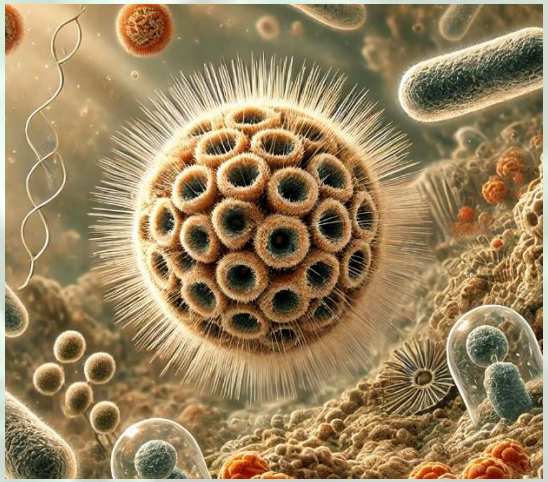
Bacterial Spore-Based Delivery System: 20 Years of a Versatile Approach for Innovative Vaccines

Presented by:

M.Zolfaghari

Importance of Vaccines in Human Health

  - COVID-19 pandemic impact: 359 million infections, 5.6 million deaths.
  - Vaccines' role in preventing widespread devastation.
- **Types of Vaccines**
  - Live, attenuated or killed viruses/bacteria.
  - Subunit vaccines: polysaccharides, nucleic acids, proteins.
- **Challenges in Vaccine Development**
  - Supply chain problems, storage complications, and high costs.



Challenges in Vaccine Design and Mucosal Immunity

- **Key Challenge in Vaccine Design**
  - Balancing systemic immunity and protective mucosal responses.
- **Importance of Mucosal Immunity**
  - Secretory IgA (sIgA) synthesis is critical to block pathogens at the entry site.
  - Traditional vaccines induce low mucosal immunity when delivered systemically.
- **Advantages of Oral/Nasal Vaccine Delivery**
  - Enhances local immunity (e.g., mucosal surfaces).
  - Non-invasive
  - Eliminates needle-related risks and bypasses hepatic metabolism.
  - Generation of cross-reactive immune response

- **Current Limitations**
  - Only 9 mucosal vaccines approved for human use.
  - Mostly live attenuated or whole-cell inactivated vaccines
  - Limited due to greater tolerability of orally administered whole-cell killed antigens
  - Instability of antigens and susceptibility to proteases/endonucleases.

	Trade Name	*Approved*	*Antigen*	*Type of vaccine*	*Form/Adm*	*Immunological mechanism*
 era	**Dukoral®**	2003 CANADA	*Vibrio cholerae* +CTB	Live attenuated	Aqueous/Oral	Antibacteria, toxin -specific and LPS-specific IgA
	Euvichol Shanchol	2013 WHO	*Vibrio cholerae*	Inactivated	Aqueous/Oral	
	Vaxchora™	2015 FDA	*Vibrio cholerae*	Live attenuated	Aqueous/Oral	
 Acute gastroenteritis	**Vivotif®**	2013 FDA	*Salmonella typhimurium*	Live attenuated	Capsule/Oral	Mucosal IgA, systemic IgG and CTL responses
 Influenza	**Fluenz™/FluMist®**	2003 FDA	Influenza A and B viruses	Live attenuated	Spray/Nasal	Mucosal IgA and CTL responses
 Polio	**OPV (b/m/tOPv)**	1961 FDA	Poliovirus	Live attenuated	Aqueous/Oral	Mucosal IgA, systemic IgG
 nfant iarrhea	**RotaTeq®**	2006 FDA	Rotavirus	Live reassortant	Aqueous/Oral	Mucosal IgA, systemic neutralizing IgG
	Rotarix	2008FDA	*RIX4414 strain*	Live attenuated	Aqueous/Oral	
 respiratory disease	**Adenovirus Type 4 and 7 Vaccine**	2015 FDA	Adenovirus Type 4 and 7	Adenovirus vector vaccine	Aqueous/Oral	

Critical Needs for Advancing Mucosal Vaccines

- Development of safe and effective mucosal adjuvants.
- Innovative antigen delivery strategies required.

Sporulation and Intestinal Life Cycle

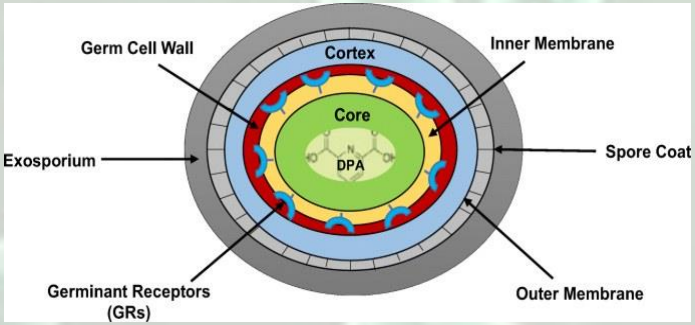
- **Germination and Gut Interaction**:
- Spores pass through the stomach and germinate in the small intestine.
- May sporulate again in the lower intestine.

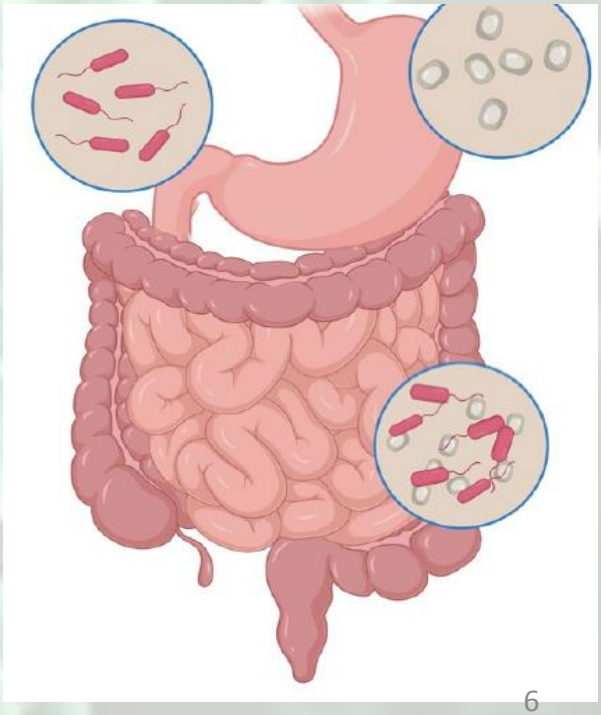
- Contribution to balance of gut Microbiota and facilitating digestion

And support gut health.

- Help modulate the immune response with

secrete hydrolytic enzymes antioxidants vitamins and peptides and antimicrobial compounds





Advantages of Spore-Based Vaccine Systems

- **Spore Stability:** Bacillus spores have extreme resistance to high temperatures, acidic pH, and chemicals, making them ideal for vaccine formulation.

- **Extreme Stability:** Spores with stand processing, storage, and extreme conditions, ideal for vaccines in developing regions.

- **Safety:** Spore formers are safe for human and animal use, commonly found in probiotics and promoting gut health.

- **Efficient Antigen Display on Spore Surfaces :** All coat proteins are synthesized during sporulation, eliminating the need for complex translocation steps.

- **Large Protein Display:** Large antigens can be fused to spore coat proteins without affecting spore structure, resistance, or germination.

- **Vaccine Development Potential:** This system shows promise for developing stable, safe, and effective mucosal vaccines.

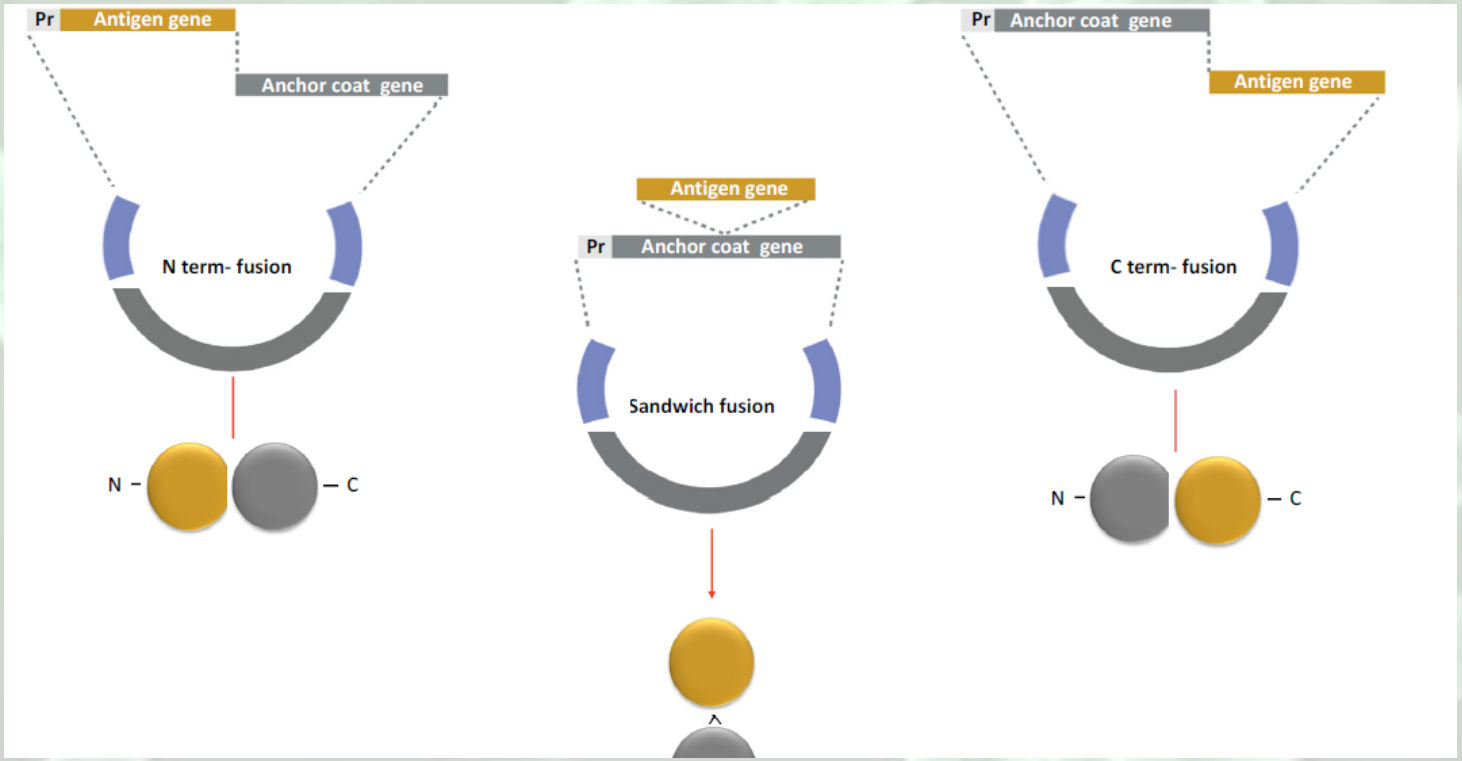
Antigen Display Strategy on Spore Surface

- **Recombinant vaccines:** Immunogenicity depends on factors like protein stability and surface exposition of the antigen.

- **Anchor Protein :**
- ensure proper attachment of the antigen to the spore surface
- allow correct epitope presentation.
- maintain the recombinant antigen's structural stability under stress (pH, temperature, redox conditions) to prevent degradation by proteases.

Antigen Display Strategy on Spore Surface

- **Fusion Types:** occur at the N-terminus, C-terminus, or internally (sandwich fusion) of the anchor protein.



- **Anchor Proteins**
- CotB, CotC, and CotG proteins are used as anchors to display antigens on the spore surface.
- **CotB:** First used in surface display technology with tetanus toxin fragment (TTFC) → induced production of sIgA and IgG in animal → protected mice against tetanus toxin
- **CotC** :display proteins like *Clostridium difficile* toxins
- **CotG:** displaying viral antigens , proteins such as UreA from *H. acinonychis* and a fragment of the flagellar cap protein FliD in *C. difficile*

Other Anchor Proteins (CotZ and CgeA)

- CotZ and CgeA have also been used as anchor proteins.
- CotZ :display *Clostridium difficile* proteins,
- CgeA: Expression of the cytotoxin-associated protein CagA gene of *H. pylori* proteins
- Using different anchor proteins for antigen display in spore-based vaccines shows great potential.

Strategies for Optimizing Antigen Exposure on Spore Surface

1: **Linker Peptides**: various linker peptides (5-11 amino acids)

- ✓ Increase Stability and Flexibility
- ✓ improve antigen exposure
- ✓ Improving Immunogenicity

- ✓ For example:increased the efficiency of antigen display with linkers in **CgeA-CagA** and **CotB-UreA** systems.

Cont....

The choice of linker is critical for the stable and effective display of antigens on spore surfaces.

- ✓ **Longer linkers like GGGEAAAKGGG** : improving the display of the antigen on the spore surface with stable α-helical structures
- ✓ **shorter linkers like GGGGS** :fail to support secondary structures, leading to instability and reduced antigen display.
- ✓ Antigens like **FliD** and **UreA** showed better exposure with flexible, longer linkers.

2. Multi-Antigen Spore-Based Mucosal Vaccine

- **Multifunctional Immune Activation:**
  Display various molecules (antigens, adjuvants) on spore surfaces to activate multiple immune cell types for combating aggressive pathogens.

- **Plasmid Library for Antigen Display:**
  A library of 16 constructs fusing different coat proteins (CotB, CotC, CotG, CotZ, CgeA) with antigens at both N-terminal and C-terminal positions.

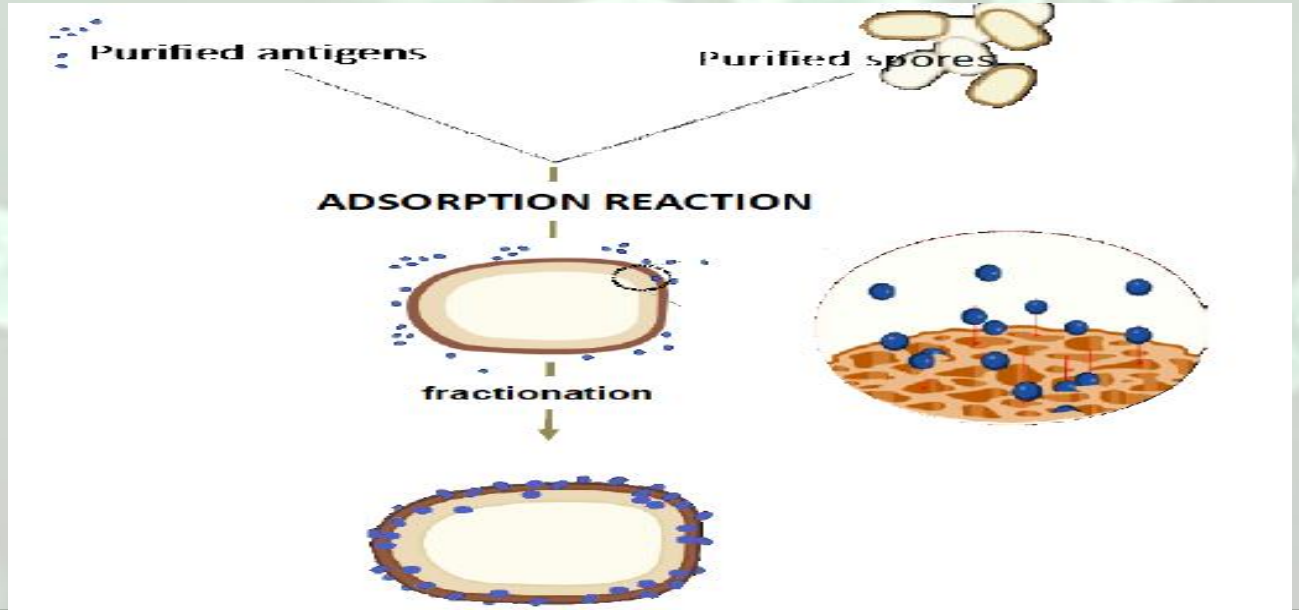
- **Example - *H. acinonychis* UreB Fragment:**
  Spores expressing IL-2 and *H. acinonychis* UreB fragment induced a stronger immune response in mice.

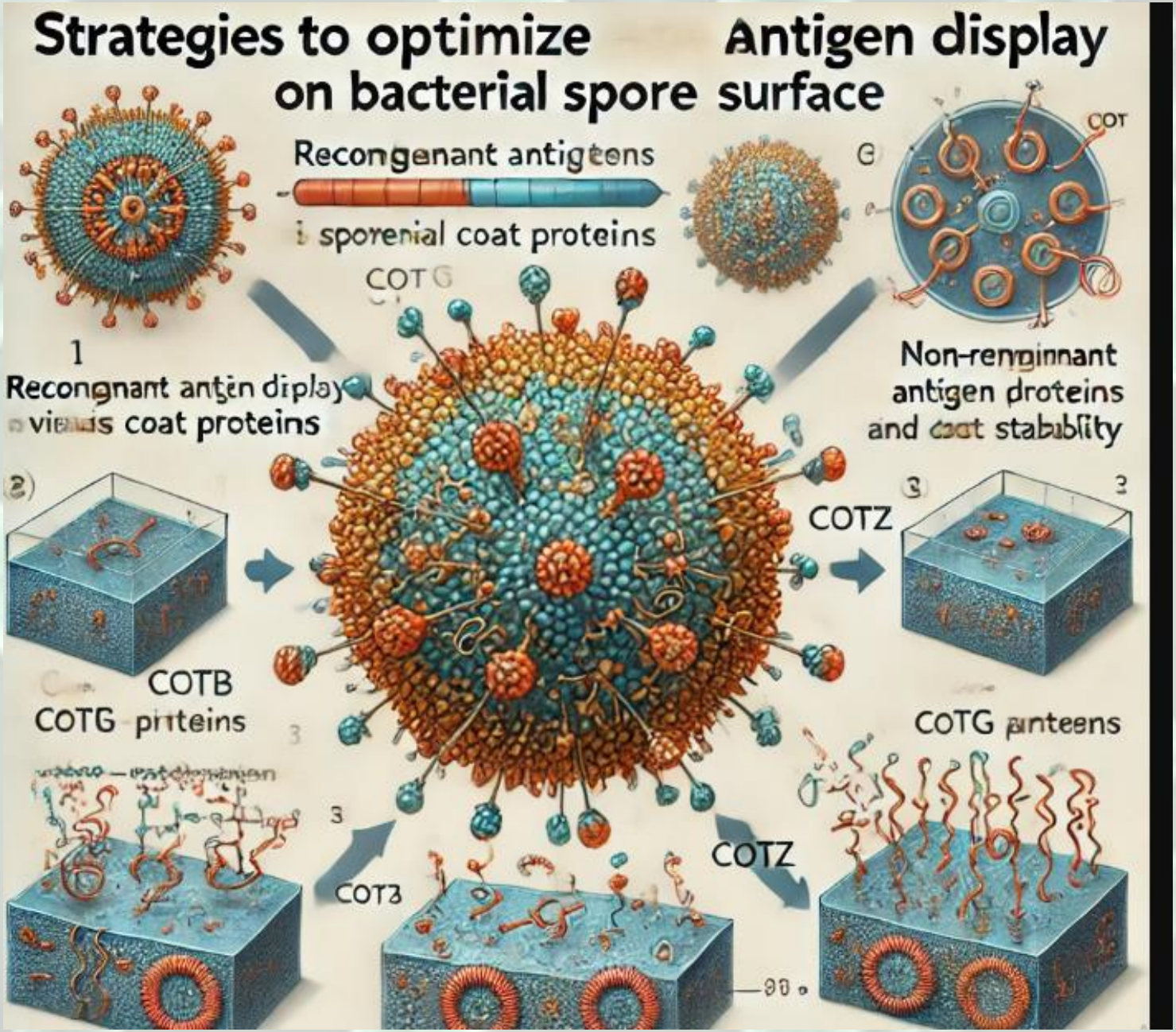
3. Non-Recombinant Antigen Display

- **Non-GMO Approach:**
  Antigens can be non-recombinantly adsorbed onto the spore surface.
- **Spore Properties:**
  Spores' negatively charged and hydrophobic nature aids adsorption.
- **Optimal Conditions:**
  stable at high temperatures and low pH

✓ display antigens in inactivated spores

✓ Control of Antigen Display by Temperature-Dependent Coat Protein Expression in Spores(e.g., producing at 25°C vs. 37–42°C).




Strategies to optimize Antigen display
on bacterial spore surface
Recongenant antigens
sporenial coat proteins
COT G
COT
1
Recongnant antgn diplay
viruis coat proteins
Non-rengginnant
antigen droteins
and coat stablility
COTZ
COTB
COTG
COTG anteens
COTZ
COTB

Conclusions

Health Concern: Mucosal infections are a significant global health issue

Limited Vaccines: Few mucosal vaccines are available due to challenges in delivery systems

Spores:High stability due to remarkable resistance and Safety records of many spore-forming species

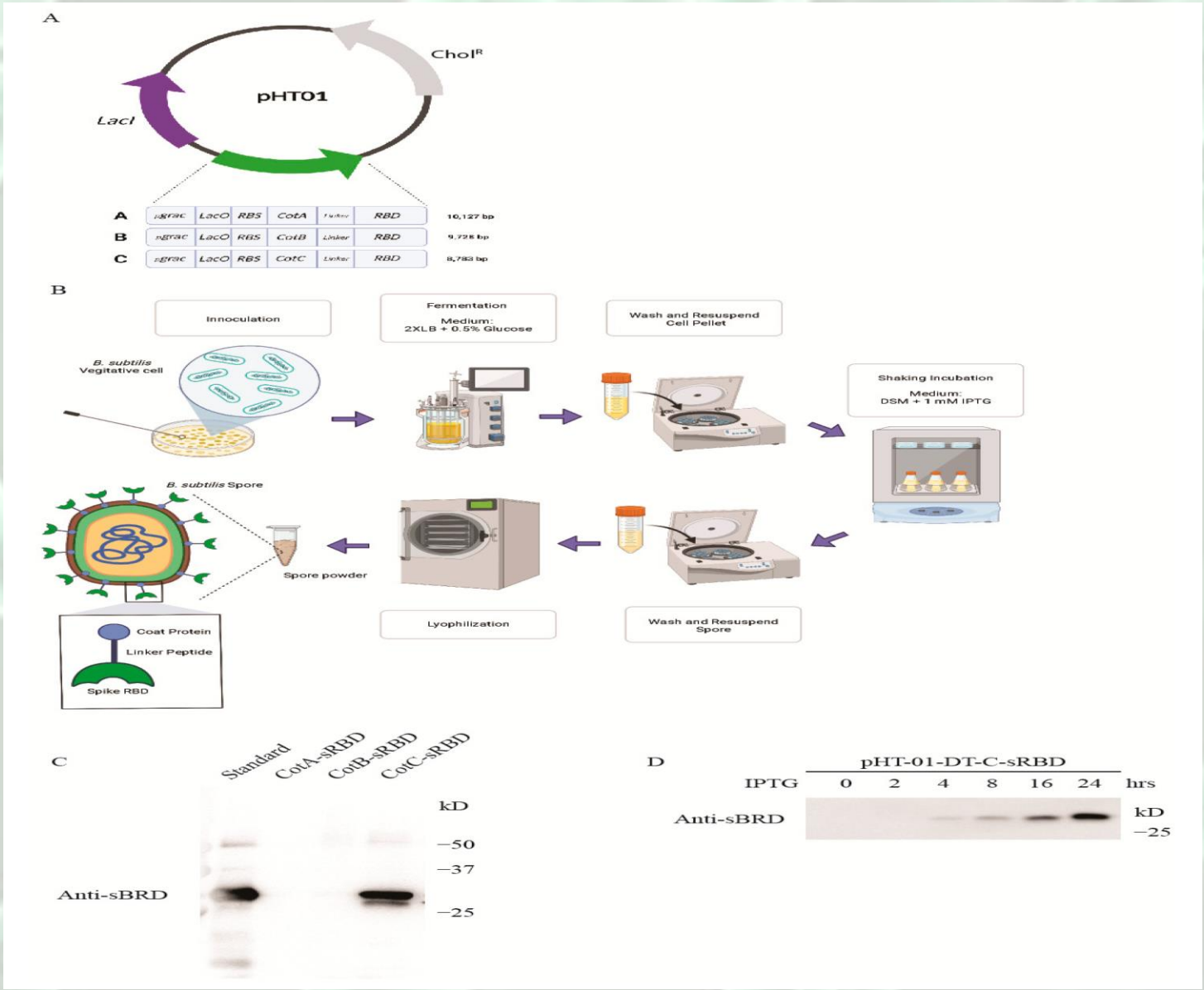
- **Research Examples**:
- Potocki et al. (2017) increased immune response against C. difficile in mice using IL-2-expressing spores.
- Nguyen et al. (2013) demonstrated binding of recombinant spores to tumor cells for targeted drug delivery.

Future Potential

- **Clinical Trials Needed:**
  Spores for antigen display should be further tested in advanced clinical trials.

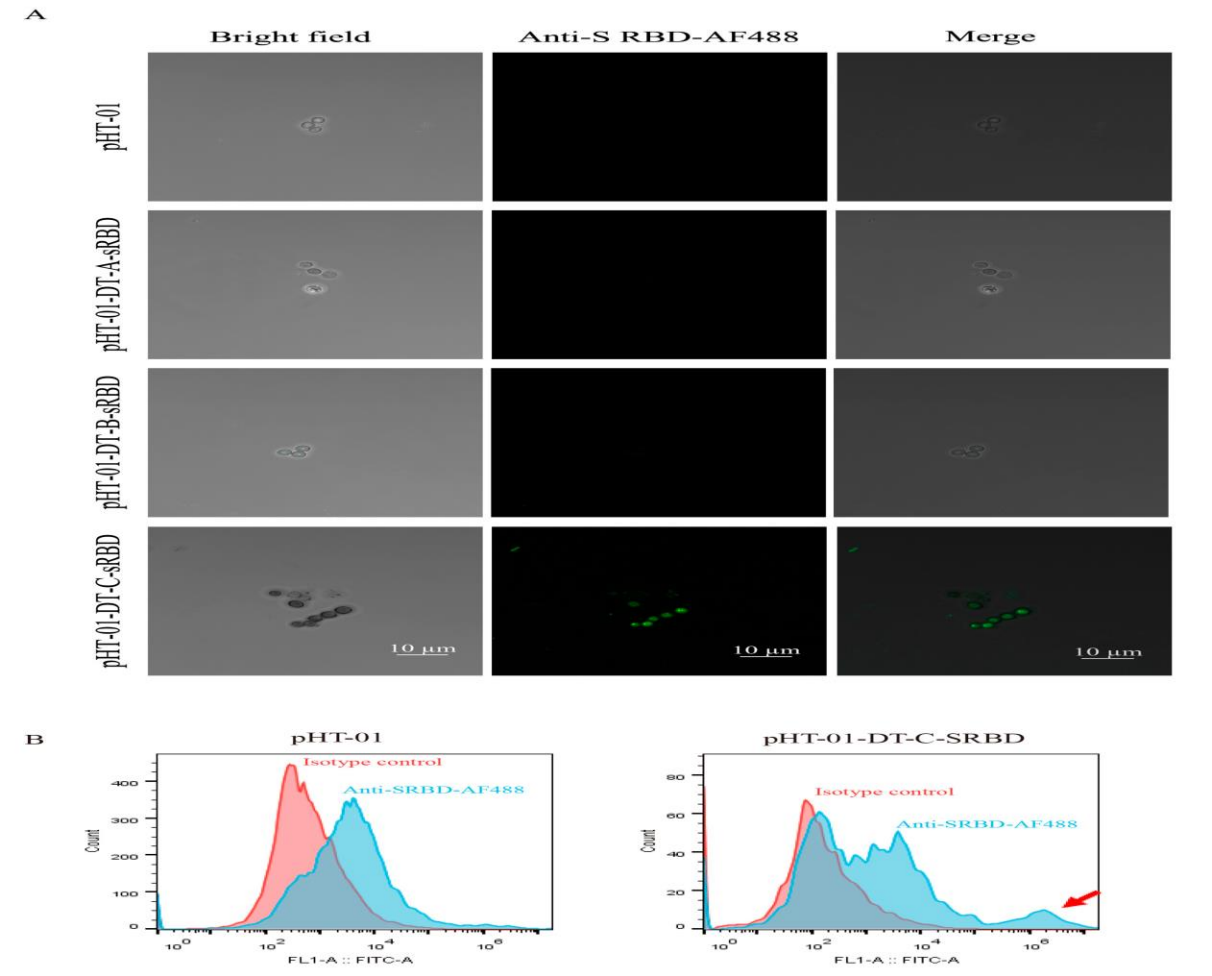
- **Vaccine Benefits:**
  Spores offer a safe, economical, and targeted approach for mucosal vaccines.

Method


A
pHT01
CholR
LacI
A
B
C
LacO
RBS
CotA
CotB
CotC
RBD
10,127 bp
B
Innoculation
Fermentation
Medium:
2XLB + 0.5% Glucose
Wash and Resuspend
Cell Pellet
B. subtilis
Vegitative cell
Shaking Incubation
Medium:
DSM + 1 mM IPTG
B. subtilis Spore
Spore powder
Coat Protein
Linker Peptide
Spike RBD
Lyophilization
Wash and Resuspend
Spore
C
Standard
CotA-sRBD
CotB-sRBD
CotC-sRBD
kD
50
37
25
Anti-sBRD
D
pHT-01-DT-C-sRBD
IPTG
0
2
4
8
16
24
hrs
Anti-sBRD
kD
25

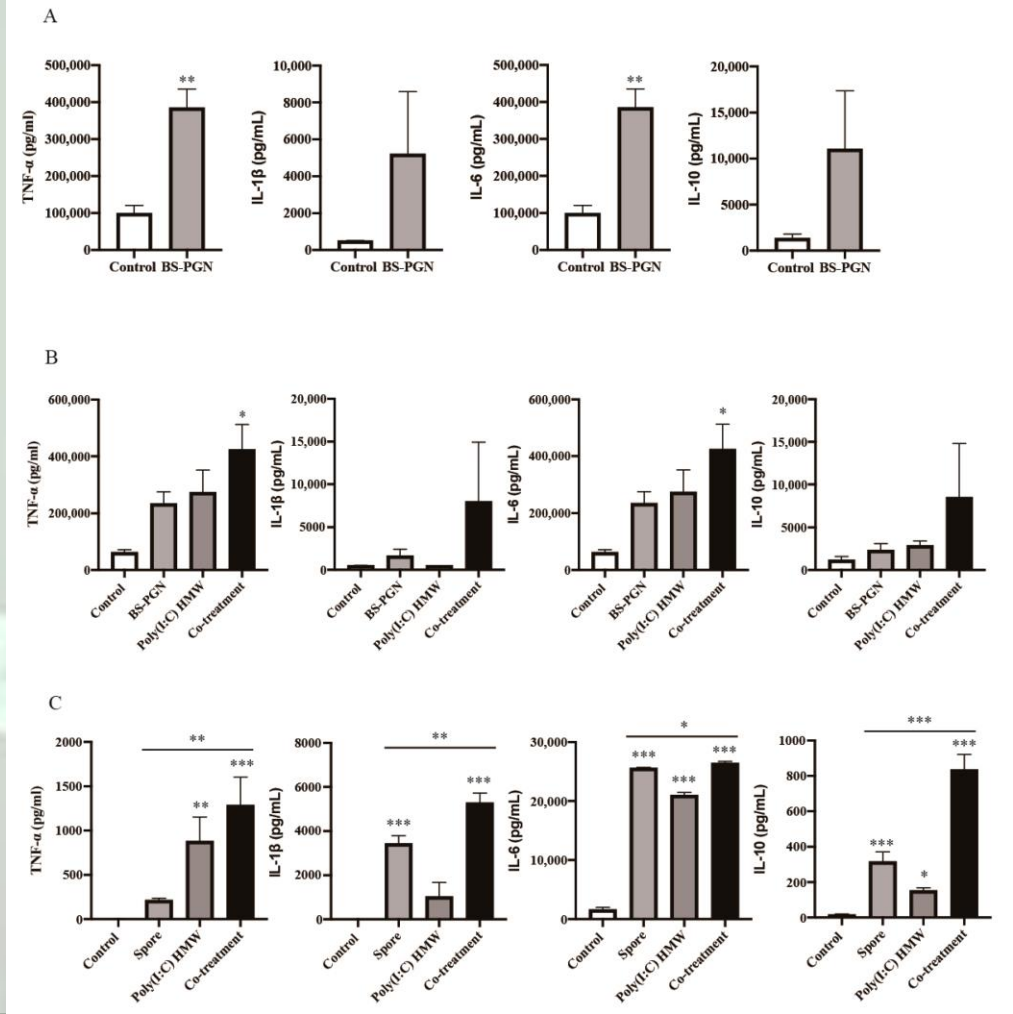
Result

Immunofluorescent staining

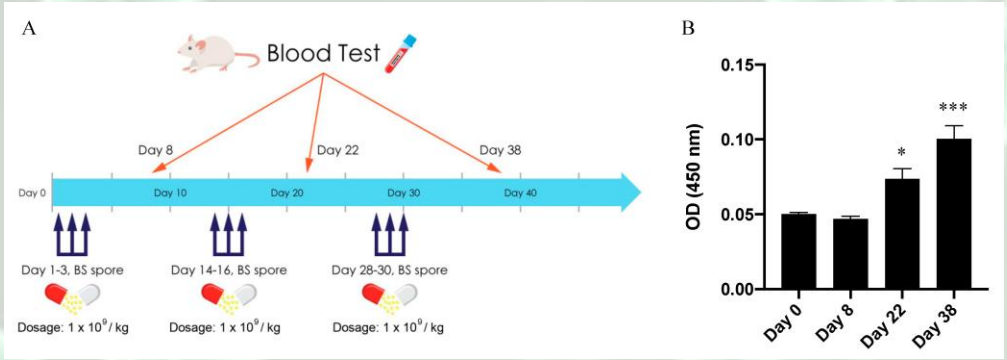


Result

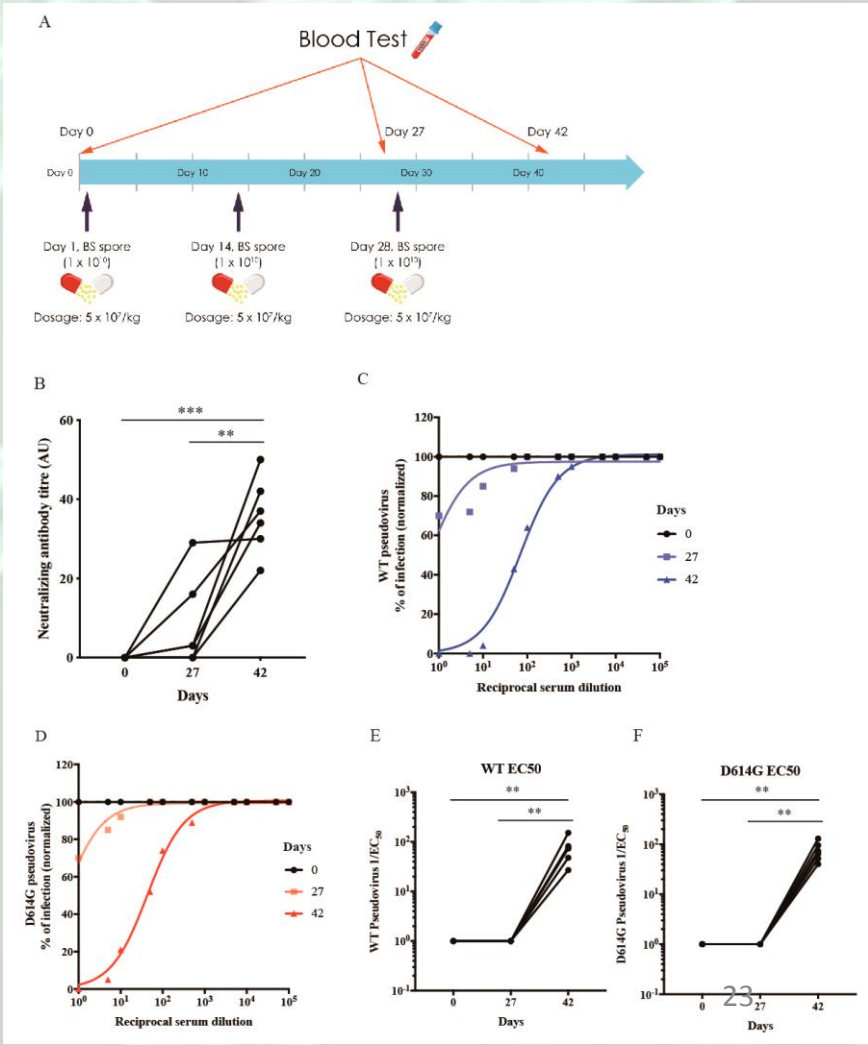
Dendritic cell cultures



Result



Mouse test"



human test

References

I. Isticato R. Bacterial Spore-Based Delivery System: 20 Years of a Versatile Approach for Innovative Vaccines. Biomolecules. 2023 Jun 6;13(6):947. doi: 10.3390/biom13060947. PMID: 37371527; PMCID: PMC10296236.

I. Saggese A, Baccigalupi L, Donadio G, Ricca E, Isticato R. The Bacterial Spore as a Mucosal Vaccine Delivery System. Int J Mol Sci. 2023 Jun 29;24(13):10880. doi: 10.3390/ijms241310880. PMID: 37446054; PMCID: PMC10341597

II. Sung JC, Liu Y, Wu KC, Choi MC, Ma CH, Lin J, He EIC, Leung DY, Sze ET, Hamied YK, Lam DM, Kwong KW. Expression of SARS-CoV-2 Spike Protein Receptor Binding Domain on Recombinant *B. subtilis* on Spore Surface: A Potential COVID-19 Oral Vaccine Candidate. Vaccines (Basel). 2021 Dec 21;10(1):2..


THANK YOU
FOR YOUR
ATTENTION