Review Article

iMedPub Journals
http://www.imedpub.com

Archives of Clinical Microbiology
ISSN 1989-8436

2021
Vol. 12 No. S2:149

Potential for Mucosal Delivery of *Bacillus subtilis* Spore Vaccines Displaying Immunogenic Protein on the Surface

Yeonsu Oh*

Department of Veterinary Pathology, College of Veterinary Medicine & Institute of Veterinary Science, Kangwon National University, Chuncheon 24341, Republic of Korea

Abstract

Vaccines are the most effective measures to prevent infectious diseases, and most of these infections occur through the mucous membranes that cover the surfaces of the vital organs of our body. Mucosal vaccination is, however, challenging. The numerous natural defense mechanisms at mucosal surfaces, including the acidic and enzyme-rich environment and the thick and firm mucus layer make the delivery of vaccines across these natural barriers challenging.

Probiotic-grade *B. subilis* spores could be utilized by the surface display technology as a mucosal vaccine delivery system and simultaneously as an adjuvant for mucosal immunity for the following reasons:

1. *B. subtilis* spores are resistant at ambient temperatures but remain viable.
2. *B. subtilis* spores are safe enough for consumption by humans as food components, probiotics, or therapeutics.
3. *B. subtilis* can be genetically manipulated, making it possible to engineer bacteria that express and display immunogens on the spore surface or in vegetative cells.
4. *B. subtilis* spores can serve as non-invasive vaccine delivery systems.

As a vaccine with 'needle-free' administration that is easy to store and transport under extreme conditions and does not require injection, the need for development is deemed to be high, as it has high application value for mass vaccination such as in a widespread disease outbreaks. A high-efficiency expression system on *Bacillus* spore, the control of proteolytic enzymes and redesign of genes encoding target proteins have been rapidly advanced.

It would be interesting to explore whether there are any particular spore types or *Bacillus* strains that show enhanced immunity with antigens displayed on the spore surface. Further discussion should be dedicated to other promising specific antigens and immunization routes that may lead to longer-lasting and more-efficacious vaccines with available technology.

Keywords: *Bacillus subtilis*; Spore; Surface display; Mucosal vaccine

***Corresponding author:** Oh Y,

Department of Veterinary Pathology, College of Veterinary Medicine & Institute of Veterinary Science, Kangwon National University, Chuncheon 24341, Republic of Korea.

yeonoh@kangwon.ac.kr

Citation: Oh Y (2021) Potential for Mucosal Delivery of *Bacillus subtilis* Spore Vaccines Displaying Immunogenic Protein on the Surface. Arch Clin Microbio Vol.12 S2: 149

Introduction

Vaccines are the most effective measures to prevent infectious diseases, and most of these infections occur through the mucous membranes that cover the surfaces of the respiratory, gastrointestinal and urogenital systems as well as the conjunctiva [1,2]. The delivery of vaccines across mucosal surfaces has the potential to stimulate the synthesis of pathogen-specific mucosal immune responses at the site of entry [2]. Mucosal vaccination is, however, challenging. In general, vaccines delivered *via* mucosal routes are poorly immunogenic because these antigens are easily degraded by the numerous natural defense mechanisms of hosts at mucosal surfaces including the acidic and enzyme-rich environment of the stomach and the thick and firm mucus layer in all mucous membranes [3]. However, considering the route of infection, the development of a mucosal adjuvant and/or a

delivery vehicle for mucosal immunity is critical for manufacturing a vaccine that acts against many pathogens. Success in generating this first-line of defense on mucosal surfaces and ultimately systemic immunogenicity will represent a major advance in vaccinology [4].

The Surface Display Technology is Promising

The surface display system is a protein engineering technique used for the functional evolution of proteins *in vitro*/*in vivo*. Since the first successful display of an antibody and its library on a phage surface [5], remarkable achievements have been made in a wide variety of applications, including peptide and/or antibody library screening [6,7], mass production of biological products such as enzymes [8], and the creation of novel vaccines.

Many types of bacteria express surface proteins to attach to and invade host cells. These proteins can be recognized by host cells, inducing immunogenicity and enabling the production of functionally upgraded immunological products. Vaccine delivery has become a very common application of bacterial surface displays, and the bacteria used can be divided into two broad categories: weakened bacteria that are no longer pathogenic and commensal or food-grade probiotic bacteria that are generally recognized as safe (GRAS) [9].

Ancient Traditional Food Now Becomes a Safe and Effective Vaccine

B. subtilis is a common Gram-positive, rod-shaped soil bacterium that is often used as a model organism for the study of cellular differentiation and morphogenesis in microbiology. *B. subtilis* spores are dormant forms of this microorganism that are well known for their resistance to harsh environmental conditions. *B. subtilis* spores have been consumed through traditional fermented food such as soybean paste, cheonggukjang, and natto for a long time in the eastern cuisine culture of the Earth, and have successfully been used as probiotics for both humans and animals [9,10]. One of the most interesting applications is the use of *B. subtilis* spores as stable carriers of antigens for use as mucosal vaccines with those properties [11]. Especially, proteins displayed on the surface of *B. subtilis* spores have unique resistance to harsh conditions and can easily pass through the gastrointestinal barrier, making them excellent vehicles for orally administered vaccines. Historically, the spore surface display technique was first applied in the field of oral vaccines. A vaccine precursor that expressed the 459-amino-acid C-Terminal Fragment of the Tetanus Toxin (TTFC) was developed with the aid of the CotB fusion partner [12]. This provided the first evidence that an efficient surface expression system using the properties of *B. subtilis* (simple purification, high stability and safety) may be promising for the preparation of bioactive components. Soon afterwards, exogenous TTFC proteins were successfully displayed on the spore for use as an oral vaccine [13-15]. Meanwhile, the VP28 envelope protein of the white spot syndrome virus is considered a candidate antigen for resisting shrimp pathogens [16,17].

Many licensed vaccines delivered by a needle and syringe, induce only suboptimal immunity, rather than strong pathogen-specific mucosal immunity, and require multiple boosts to induce a robust immune response. Some vaccines demand more than three multiple injections intramuscularly and leave local reactogenicity at the injection site, such as the anthrax, tetanus, pertussis and diphtheria vaccines [18-21].

Issues such as the development of a high-efficiency expression system that enables spore display have posed major challenges in the implementation of spore display technology to date, and the control of proteolytic enzymes and redesign of genes encoding target proteins to be expressed in *Bacillus* have been rapidly advanced. Most of these problems can be solved. In other words, genome library screening, ultrafast screening for expression, and advanced in synthetic biology have dramatically improved the above mentioned problem-solving capabilities, allowing the optimization of entry conditions and timing, which is a challenge in technological development [6-8].

Long-term study of the *Bacillus* fermentation process has shown that the production is relatively simple and economical. *Bacillus* spores are stable even after production and do not require a cold-chain system during distribution. Those characteristics have attracted attention for a long time in terms of favorable culture conditions for large-scale fermentation and superior capacity for protein secretion [22].

Spore Vaccines Offer a Myriad of Advantages

In vaccine delivery, displaying antigens on GRAS probiotic bacteria has advantages over conventional vaccine design for many reasons. One of the reasons is that the probiotic-bacteria themselves have useful functions within the treated host, such as adjuvant-like action [23]. These so-called multifunctional adjuvants are urgently needed as highly sophisticated and novel vaccine antigens are developed such as messenger RNA vaccines against SARS-CoV-2.

Spore vaccines offer a myriad of advantages such as aid in mass vaccinations by increasing the ease and speed of delivery, decreased costs by elimination of purification steps, and flexible administration *via* mucosal and/or oral routes, thus providing 'needle-free' and 'refrigeration-free' vaccine delivery systems [23,24]. As mucosal vaccines, spore vaccines could also be self-administered, reducing the burden on healthcare professionals [4] and reducing needle phobia especially in pediatrics.

Bacillus Spore as an Immune Enhancer

Another unique feature is that spores have sub-micron scale nanostructures, allowing them to serve as effective particulate adjuvants [25]. Particulate adjuvants, such as liposomes, virosomes, virus-like particles, Poly-Lactide-Co-Glycolide (PLG) microspheres and immune-stimulating complexes (ISCOMS),

sufficiently target Antigen Presenting Cells (APCs) and once internalized within the cell are processed by the class I and class II Major Histocompatibility Complex (MHC) pathways, leading to antigen presentation on the surface of the APC [26]. Studies investigating the adjuvanticity of *B. subtilis* spores suggested that strong auxiliary effects were observed when the spores were co-administered with protein antigens that were either admixed or adsorbed on the spore coat surface [25].

In addition, as *Bacillus* spores own immunity-enhancing effects began to be known, it was found that the spore particle size was suitable for enhancing mucosal immunity. Optimization of spore displays has allowed the development of effective immunity-enhancing vaccine agents.

Probiotic *B. subtilis* spores have been mentioned to be immunostimulatory in many other studies [27,28-30]. Interestingly, when given at a high dose, spores alone were able to protect against H5N1 influenza virus challenge in a mouse model [29,30], and mice treated with spores alone presented meaningful antibody isotype profiles in sera together with mice treated with the Protective Antigen (PA) of *B. anthracis* displayed spore. Particularly notable was the increased anti-PA-specific IgA response observed in the saliva. The results are consistent with other studies showing that needle-free mucosal vaccines can induce immune responses at both the systemic and mucosal levels [31]. In addition, *B. subtilis* spore vaccines displaying the PA were successfully delivered across mucosal surfaces orally and induced systemic immune responses similar to those induced by conventional needle-and-syringe-based vaccination [21]. The *B. subtilis* spore vaccine efficiently instructed and augmented polyvalent antigen-specific CD4+ and CD8+ T cell effector responses in the presence of antigen. The adjuvanticity of the spores drove a 'balanced' T helper response, and the strength of which could be increased by repeated boosting in the presence of spores. Mucosal administration led to enhanced mucosal and systemic IgA and IgG levels in response to the co-administered antigen, and presented IgA is more broadly protective than other immune molecules against foreign invaders entering our bodies through mucosal barriers [32].

Other studies have demonstrated that orally administered *B. subtilis* spores germinate in the murine gut, disseminate to the Gut-Associated Lymphoid Tissue (GALT), and enter Peyer's patches and mesenteric lymphoid tissues [13,33]. Spores displaying antigens were also shown to confer a germination-independent immune response [34].

Conclusion

The resilience of spores, coupled with a mucosal route of delivery, makes spore vaccines promising candidates especially when mass production is urgently required and large-scale vaccination is needed. The development of vaccines, which is still ongoing, is progressing towards vaccines being less invasive, more patient friendly, and easy to store and transport, and multiple adverse effects can be expected from a single dose.

Funding

This research was supported by the Technology Development Program for Bio-industry (Project No. 1116043-1), Ministry for Agriculture, Food and Rural Affairs, Republic of Korea.

References

1. Chevalier-Cottin EP, Ashbaugh H, Brooke N, Gavazzi G, Santillana M, et al. (2020) Communicating benefits from vaccines beyond preventing infectious diseases. Infect Dis Ther 9: 467-480.
2. Holmgren J, Czerkinsky C (2005) Mucosal immunity and vaccines. Nat Med 11: S45-S53.
3. Tamura S, Ito Y, Asanuma H, Hirabayashi Y, Suzuki Y, et al. (1992) Cross-protection against influenza virus infection afforded by trivalent inactivated vaccines inoculated intranasally with cholera toxin B subunit. J Immunol 149(3): 981–8.
4. Shakya AK, Chowdhury MYE, Tao W, Gill HS (2016) Mucosal vaccine delivery: current state and a pediatric perspective. J Control Release 240: 394-413.
5. Smith GP (1985) Filamentous fusion phage: Novel expression vectors that display cloned antigens on the virion surface. Science 228: 1315-1317.
mucosal
6. Freudl A, Maclntyre S, Degen M, Henning U (1986) Cell surface exposure of the outer membrane protein OmpA of Escherichia coli K-12. J Mol Biol 188: 491-494.
7. Charbit A, Boulain JC, Ryter A, Hofnung M (1986) Probing the topology of a bacterial membrane protein by genetic insertion of a foreign epitope; expression at the cell surface. EMBO J 5: 3029-3037.
8. Georgiou G, Stathopoulos C, Daugherty PS, Nayak AR, Iverson BL, et al. (1997) Display of heterologous proteins on the surface of microorganisms: from the screening of combinatorial libraries to live recombinant vaccines. Nat Biotechnol 15: 29-34.
9. Cutting SM (2011) Bacillus probiotics. Food Microbiol 28: 214–220.
10. Inatsu Y, Nakamura N, Yuriko Y, Fushimi T, Watanasiritum L, et al. (2006) Characterization of Bacillus subtilis strains in Thua nao, a traditional fermented soybean food in northern Thailand. Lett Appl Microbiol 43: 237-242.
11. Potocki W, Negri A, Peszyńska-Sularz G, Hinc K, Obuchowski M, et al. (2017) The combination of recombinant and non-recombinant Bacillus subtilis spore display technology for presentation of antigen and adjuvant on single spore. Microb Cell Fact 16: 151.
12. Isticato R, Cangiano G, Tran HT, Ciabattini A, Medaglini D, et al. (2001) Surface display of recombinant proteins on Bacillus subtilis spores. J Bacteriol 183: 6294-6301.
13. Duc le H, Hong HA, Fairweather N, Ricca E, Cutting SM (2003) Bacterial spores as vaccine vehicles. Infect Immun 71: 2810-2818.
14. Uyen NQ, Hong HA, Cutting SM (2007) Enhanced immunisation and expression strategies using bacterial spores as heat-stable vaccine delivery vehicles. Vaccine 25: 356-365.
15. Ciabattini A, Parigi R, Isticato R, Oggioni MR, Pozzi G (2004) Oral priming of mice by recombinant spores of Bacillus subtilis. Vaccine 22: 4139-4143.

16. Van Hulten MCW, Witteveldt J, Snippe M, Vlak JM (2001) White spot syndrome virus envelope protein VP28 is involved in the systemic infection of shrimp. Virology 283: 228-233.

17. Yi G, Wang Z, Qi Y, Yao L, Qian J, et al. (2004) Vp28 of shrimp white spot syndrome virus is involved in the attachment and penetration into shrimp cells. J Biochem Mol Biol 37: 726-734.

18. Skowera A, de Jong EC, Schuitemaker JH, Allen JS, Wessely SC, et al. (2005) Analysis of anthrax and plague biowarfare vaccine interactions with human monocyte-derived dendritic cells. J Immunol 175: 7235-7243.

19. Storsaeter J, Wolter J (2006) Is there a need for a new generation of vaccines against pertussis? Expert Opin Emerg Drugs 11: 195-205.

20. Trollfors B, Knutsson N, Taranger J, Mark A, Bergofors E, et al. (2006) Diphtheria, tetanus and pertussis antibodies in 10-year-old children before and after a booster dose of three toxoids: implications for the timing of a booster dose. Eur J Pediatr 165: 14-18.

21. Oh Y, Kim JA, Kim CH. Choi SK, Pan JG (2020) Bacillus subtilis spore vaccines displaying protective antigen induce functional antibodies and protective potency. BMC Vet Res 16:259.

22. Zhang X, Al-Dossary A, Hussain M, Setlow P, Li J (2020) Application of Bacillus subtilis spores in biotechnology and advanced materials. Appl Environ Microbiol 86: e01096-20.

23. Amuguni H, Tzipori S (2012) Bacillus subtilis: A temperature resistant and needle free delivery system of immunogens. Hum Vaccin Immunother 8: 979–986.

24. Pan JG, Kim EJ, Yun CH (2012) Bacillus spore display. Trends Biotechnol 30:610-612.

25. Barnes AG, Cerovic V, Hobson PS, Klavinskis LS (2007) Bacillus subtilis spores: A novel microparticle adjuvant which can instruct a balanced Th1 and Th2 immune response to specific antigen. Eur J Immunol 37: 1538–1547.

26. Singh M, O'Hagan D (1999) Advances in vaccine adjuvants. Nat Biotechnol 17:1975–1981.

27. Robinson K, Chamberlain LM, Schofield KM, Wells JM, Le Page RWF (1997) Oral vaccination of mice against tetanus with recombinant Lactococcus lactis. Nat Biotech 15:653–657.

28. Kawamura F, Doi RH (1984) Construction of a Bacillus subtilis double mutant deficient in extracellular alkaline and neutral proteases. J Bacteriol 160: 442–444.

29. Jeong H, Jeong DE, Park SH, Kim SJ, Choi SK (2018) Complete genome sequence of Bacillus subtilis strain WB800N, an extracellular protease-deficient derivative of strain 168. Microbiol Resourc Announc 7: e01380–18.

30. Ricca E, Baccigalupi L, Cangiano G, De Felice M, Isticato R (1984) Mucosal vaccine delivery by non-recombinant spores of Bacillus subtilis. Microb Cell Factories 13:115.

31. Jeong DE, So Y, Park SY, Park SH, Choi SK (2018) Random knock-in expression system for high yield production of heterologous protein in Bacillus subtilis. J Biotechnol 266: 50-58.

32. Challacombe SJ (1983) Salivary antibodies and systemic tolerance in mice after oral immunization with bacterial antigens. Ann N Y Acad Sci 409: 177-192.

33. Duc le H, Hong HA, Atkins HS, Flick-Smith HC, Durrani Z, et al. (2007) Immunization against anthrax using Bacillus subtilis spores expressing the anthrax protective antigen. Vaccine 25: 346-55.

34. Mauriello EM, Cangiano G, Maurano F, Saggese V, De Felice M, et al. (2007) Germination-independent induction of cellular immune response by Bacillus subtilis spores displaying the C fragment of the tetanus toxin. Vaccine 2:788-793.