments, Liu et al. showed that, by targeting both the spike protein (S1) and nucleocapsid (N) via surface expression (rather than via TABLE 2 (Continued)

| Pathogen                          | Lactobacillus species | Antigen(s) expressed | Expression | Result(s)                                      | Intended host(s) | Reference |
|----------------------------------|-----------------------|----------------------|------------|-----------------------------------------------|------------------|-----------|
| *Escherichia coli* (ETEC)        | L. plantarum         | Fimbrial adhesin     | Unknown    | Serum IgG, intestinal IgA, challenge          | Swine, ruminant, human | 131       |
| *Escherichia coli* (UPEC)        | L. reuteri            | PapG                 | Surface    | Stability                                     | Human            | 132       |
| *Helicobacter pylori*            | L. acidophilus        | Hp0410               | Unknown    | Serum IgG, mucosal IgA, challenge             | Human            | 85        |
| *Helicobacter pylori*            | L. plantarum         | UreB                 | Surface    | Stability                                     | Human            | 134       |
| *Mycobacterium avium* (MAP)      | L. salivarius         | MMP                  | Surface    | Stability                                     | Ruminant         | 135       |
| *Mycobacterium tuberculosis*     | L. plantarum         | Ag85B, ESAT-6        | Surface    | Mucosal IgA, T cell response                  | Human            | 137       |
| *Salmonella enterica* (SE)       | L. casei              | FliC, SipC           | Surface    | Serum IgG, T cell response                    | Human, animal    | 138       |
| *Salmonella enterica* (SE)       | L. casei              | FliC                 | Surface    | Challenge                                     | Human            | 139       |
| *Streptococcus mutans*           | L. zeae               | scFv                 | Surface, secreted | Challenge                                 | Human            | 140       |
| *Streptococcus pneumoniae*       | L. casei              | PspC                 | Surface, intracell. | Mucosal IgA, challenge                  | Human            | 141       |
| *Streptococcus pneumoniae*       | L. casei              | PspA, PspC           | Surface    | Serum IgG, mucosal IgA                        | Human            | 81        |
| *Streptococcus pneumoniae*       | L. casei              | PspA                 | Surface    | Serum IgG, challenge                          | Human            | 80        |
| *Streptococcus pneumoniae*       | L. casei, L. plantarum, L. helveticus | PspA | Surface | Serum IgG, mucosal IgA, challenge              | Human            | 79        |
| *Streptococcus pneumoniae*       | L. casei              | PsaA, PspA<sub>1</sub>, PspA<sub>3</sub> | Intracell, secreted | Stability                                  | Human            | 142       |
| *Streptococcus pyogenes*         | L. gasseri            | CRR6                 | Unknown    | Serum IgG, mucosal IgA, challenge             | Human            | 143       |
| *Streptococcus pyogenes*         | L. sake, L. fermentum | M6                   | Secreted, surface | Stability                                  | Human            | 144       |
| *V. cholerae*                    | L. casei, L. reuteri  | CTB                  | Intracell, secreted | Serum IgG                                  | Human            | 145       |
| *Vibrio parahaemolyticus*        | L. rhamnosus          | MAM-7                | Unknown    | MAM-7 expression (reduced *Lactobacillus* ability to inhibit pathogen) | Human            | 146       |
| *Vibrio parahaemolyticus*        | L. rhamnosus          | MAM-7                | Unknown    | Stability                                     | Human            | 146       |
| *Yersinia pestis*                | L. plantarum         | LcrV                 | Surface    | Serum IgG, mucosal IgA, T cell response       | Human            | 84        |
| *Yersinia pseudotuberculosis*    | L. plantarum         | D1-D5, D4-D5         | Surface    | Stability                                     | Human            | 147       |

<sup>4</sup>FHA, filamentous hemagglutinin adhesin; BoNT, clostridial botulinum neurotoxin; TTFC, tetanus toxin fragment C; FP, fusion protein; MMP, mucous membrane pemphigoid; intracell., intracellular; Neutr. Ab, neutralizing antibody.
secretion), levels of anti-S1 and anti-N antibodies were significantly increased, even in atypically studied secretions such as ophthalmic and nasal secretions (44). Interestingly, they observed a synergy against the spike protein, but not against the nucleocapsid, in mice vaccinated against both proteins.

To improve the immune response against TGEV’s core neutralizing epitope (COE), Ge et al. fused the COE with *E. coli* enterotoxin B (LTB), with results which showed some statistical significance, particularly with respect to splenocyte IFN-γ and IL-4 secretion (45). In perhaps the most directly useful study, Hou et al. observed the increased presence of anti-nucleocapsid antibodies in the milk and colostrum of nursing sows, correlating with increased anti-N serum IgG levels in suckling piglets (46). A recent set of experiments by Jiang et al. delved deeper into the immune response generated by *L. casei*, highlighted by strong mucosa-dependent protection from infection, stimulation of the IL-17 pathway, and an imbalance between the Th1 and Th2 responses, as indicated by variations in numbers of CD4+ T cells containing either intracellular IFN-γ or IL-4 (47). Interestingly, some *Lactobacillus* species have been shown to downregulate IL-17 responses (48), but this simply points to the delicate balance that Th17 cells must strike between pathogen-stimulated inflammation and the potential damage of errant autoimmune inflammation (49). It is clear that homeostasis with respect to inflammation, immunity, lactobacilli, and Th17 cells is a complex subject and is dependent on a number of factors, including host genetics, pathogen, *Lactobacillus* strain, and adjuvants.

**Rotavirus.** Diarrheal disease is the second leading cause of death in children under the age of 5 worldwide, with rotavirus responsible for 40% of hospitalizations due to diarrheal illness (50). It is estimated that rotavirus killed approximately 215,000 children in 2013. The World Health Organization recommends inclusion of a rotavirus vaccine in all global vaccination protocols, and there are currently two modified live vaccines licensed worldwide (51). The global implementation is ongoing, but in countries where data are available, vaccination has resulted in a 33% reduction in hospitalization due to rotavirus morbidities. Unfortunately, both vaccines have limited (50% to 60%) efficacy in developing countries and are associated with a low-level risk of intussusception (52). A recombinant *Lactobacillus*-based vaccine could address the need for a subunit rotavirus vaccine that provides the benefits of a probiotic and the appropriate safety profile for use in neonates and infants. Two main avenues of lactobacillus-based rotavirus protection have been attempted in mice. The first avenue used typical oral vaccination with *L. casei*, inducing mucosal IgA and neutralizing serum IgG against porcine Rotavirus major protective antigen (PA) VP4 in mice (53). The second used antibody fragments to confer protection. Álvarez et al. expressed a protective anti-rotavirus llama antibody fragment on the surface of *L. rhamnosus*, protecting against diarrhea in a mouse pup model (54). Another group adapted the use of anti-rotavirus hyperimmune bovine colostrum (HBC) in the same model system, expressing an anti-HBC protein from *Streptococcus*, which binds HBC antibodies, thus conferring protection when orally dosed (55).

**Fish-related viruses.** Aquaculture is an important food supply paradigm, and with it comes the typical pathogen problems that large-scale animal farms encounter. Vaccination against fish pathogens can be performed by intraperitoneal administration (which can be cost-prohibitive), by immersion, or orally via feed, with the latter two options suffering from a lack of vaccine persistence in water and from the particularly strong mucosal tolerance observed in fish. For a comprehensive summary of vaccination attempts in fish, see the excellent review by Embregts and Forlenza (56). *Lactobacillus* vaccine vectors can provide an effective and easily administered system for pisciculture. The first set of studies targeted infectious pancreatic necrosis virus (IPNV), a birnavirus that affects rainbow trout. Direct oral administration with *L. casei* expressing portions of viral capsid generated significant serum IgM and afforded challenge protection in two studies by the same group (57, 58). Two viruses that primarily affect carp, *Cyprinid herpesvirus 3* (Koi herpesvirus [KHV]) and *Rhabdovirus carpio* (spring...
viremia of carp virus [SVCV]), have also been studied. The two antigens (KHV ORF81 and SVCV glycoprotein) were expressed together in *L. plantarum* and dosed orally in carp and koi. The resulting serum IgM and challenge survival data were promising, particularly for a vaccine that offers dual protection (59). Further *Lactobacillus* studies must be conducted, looking in particular at cellular mucosal immunity in fish, as well as at the potential for multiple pathogens to be addressed with a single modified *Lactobacillus* vaccine.

**Other viruses.** In addition to the categories already addressed, a large and diverse number of viruses have been targeted using *Lactobacillus* vector systems. A few are highlighted here, with the rest detailed in Table 1. Classical swine fever virus (CSFV), a flavivirus affecting pigs, has been tested in rabbits, mice, and pigs, with all tests resulting in production of serum and mucosal antibodies (60, 61). Importantly, addition of thymosin α-1, a T cell-stimulating peptide, was able to increase levels of IgG, IgA, IFN-γ, IL-2, and tumor necrosis factor alpha (TNF-α) in pigs (62). *Porcine parvovirus* has been studied in BALB/c mice and pigs, with excellent IgG and IgA responses, as well as challenge protection and virus neutralization (60, 63, 64). A recent study observed strong protective immune responses in chickens against *Newcastle disease virus*, a paramyxovirus primarily afflicting poultry, which were improved by the addition of DC-pep, which not only boosted mucosal and serum antibody levels but also increased levels of T helper cells in the spleen and peripheral blood versus the results seen with bacteria without DC-pep (65). Foot-and-mouth disease virus, a *Picornavirus* afflicting cloven-hooved animals, was investigated in a comprehensive dosing study that assessed antcapsid immune responses resulting from administration of recombinant *L. acidophilus* via the intramuscular, intraperitoneal, intranasal, or oral route. Of note, this vaccine strategy utilized the bacteria as a delivery vehicle for a capsid-expressing DNA vaccine plasmid, in contrast to utilization of expression of heterologous proteins by the bacteria. The resulting antibody responses were thus much higher via intramuscular and intraperitoneal administration than via mucosal delivery (66). As the ease of use and awareness of *Lactobacillus* expression systems and their abilities to induce excellent mucosal and systemic immune responses increase, the number and variety of pathogens addressed will likely increase in the future.

**BACTERIA**

*Bacillus anthracis*. Though infections are relatively rare, the prevalence of natural *Bacillus anthracis* in soil and its potential as a bioterrorist agent gives antianthrax vaccines some priority. Protective antigen (PA), the only antigen used in *Lactobacillus* vaccinations, is well studied and has been tested in other vaccine systems with various degrees of success (67). One of the earliest proof-of-concept *Lactobacillus* experiments involved dosing BALB/c mice with *L. casei* either orally or intranasally. That early study showed that the antibody responses against heterologous protein exceeded the antibody responses against the bacteria itself (68). Ten years later, Mohamadzadeh et al. combined an *L. acidophilus* or *L. gasseri* strain with DC-pep, resulting in neutralizing antibodies and challenge survival in A/J mice (69, 70). That same group later observed colonic DC activation, Th17 and regulatory T cell (Treg) upregulation, and upregulation of pattern recognition receptor genes with a single vaccine dose (71).

*Escherichia coli*. Enteric *Escherichia coli* bacteria are a major cause of diarrheal morbidity and mortality, particularly for children in developing countries. The most common antigens targeted for *E. coli* vaccination are fimbrial proteins, which are bacterial adhesins that aid in host cell binding. Most experiments mentioned here, except one, have targeted enterotoxigenic *E. coli* (ETEC). A prolific group from China utilized several fimbrial protein antigens (F41, K99, K88) over several years and in several models (BALB/c, C57BL/6, BALB/c pups), all using *L. casei*. Among their many findings, an increase in levels of several subclasses of serum IgG (IgG1, IgG2a, IgG2b) followed oral dosing, along with increased IL-4 levels and a lesser increase of IFN-γ levels measured by CD4+ T cell enzyme-linked immunosorbent spot (ELISPOT) assays. Intestinal and bronchial IgA levels were increased, and challenge with standard ETEC
resulted in protection of >80% of mice challenged with a lethal dose (72). The studies were repeated using intranasal dosing, which resulted in decreased intestinal IgA levels but increased bronchial IgA levels compared to oral delivery (73). Dosing in C57BL/6 mice induced similar IgG and IgA responses, as well as T cell proliferation and challenge protection (74). Challenge protection was conferred to mouse pups born to orally or intranasally immunized dams (75). Wu and Chung targeted two enterotoxins (ST and LT-B), rather than fimbrial proteins, with a secreted green fluorescent protein (GFP)/enterotoxin fusion protein. Similar increases in IgG and IgA levels were observed as well as challenge protection in a patent mouse gut assay (76). Ferreira et al. were the only group to target enteropathogenic E. coli (EPEC) and attempted the only sublingual dosing regimen. Experiments using L. casei expressing a portion of bacterial β-intimin (a cell surface protein that aids in attachment to the host cell) resulted in serum IgG and fecal IgA responses, though, interestingly, oral dosing did not generate an IgG response. Splenocytes also secreted elevated levels of IL-6 and IFN-γ, though only the results from the sublingual vaccination were reported (77). While Ferreira et al. performed their studies in C57BL/6 mice, they used C3H/HePas mice as their challenge model, due to that strain’s susceptibility to Citrobacter rodentium, a commonly used strain that shares some pathology with EPEC (78). Ferreira et al. observed an increase in survival time, though animals eventually succumbed to disease.

**Streptococcus pneumoniae.** Most Lactobacillus experiments involving Streptococcus pneumoniae have been performed by the Oliveira laboratory and have focused on pneumococcal surface proteins (either PspA or PspC), with immunity studies conducted in C57BL/6 mice. Early work noted significant increases in bronchial IgA but not IgG levels following intranasal administration, with some variations due to bacterial strain differences (79). Strategies to increase antigen expression resulted in increased IgG levels (IgA levels were not measured), with enhancement of multiple IgG subsets (IgG1, IgG2a, IgG2b, IgG3). Challenge survival was improved compared to that seen with controls inoculated with saline solution alone, but no differences from the results seen with animals immunized with bacteria expressing the empty vector plasmid were observed (80). Further experiments identified a propensity for responses involving IgG1 versus IgG2a, which, along with increased IFN-γ levels and low levels of IL-5, indicated Th1 polarization. The levels of IL-17 secretion and neutrophil recruitment in the lungs varied by route of administration, adding to the idea of the importance of the manner in which vaccines are administered and not just of their expression of antigens (81). A final set of experiments failed to induce significant levels of IgA prior to challenge, but the researchers noted that challenge with S. pneumoniae did induce a significant IgA response, which correlated with reduced bacterial loads.

**Other bacteria.** Very few of the large number of pathogenic bacterial species have been targeted with lactobacilli, and such studies have been reported in only a few research publications. A few are highlighted here, with the rest addressed in Table 2. Borrelia burgdorferi, the causative agent of Lyme disease, was targeted with an L. plantarum system, resulting in 100% protection following a B. burgdorferi-infected tick challenge (82). Those authors also identified what has become an interesting theme with lactobacillus vaccinations, i.e., that of dual Th1 and Th2 induction. In vitro work with human cells resulted in Th1 and Th2 cytokine responses, and oral administration in C3H-HeJ mice resulted in induction of both IgG1 (Th2) and IgG2a (Th1) (83). The same authors also targeted Yersinia pestis with L. plantarum, observing once again both inflammatory (TNF-α, IL-12, IFN-γ, and IL-6) and anti-inflammatory (IL-10) cytokines, indicating stimulation of both Th1 and Th2 responses (84). Importantly, however, as with the previous experiment, those were human ex vivo cytokine studies whose results were not confirmed in vivo. A vaccine targeting Helicobacter pylori, a common cause of stomach ulcers, would be extremely beneficial. By targeting H. pylori adhesin Hp0410 with an L. acidophilus strain, Hongying et al. generated anti-adhesion serum IgG and intestinal IgA that reduced bacterial loads.
load and gastric inflammation following challenge (85). Antibodies against the ε-toxoid of Clostridium perfringens were identified in BALB/c mice following oral L. casei administration, and though the statistical significance of the antibody levels was unclear, the animals survived challenge (86).

**CONCLUSIONS**

In order to combat most pathogens at their main point of entry, next-generation vaccines must establish protective mucosal immunity (87). Lactic acid bacteria, particularly species of genus Lactobacillus, have shown great promise as mucosal vectors that are capable of driving both systemic and mucosal responses, especially in combination with adjuvants. The number of studies involving lactobacilli has steadily increased over the last 20 years, and as data accumulate, key concepts regarding the immune responses that these vectors elicit have emerged. Interestingly, coinduction of Th1 and Th2 cytokines points to the complexity of T cell subsets in the mucosa. A growing number of studies have suggested that T cell effector plasticity in the mucosa, especially in the gut, is the norm and that the gut must strike a balance between tolerance and inflammation (88). This appears to be one major factor arising from these Lactobacillus studies, since evidence of Th17 inflammation, as well as of Treg-based tolerance, points to a complex T cell response. In terms of mucosal vaccination, this reiterates the importance of maintaining a balanced and well-characterized approach to immunogenicity. More work must be done to identify the contributing immune pathways within the mucosa, especially the routes of bacterial uptake into immune inductive sites (M cells, DCs).

There are several major takeaways as development of LAB vaccine platforms continues. While the safety of LAB is an important strength, enhancing protective immunogenicity is a key challenge. Several studies have explored strategies to express adjuvants such as cytokines, pathogen-associated molecular patterns, toxins, and targeting molecules for M cells and DCs. A mechanistic understanding of each of these strategies is necessary to design the right combination of immunogens and adjuvants that will result in protection. The route of administration, while typically oral for LAB, can have an effect on the type of response elicited due to differences in mucosal inductive sites. The intrinsic differences between strains of lactobacilli, as well as the location of antigen expression (surface display, intracellular, secreted), can alter the resulting immune response, and the strains must therefore be properly selected for specific antigens (89). Boosting is also clearly a component of successful vaccination, and there is evidence that heterologous prime-boost strategies may improve, or at least alter, the resulting immune response (90). As always, the model system must be taken into consideration, especially in light of new evidence for mucosal immune differences between the two most common mouse models (BALB/c and C57BL/6) (91). On the basis of their safety and efficacy, as well as their overall cost, Lactobacillus vaccine vectors hold great promise as mucosal vaccines. It is anticipated that the use of clustered regularly interspersed short palindromic repeat (CRISPR)/Cas9 analysis will allow a more sophisticated approach to engineering vaccine candidates (92). Ultimately, it is critical for one of these candidates to successfully navigate the regulatory gauntlet and demonstrate efficacy in a target population.

**REFERENCES**

1. Rosales-Mendoza S, Angulo C, Meza B. 2016. Food-grade organisms as vaccine biofactories and oral delivery vehicles. Trends Biotechnol 34: 124–136. [https://doi.org/10.1016/j.tibtech.2015.11.007](https://doi.org/10.1016/j.tibtech.2015.11.007).

2. Wells JM, Mercenier A. 2008. Mucosal delivery of therapeutic and prophylactic molecules using lactic acid bacteria. Nat Rev Microbiol 6:349–362. [https://doi.org/10.1038/nrmicro1840](https://doi.org/10.1038/nrmicro1840).

3. Lee SF, Halbein SA. 2016. Development of a gene delivery system in Streptococcus gordonii using thymidylate synthase as a selection marker. J Microbiol Methods 125:43–48. [https://doi.org/10.1016/j.mimet.2016.04.003](https://doi.org/10.1016/j.mimet.2016.04.003).

4. Pontes DS, de Azevedo MS, Chatel JM, Langella P, Azevedo V, Miyoshi A. 2011. Lactococcus lactis as a live vector: heterologous protein production and DNA delivery systems. Protein Expr Purif 79:165–175. [https://doi.org/10.1016/j.pep.2011.06.005](https://doi.org/10.1016/j.pep.2011.06.005).

5. Wyszyn’ ska A, Kobieracka P, Bardowski J, Jagusztyn-Krynicka EK. 2015. Lactic acid bacteria—20 years exploring their potential as live vectors for mucosal vaccination. Appl Microbiol Biotechnol 99:2967–2977. [https://doi.org/10.1007/s00253-015-6498-0](https://doi.org/10.1007/s00253-015-6498-0).

6. Bermúdez-Humarán LG, Kharrat P, Chatel JM, Langella P. 2011. Lactococci and lactobacilli as mucosal delivery vectors for therapeutic pro-
Minireview

19. Marcobal A, Liu X, Zhang W, Dimitrov AS, Jia L, Lee PP, Fouts TR, Parks TP, Lagenaur LA, Sanders-Beer BE, Brichacek B, Pal R, Liu X, Liu Y, Yu R, Venzon D, Lee PP, Hamer DH. 2015. Mucosal immunogenicity of genetically modified Lactobacillus acidophilus expressing an HIV-1 epitope within the surface layer protein. PLoS One 10:e014173. https://doi.org/10.1371/journal.pone.014173.

20. Lowy DR, Schiller JT. 2012. Reducing HPV-associated cancer globally. Cancer Prev Res (Phila) 5:18–23. https://doi.org/10.1158/1940-6277.CAPR-11-0542.

21. Basu P, Banerjee D, Singh P, Bhattacharya C, Biswas J. 2013. Efficacy and safety of human papillomavirus vaccine for primary prevention of cervical cancer: a review of evidence from phase III trials and national programs. A Asia J Cancer 2:187–192. https://doi.org/10.4103/2237-3033.119877.

22. Keating KM, Brewer NT, Gottlieb SL, Liddon N, Ludema C, Smith JS. 2015. Human papillomavirus type 16 E7 is an effective strategy to induce mucosal cytotoxic lymphocytes against HPV16 E7. Vaccine 28:2810–2817. https://doi.org/10.1016/j.vaccine.2010.02.005.

23. Shim H, Yang WT, Yang GL, Cong YL, Huang HB, Wang Q, Cai RP, Ye LP, Hu JT, Zhou YJ, Wang CF. 2016. Lactobacillus plantarum vaccine vector expressing hemagglutinin in BALB/c mice. Virology 464–465:166–176. https://doi.org/10.1016/j.virol.2014.07.011.

24. Wang Z, Yu Q, Gao J, Yang Q. 2012. Mucosal and systemic immune responses induced by recombinant Lactobacillus spp. expressing the hemagglutinin of the avian influenza virus H5N1. Clin Vaccine Immunol 19:174–179. https://doi.org/10.1128/CVI.00518-11.

25. Wang Z, Yu Q, Fu J, Liang J, Yang Q. 2013. Immune responses of chickens inoculated with recombinant Lactobacillus expressing the haemagglutinin of the avian influenza virus. J Appl Microbiol 115:1269–1277. https://doi.org/10.1111/jam.12235.

26. Chowdhury MY, Li R, Kim JH, Park ME, Kim TH, Pathinayake P, Weeratunga P, Song MK, Son HY, Hong SP, Sung MH, Lee JS, Kim CJ. 2014. Mucosal vaccination with recombinant Lactobacillus casei-displayed CTA1-conjugated consensus matrix protein-2 (sm2) induces broad protection against divergent influenza subtypes in BALB/c mice. PLoS One 9:e904051. https://doi.org/10.1371/journal.pone.0090451.

27. Li R, Chowdhury MY, Kim JH, Kim TH, Pathinayake P, Koo WS, Park ME, Yoon JE, Roh JB, Hong SP, Sung MH, Lee JS, Kim CJ. 2015. Mucosally administered Lactobacillus surface-displayed influenza antigens (sm2 and HA2) with cholera toxin subunit A1 (CTA1) induce broadly protective immune responses against divergent influenza subtypes. Vet Microbiol 179:250–263. https://doi.org/10.1016/j.vetmic.2015.07.020.

28. Fehr AR, Perlman S. 2015. Coronaviruses: an overview of their replication and pathogenesis. Methods Mol Biol 1281:1–23. https://doi.org/10.1007/978-1-4939-2438-7_1.

29. Ho PS, Kwang J, Lee YK. 2005. Intragastric administration of Lactobacillus casei expressing transmissible gastroenteritis coronavirus spike proteins and DNA vaccines. Microb Cell Fact 10 Suppl 1:S4. https://doi.org/10.1186/1475-2859-10-51-S4.

30. Lin JY, Van TT, Smooker PM. 2015. Live-attenuated bacterial vectors: tools for vaccine and therapeutic agent delivery. Vaccines 3:940–972. https://doi.org/10.3390/vaccines3040490.

31. Song JA, Kim HJ, Hong SK, Lee DH, Lee SW, Song CS, Kim KT, Choi IS, Lee JB, Park SY. 2016. Oral intake of Lactobacillus rhamnosus M21 enhances the survival rate of mice lethally infected with influenza virus. J Microbiol Immunol Infect 49:16–23. https://doi.org/10.1016/j.jmii.2014.07.011.

32. Beerepoort M, Geerlings S. 2016. Non-antibiotic prophylaxis for urinary tract infections. Pathogens 5:S36. https://doi.org/10.3390/pathogens5020036.

33. Logothetidis S, Iliadou A, Fotheringham A, Fotheringham M. 2018. Cancer vaccines: an overview. Curr Cancer Drug Targets 18:491–500. https://doi.org/10.2174/1568009X178661802281630742.

34. Jokinen M, Penttilä M, Poutanen K, Hovi T. 2016. Efficacy and safety of a single-dose influenza virus based on lactic acid bacteria. Vaccine 34:6937–6944. https://doi.org/10.1016/j.vaccine.2016.06.062.

35. Ribeille P, Benouziane B, Langella P, Suárez JE, Bermúdez-Humarán LG. 2013. Protection against human papillomavirus type 16-induced tumours in mice using non-genetically modified lactic acid bacteria displaying E7 antigen at its surface. Appl Microbiol Biotechnol 97:1231–1239. https://doi.org/10.1007/s00253-012-4575-1.

36. Cortes-Perez NG, Lefèvre C, Cortiher G, Adel-Patient K, Langella P, Bermúdez-Humarán LG. 2007. Influence of the route of immunization and the nature of the bacterial vector on immunogenicity of mucosal vaccines based on lactic acid bacteria. Vaccine 25:6581–6588. https://doi.org/10.1016/j.vaccine.2007.01.027.

37. Wang Z, Yu Q, Gao J, Yang Q. 2012. Mucosal and systemic immune responses induced by recombinant Lactobacillus spp. expressing the hemagglutinin of the avian influenza virus H5N1. Clin Vaccine Immunol 19:174–179. https://doi.org/10.1128/CVI.00518-11.

38. Chowdhury MY, Li R, Kim JH, Park ME, Kim TH, Pathinayake P, Weeratunga P, Song MK, Son HY, Hong SP, Sung MH, Lee JS, Kim CJ. 2014. Mucosal vaccination with recombinant Lactobacillus casei-displayed CTA1-conjugated consensus matrix protein-2 (sm2) induces broad protection against divergent influenza subtypes in BALB/c mice. PLoS One 9:e904051. https://doi.org/10.1371/journal.pone.0090451.

39. Li R, Chowdhury MY, Kim JH, Kim TH, Pathinayake P, Koo WS, Park ME, Yoon JE, Roh JB, Hong SP, Sung MH, Lee JS, Kim CJ. 2015. Mucosally administered Lactobacillus surface-displayed influenza antigens (sm2 and HA2) with cholera toxin subunit A1 (CTA1) induce broadly protective immune responses against divergent influenza subtypes. Vet Microbiol 179:250–263. https://doi.org/10.1016/j.vetmic.2015.07.020.

40. Fehr AR, Perlman S. 2015. Coronaviruses: an overview of their replication and pathogenesis. Methods Mol Biol 1281:1–23. https://doi.org/10.1007/978-1-4939-2438-7_1.

41. Ho PS, Kwang J, Lee YK. 2005. Intragastric administration of Lactobacillus casei expressing transmissible gastroenteritis coronavirus spike proteins and DNA vaccines. Microb Cell Fact 10 Suppl 1:S4. https://doi.org/10.1186/1475-2859-10-51-S4.
glycoprotein induced specific immune production. Vaccine 23: 1335–1342. https://doi.org/10.1016/j.vaccine.2004.09.015.

40. Di-Qiu L, Xin-Yuan Q, Jun-Wei G, Li-Jie T, Yan-Ping Y, Yi-Jing L. 2011. Construction and characterization of Lactobacillus penosus expressing the D antigenic site of the spike protein of Transmissible gastroenteritis virus. Can J Microbiol 57:392–397. https://doi.org/10.1139/w11-027.

41. Jiang X, Yu M, Qiao X, Liu M, Tang L, Jiang Y, Cui W, Li Y. 2014. Up-regulation of MDP and tufsin gene expression in Th1 and Th17 cells as an adjuvant for an oral Lactobacillus casei vaccine against anti-transmissible gastroenteritis virus. Appl Microbiol Biotechnol 98: 8301–8312. https://doi.org/10.1007/s00253-014-5893-2.

42. Lee JS, Poo H, Han DP, Hong SP, Kim K, Cho MW, Kim E, Sung MH, Kim CJ. 2006. Mucosal immunization with surface-displayed severe acute respiratory syndrome coronavirus spike protein on Lactobacillus casei induces neutralizing antibodies in mice. J Virol 80:4079–4087. https://doi.org/10.1128/JVI.80.7.4079-4087.2006.

43. Song D, Park B. 2012. Porcine epidemic diarrhea virus: a comprehensive review of molecular epidemiology, diagnosis, and vaccines. Virus Genes 44:167–175. https://doi.org/10.1007/s11262-012-0713-1.

44. Liu DQ, Ge JW, Qiao XY, Liu SM, Li YJ. 2012. Construction of recombinant lactobacilli expressing G protein of spring viremia of carp virus (SVCV) combined with ORF81 protein of koi herpesvirus (KHV): a promising way to induce protective immunity against SVCV and KHV infection in cyprinid fish via oral vaccination. Vaccine 33:3092–3099. https://doi.org/10.1016/j.vaccine.2015.05.002.

45. Hou XL, Yu LY, Liu J, Wang GH. 2007. Surface-displayed porcine epidemic diarrhea viral (PEDV) antigens on lactic acid bacteria. Vaccine 25:24–31. https://doi.org/10.1016/j.vaccine.2006.09.034.

46. Xu YG, Cui LC, Ge JW, Zhao LL, Li YJ. 2007. The oral immune efficacy of recombinant Lactobacillus casei casei expressing CSFV E290 peptide and elicited specific CTL response. Sheng Wu Gong Cheng Xue Bao 23: 930–934. (In Chinese.).

47. Xu YG, Yu GN, Liu ZM, Tian CY, Cui LC. 2015. Immunogenicity in swine of orally administered recombinant Lactobacillus plantarum expressing classical swine fever virus E2 protein in conjunction with thyminosin alpha-1 as an adjuvant. Appl Environ Microbiol 81: 3745–3752. https://doi.org/10.1128/AEM.00127-15.

48. Hou XL, Liu DQ, Li YJ. 2012. Construction of recombinant lactic acid bacterium expressing the core neutralizing epitope (COE) of porcine epidemic diarrhea virus and a fusion protein consisting of COE and Escherichia coli heat-labile enterotoxin B, and comparison of the immune responses by oro gastric immunization. Can J Microbiol 58:1258–1267. https://doi.org/10.1139/w12-098.

49. Xing RL, Qiang Z, Yu P, Zhi Y, Yu Y, Wang B. 2011. Immune response of Williams’ pigs orally challenged with Lactobacillus casei vaccine polarizes Th2 cell immunity against transmissible gastroenteritis virus. Can J Microbiol 57:392–397. https://doi.org/10.1139/w11-027.

50. Hou XL, Yu LY, Zhang G, Huo G, Tang L, Li Y. 2011. Immunogenicity of recombinant classic swine fever virus E2 protein expressing classical swine fever virus VP2 protein. Vaccine 29:7004–7007. https://doi.org/10.1016/j.vaccine.2011.05.002.

51. Hou XL, Qiao X, Liu M, Tang L, Li J, Yang C. 2009. The oral immune efficacy of recombinant Lactobacillus casei vaccine expressing CSFV E290 peptide and elicited specific CTL response. Sheng Wu Gong Cheng Xue Bao 23: 930–934. (In Chinese.).

52. Xu YG, Yu GN, Liu ZM, Tian CY, Cui LC. 2015. Immunogenicity in swine of orally administered recombinant Lactobacillus plantarum expressing classical swine fever virus E2 protein in conjunction with thyminosin alpha-1 as an adjuvant. Appl Environ Microbiol 81: 3745–3752. https://doi.org/10.1128/AEM.00127-15.

53. Qiao X, Li G, Wang X, Li X, Liu M, Li Y. 2009. Recombinant porcine rotavirus VP4 and VP4-LTB expressed in Lactobacillus casei induced mucosal and systemic antibody responses in mice. BMC Microbiol 9:249. https://doi.org/10.1186/1471-2180-9-249.

54. Álvarez B, Krogh-Andersen K, Tellgren-Roth C, Martinez N, Günyaydın G, Lin Y, Martín MC, Álvarez MA, Hammarström L, Marcotte H. 2015. An exopolysaccharide-deficient mutant of Lactobacillus rhamnosus GG efficiently displays a protective llama antibody fragment against rotavirus on its surface. Appl Environ Microbiol 81:5784–5793. https://doi.org/10.1128/AEM.00495-15.

55. Günyaydın G, Zhang R, Hammarström L, Marcotte H. 2014. Engineered Lactobacillus rhamnosus GG expressing IgG-binding domains of protein G: capture of hyperimmune bovine colostrum antibodies and protection against diarrhea in a mouse pup rotavirus infection model. Vaccine 32:470–477. https://doi.org/10.1016/j.vaccine.2013.11.057.

56. Embregts CW, Forlenza M. 2016. Oral vaccination of fish: lessons from humans and veterinary species. Dev Comp Immunol 64:118–137. https://doi.org/10.1016/j.dci.2016.03.024.

57. Min L, Li-Li Z, Jun-Wei G, Xin-Yuan Q, Yuan-Ying L, Di-Qiu L. 2012. Immunogenicity of Lactobacillus casei expressing VP2 and VP3 of the infectious pancreatic necrosis virus (IPNV) in rainbow trout. Fish Shellfish Immuno 32:196–203. https://doi.org/10.1016/j.fsi.2011.11.015.
Immunization with recombinant Lactobacillus casei strains producing K99, F88 fimbrial protein protects mice against enterotoxigenic Escherichia coli. Vaccine 30:3339–3349. https://doi.org/10.1016/j.vaccine.2011.08.036.

75. Liu JK, Wei CH, Hou XL, Yu LY. 2014. Passive protection of mice pups through oral or intranasal immunization of dams with recombinant Lactobacillus casei vaccine against ETEC F41. Res Vet Sci 96:283–287. https://doi.org/10.1016/j.resv.2014.01.010.

76. Wu CM, Chung TC. 2007. Mice protected by oral immunization with Lactobacilli reuteri secreting fusion protein of Escherichia coli enterotoxin subunit protein. FEMS Immunol Med Microbiol 50:354–365. https://doi.org/10.1111/j.1574-695X.2007.00255.x.

77. Ferreira PC, da Silva JB, Piazza RM, Eckmann L, Ho PL, Oliveira ML. 2011. Immunization of mice with Lactobacillus casei expressing a beta-intimin fragment reduces intestinal colonization by Citrobacter rodentium. Clin Vaccine Immunol 18:1823–1833. https://doi.org/10.1128/CVI.05262-11.

78. Collins JW, Keeney VM, Crepin VF, Fitzgerald KA, Finlay BB. 2014. Citrobacter rodentium: infection, inflammation and the microbiota. Nat Rev Microbiol 12:612–623. https://doi.org/10.1038/nrmicro3315.

79. Oliveira ML, Arêas AP, Campos IB, Monedero V, Perez-Martinez G, Miyaji EN, Leite LC, Arêas AP, Aires KA, Lee Ho P. 2006. Induction of systemic and mucosal immune response and decrease in Streptococcus pneumoniae colonization by inoculation of mice with recombinant lactic acid bacteria expressing pneumococcal surface antigen A. Microbes Infect 8:1016–1024. https://doi.org/10.1016/j.micinf.2005.10.020.

80. Campos IB, Darieux M, Ferreira DM, Miyaji EN, Silva DA, Arêas AP, Aires KA, Leite LC, Ho PL, Oliveira ML. 2008. Nasal immunization of mice with Lactobacillus casei expressing the pneumococcal surface protein A: induction of antibodies, complement deposition and partial protection against Streptococcus pneumoniae challenge. Microbes Infect 10:481–488. https://doi.org/10.1016/j.micinf.2008.01.007.

81. Ferreira DM, Darieux M, Silva DA, Leite LC, Ferreira JM, Jr, Ho PL, Miyaji EN, Oliveira ML. 2009. Characterization of protective mucosal and systemic immune responses elicited by pneumococcal surface protein PspC and PspA nasal vaccines against a respiratory pneumococcal challenge in mice. Clin Vaccine Immunol 16:636–645. https://doi.org/10.1128/CVI.00395-08.

82. del Rio B, Dattwyler RJ, Aroso M, Neves V, Aroso M, Aires KA, Leite LC, Ho PL, Oliveira ML. 2008. Oral immunization with recombinant Lactobacillus casei expressing a beta-intimin fragment reduces intestinal colonization by Citrobacter rodentium. Clin Vaccine Immunol 18:1823–1833. https://doi.org/10.1128/CVI.05262-11.

83. del Rio B, Seegers JF, Gomes-Solecki M. 2010. Immune response to Lactobacillus casei vaccine against ETEC F41. Res Vet Sci 96:283–287. https://doi.org/10.1016/j.resv.2014.01.010.

84. Hidalgo-Cantabrana C, O’Flaherty S, Barrangou R. 2017. CRISPR-based engineering of next-generation lactic acid bacteria. Curr Opin Microbiol 37:79–87. https://doi.org/10.1016/j.mib.2017.05.015.

85. Moeini H, Rahim RA, Omar AR, Shafee N, Yusoff K. 2011. Lactobacillus acidophilus as a live vehicle for oral immunization against chicken anemia virus. Appl Microbiol Biotechnol 90:77–88. https://doi.org/10.1007/s00253-010-3050-0.

86. Alimolaei M, Golchin M, Daneshvar H. 2016. Oral immunization of mice with Lactobacillus casei expressing the pneumococcal surface protein A: induction of antibodies, complement deposition and partial protection against Streptococcus pneumoniae challenge. Microbes Infect 10:481–488. https://doi.org/10.1016/j.micinf.2008.01.007.

87. Taguchi A, Kawanai K, Yokoyama T, Adachi K, Yamashita A, Tomio K, Kojima S, Oda K, Fuji T, Kozuma S. 2012. Adjuvant effect of Japanese herbal medicines on the mucosal type 1 immune responses to human papillomavirus. J Virol 86:6098–6105. https://doi.org/10.1128/JVI.05262-11.

88. Yang WT, Yang GL, Wang Q, Huang HB, Jiang YL, Shi CW, Hou XL, Yu LY. 2014. Passive protection of mice pups against Clostridium perfringens epsilon toxin with a Lactobacillus casei strain expressing epsilon toxoid. Infect Genet Evol 40:282–287. https://doi.org/10.1016/j.mib.2017.05.015.

89. Pouwels PH, Leer RJ, Boersma WJ. 1996. The potential of Lactobacillus as a carrier for oral immunization: development and preliminary characterization of vectors for targeted delivery of antigens. J Biotechnol 44:183–192. https://doi.org/10.1016/1666-9556/95/00110-9.

90. Liu YY, Yang WT, Shi SH, Li YJ, Zhao L, Shi CW, Zhou FY, Yang YL, Hu JT, Gu W, Yang GL, Wang CF. 2017. Immunogenicity of recombinant Lactobacillus plantarum NC8 expressing goose parvovirus P2P protein in BAB/c mice. J Vet Sci 18:159–167. https://doi.org/10.4142/jvs.2017.18.2.159.

91. Turner MS, Giffard PM. 1999. Expression of Chlamydia pittaci and human immunodeficiency virus-derived antigens on the cell surface of Lactobacillus fermentum BR11 as fusions to bspA. Infect Immun 67:5486–5489.

92. Taguchi A, Kawanai K, Yokoyama T, Adachi K, Yamashita A, Tomio K, Kojima S, Oda K, Fuji T, Kozuma S. 2012. Adjuvant effect of Japanese herbal medicines on the mucosal type 1 immune responses to human papillomavirus. J Virol 86:6098–6105. https://doi.org/10.1128/JVI.05262-11.

93. Cortes-Perez NG, Azevedo V, Alcocer-González JM, Rodriguez-Padilla C, Tamez-Guerra RS, Cortiher G, Gruss A, Langella P, Bernadez-Humarin LG. 2005. Cell-surface display of E7 antigen from human papillomavirus type-16 in Lactobacillus and in Lactobacillus plantarum using a cell-surface display system. Yakugaku Zasshi 129:1327–1332. (In Japanese.) https://doi.org/10.1248/yakushi.129.1327.

94. Xu YG, Cui LC, Ge JW, Zhao LL, Li YJ. 2014. Co-expression of CSFV T cell epitope E290 peptide and PPV VP2 protein in Lactobacillus casei and determination of specific antibodies in immunized mice. Wei Sheng Wu Xue Bao 47:667–672.

95. Yang WT, Yang GL, Wang Q, Huang HB, Jiang YL, Shi CW, Hou XL, Yu LY. 2014. Passive protection of mice pups against Clostridium perfringens epsilon toxin with a Lactobacillus casei strain expressing epsilon toxoid. Infect Genet Evol 40:282–287. https://doi.org/10.1016/j.mib.2017.05.015.

96. Yang WT, Yang GL, Wang Q, Huang HB, Jiang YL, Shi CW, Wang JZ, Huang KY, Jin YB, Wang CF. 2017. Protection of chickens against H9N2 avian influenza virus challenge with recombinant Lactobacillus plantarum expressing conserved influenza virus M2e antigen expressed by Lactobacillus casei. Int Microbiol 20:36–40. https://doi.org/10.1007/s10025-010-3050-0.

97. Huang KY, Jin YB, Wang CF. 2017. Protective efficacy of Fc targeting vector vaccine expressing pneumococcal surface protein A, delivered orally with Lactobacillus plantarum. Antiviral Res 138:9–21. https://doi.org/10.1016/j.antiviral.2016.03.013.

98. Huang KY, Jin YB, Wang CF. 2017. Protective efficacy of Fc targeting vector vaccine expressing pneumococcal surface protein A, delivered orally with Lactobacillus plantarum. Antiviral Res 138:9–21. https://doi.org/10.1016/j.antiviral.2016.03.013.
expressing conserved antigens. Appl Microbiol Biotechnol 101: 4593–4603. https://doi.org/10.1007/s00253-017-8230-8.

107. Yang WT, Shi SH, Yang GL, Jiang YL, Zhao L, Li Y, Wang CF. 2016. Cross-protective efficacy of dendritic cells targeting conserved influenza virus antigen expressed by Lactobacillus plantarum. Sci Rep 6:39665. https://doi.org/10.1038/srep39665.

108. Tan TS, Syed Hassan S, Yap WB. 2017. Expression of surface-bound nonstructural 1 (NS1) protein of influenza virus A H5N1 on Lactobacillus casei strain C1. Lett Appl Microbiol 64:446–451. https://doi.org/10.1111/lam.12736.

109. Martin MC, Fernández M, Martin-Alonso JM, Parra F, Boga JA, Alvarez MA. 2004. Nisin-controlled expression of Norwalk virus VP60 protein in Lactobacillus casei. FEMS Microbiol Lett 237:385–391. https://doi.org/10.1111/j.1574-6966.2004.tb09721.x.

110. Li X, Xia C, Li Y. 2009. Induced expression of alpha-toxin gene of Clostridium perfringens in recombinant Lactobacillus casei and their immunoprotective in mice. Wei Sheng Wu Xue Bao 49:1115–1120. (In Chinese).

111. Hu J, Wang C. 2008. Expression and immunogenicity analysis of recombinant plasmin plW42Set-Vpf of porcine rotavirus A in Lactobacillus. Wei Sheng Wu Xue Bao 48:1514–1519. (In Chinese).

112. Yin JY, Guo CQ, Wang Z, Yu ML, Gao S, Bukhari SM, Tang LJ, Xu YG, Li YJ. 2016. Directed chromosomal integration and expression of porcine rotavirus outer capsid protein VLP in Lactobacillus casei ATCC3939. Appl Microbiol Biotechnol 100:9593–9604. https://doi.org/10.1007/s00253-016-7779-y.

113. Günaydin G, Alvarez B, Lin Y, Hammarström L, Marcotte H. 2014. Co-expression of anti-rotavirus proteins (llama VH4 antibody fragments) in Lactobacillus: development and functionality of vectors containing two expression cassettes in tandem. PLoS One 9:e96409. https://doi.org/10.1371/journal.pone.0096409.

114. O’Flaherty S, Kammerer TR. 2016. Multivalent chromosomal expression of the Clostridium botulinum serotype A neurotoxin heavy-chain antigen and the Bacillus anthracis protective antigen in Lactobacillus acidophilus. Appl Environ Microbiol 82:6091–6101. https://doi.org/10.1128/AEM.01533-16.

115. Colombo D, Oliveira ML, Campos IB, Monedero V, Pérez-Martínez G, Ho PL. 2006. Haemagglutination induced by Bordetella pertussis filamentous haemagglutinin ( FHA) is inhibited by antibodies produced against FHA(430-873) fragment expressed in Lactobacillus casei. Curr Microbiol 53:462–466. https://doi.org/10.1007/s00284-005-0388-0.

116. Zhao L, Guo Z, Liu J, Wang Z, Wang R, Li Y, Wang L, Li Y, Tang L, Qiao X. 2017. Recombinant Lactobacillus casei expressing Clostridium perfringens toxins alpha, epsilon and beta1 gives protection against Clostridium perfringens in rabbits. Vaccine 35:4010–4021. https://doi.org/10.1016/j.vaccine.2017.05.076.

117. Alimolai M, Golchin M, Ezatkhah M, 2017. A newly administered recombinant Lactobacillus casei vector vaccine expressing beta-toxoid of Clostridium perfringens that induced protective immunity responses. Res Vet Sci 115:332–339. https://doi.org/10.1016/j.rvsc.2017.06.018.

118. Alimolai M, Golchin M, Abshenas J, Ezatkhah M, Bafti MS. 2017. A recombinant probiotic, Lactobacillus casei, expressing the Clostridium perfringens alpha-toxoid, as an oral vaccine candidate against gas gangrene and necrotic enteritis. Probiotics Antimicrob Proteins. https://doi.org/10.1007/s12260-017-9276-8.

119. Maassen CB, Laman JD, den Bak-Glashouwer MJ, Tielen FJ, van Holten-Thole JE, Tielen FJ, Pouwels PH, Havenith CE. 2000. Engineering the microflora to vaccinate the mucosa: serum immunoglobulin G responses and activated draining cervical lymph nodes following mucosal application of tetanus toxin fragment C-expressing lactobacilli. Immunology 100:510–518. https://doi.org/10.1046/j.1365-2677.2000.00069.x.

120. Rush C, Hafner L, Timms P. 1997. Protein A as a fusion partner for the expression of heterologous proteins in Lactobacillus. Appl Microbiol Biotechnol 47:537–542. https://doi.org/10.1007/s002530050969.

121. Lin B, Zhang Y, Long B, Li JY, Xu Y, Duan S, Zhu B, Wu X, Fan H. 2017. Oral immunization with recombinant Lactobacillus acidophilus expressing espA-Tir-M confers protection against enterohemorrhagic Escherichia coli O157:H7 challenge in mice. Front Microbiol 8:417. https://doi.org/10.3389/fmicb.2017.00417.

122. Yu M, Qi R, Chen C, Yin J, Ma S, Shi W, Wu Y, Ye G, Jiang Y, Tang L, Xu Y, Li Y. 2017. Immunogenicity of recombinant Lactobacillus casei expressing F4 (K88) fimbrial adhesin FaEG in conjunction with a heat-labile enterotoxin A (LTAK63) and heat-labile enterotoxin B (LTB) of enterotoxigenic Escherichia coli as an oral adjuvant in mice. J Appl Microbiol 122:506–515. https://doi.org/10.1111/jam.13352.

123. Lu WW, Wang T, Wang Y, Xin M, Kong J. 2016. A food-grade fimbrial adhesin FaEG expression system in Lactococcus lactis and Lactobacillus casei. Can J Microbiol 62:241–248. https://doi.org/10.1139/cjm-2015-0596.

124. Chu H, Kang S, Ha S, Cho K, Park SM, Han KH, Kang SK, Lee H, Han SH, Yun CH, Choi Y. 2015. Lactobacillus acidophilus acidophilus expressing recombinant K99 adhesive fimbriae has an inhibitory effect on adhesion of enterotoxigenic Escherichia coli. Microbiol Immunol 49:941–948. https://doi.org/10.1111/1348-0421.12367.

125. Yang G, Jiang Y, Tong P, Li C, Yang W, Hu J, Ye L, Gu W, Shi C, Shan B, Wang C. 2017. Allelopathic of enterotoxigenic Escherichia coli challenge by recombinant Lactobacillus plantarum expressing a FaEG and DC-targeting peptide fusion protein. Benef Microbes 8:379–391. https://doi.org/10.3920/BM2016.0116.

126. Ashrafi F, Fallah Mehrabadi J, Siadat SD, Aghasadeghi MR. 2015. Expression and purification of the uropathogenic Escherichia coli PapG protein and its surface absorption on Lactobacillus reuteri: implications for surface display system vaccines. Jundishapur J Microbiol 8:25595. https://doi.org/10.5812/jmm.25595.

127. Zhu LF, Long BG, Luo J, Jiang R, Fang HY. 2010. Construction of a recombinant Lactobacillus acidophilus acidophilus expressing high levels of Helicobacter pylori adhesin Hp0410. Nan Fang Yi Ke Da Xue Xue Bao 30:334–337. (In Chinese).

128. Corthésy B, Boris S, Isler P, Grangerette C, Mercenier A. 2005. Oral immunization of mice with lactic acid bacteria producing Helicobacter pylori urease B subunit partially protects against challenge with Helicobacter felis. J Infect Dis 192:1441–1449. https://doi.org/10.1086/444425.

129. Johnston CD, Bannantine JP, Govender R, Endersen L, Pletzer D, Weingart H, Coffey A, O’Mahony J, Sleator RD. 2014. Enhanced expression of codon optimized Mycobacterium avium subs. paratuberculosis antigens in Lactobacillus reuteri. Front Cell Microbiol 4:120. https://doi.org/10.3389/fcimb.2014.00120.

130. Johnston C, Douarre PE, Soulunaine T, Pletzer D, Weingart H, MacSharry J, Coffey A, Sleator RD, O’Mahony J. 2013. Codon optimisation to improve expression of a Mycobacterium avium ss. paratuberculosis-specific membrane-associated antigen by Lactobacillus salivarius. Pathog Dis 66:28–38. https://doi.org/10.1111/1445-7053.12104.

131. Kurpiszewska K, Milenić CR, Minic R, Molen MF, Øverland L, Tjåland R, Carlén H, Lea T, Mathiesen G, Ejsink VG. 2017. Immunogenic properties of Lactobacillus plantarum producing surface-displayed Mycobac-

May/June 2018 Volume 3 Issue 3 e00061-18 mSphere.asm.org 14
terium tuberculosis antigens. Appl Environ Microbiol 83. https://doi.org/10.1128/AEM.02782-16.

138. Kajikawa A, Igimi S. 2010. Innate and acquired immune responses induced by recombinant Lactobacillus casei displaying flagellin-fusion antigen on the cell-surface. Vaccine 28:3409–3415. https://doi.org/10.1016/j.vaccine.2010.02.077.

139. Kajikawa A, Satoh E, Leer RJ, Yamamoto S, Igimi S. 2007. Intragastric immunization with recombinant Lactobacillus casei expressing flagellar antigen confers antibody-independent protective immunity against Salmonella enterica serovar Enteritidis. Vaccine 25:3599–3605. https://doi.org/10.1016/j.vaccine.2007.01.055.

140. Krüger C, Hu Y, Pan Q, Marcotte H, Hultberg A, Delwar D, van Dalen PJ, Pouwels PH, Leer RJ, Kelly CG, van Doiennenweerd C, Ma JK, Hammarström L. 2002. In situ delivery of passive immunity by lactobacilli producing single-chain antibodies. Nat Biotechnol 20:702–706. https://doi.org/10.1038/nbt0702-702.

141. Hernani MDL, Ferreira PC, Ferreira DM, Miyaji EN, Ho PL, Oliveira ML. 2011. Nasal immunization of mice with Lactobacillus casei expressing the pneumococcal surface protein C primes the immune system and decreases pneumococcal nasopharyngeal colonization in mice. FEMS Immunol Med Microbiol 62:263–272. https://doi.org/10.1111/j.1574-695X.2011.00809.x.

142. Oliveira ML, Monedero V, Miyaji EN, Leite LC, Lee Ho P, Pérez-Martínez G. 2003. Expression of Streptococcus pneumoniae antigens, PsA (pneumococcal surface antigen A) and PspA (pneumococcal surface protein A) by Lactobacillus casei. FEMS Microbiol Lett 227:25–31. https://doi.org/10.1016/S0378-1097(03)00645-1.

143. Mansour NM, Abdelaziz SA. 2016. Oral immunization of mice with engineered Lactobacillus gasseri N713 strain expressing Streptococcus pyogenes M6 antigen. Microbiol Immunol 60:527–532. https://doi.org/10.1111/1348-0421.12397.

144. Piard JC, Hautefort I, Fischetti VA, Ehrlich SD, Fons M, Gruss A. 1997. Cell wall anchoring of the Streptococcus pyogenes M6 protein in various lactic acid bacteria. J Bacteriol 179:3068–3072. https://doi.org/10.1128/jb.179.9.3068-3072.1997.

145. Okuno T, Kashihe N, Satho T, Irie K, Hiramatsu Y, Sharmin T, Fukumitsu Y, Iyeda S, Yamada S, Harakuni T, Miyata T, Arakawa T, Imoto M, Toda A, Nakashima Y, Mieke F. 2013. Expression and secretion of cholera toxin B subunit in lactobacilli. Biol Pharm Bull 36:952–958. https://doi.org/10.1248/bpb.b12-01021.

146. Beltran S, Munoz-Bergmann CA, Elola-Lopez A, Quintana J, Segovia C, Trombert AN. 2016. The expression of heterologous MAM-7 in Lactobacillus rhamnosus reduces its intrinsic capacity to inhibit colonization of pathogen Vibrio parahaemolyticus in vitro. Biol Res 49:2. https://doi.org/10.1186/s40659-015-0064-1.

147. Fredriksen L, Kleiveland CR, Hult LT, Lea T, Nygaard CS, Eijssink VG, Mathiesen G. 2012. Surface display of N-terminally anchored invasin by Lactobacillus plantarum activates NF-kappaB in monocytes. Appl Environ Microbiol 78:5864–5871. https://doi.org/10.1128/AEM.01227-12.