Defeating the COVID-19 pandemic with bacterial biofilms

The SARS-CoV-2 pandemic has opened a veritable race for innovative vaccination platforms. For example, mRNA vaccines have emerged as highly efficient, safe, and affordable, at least in the developed world. Another new vaccine platform, based on the commensal bacterium *B. subtilis*, may make protection from SARS-CoV-2 infection effective and affordable worldwide.

The COVID-19 pandemic has opened a race without precedent for vaccine development using different strategies, including mRNA encapsidated in liposomes, adenovirus vectors encoding proteins, individual proteins, or inactivated SARS-CoV-2 viruses. The mRNA vaccines proved remarkably successful, and their flexibility allows easy adaptation to mutant viruses. However, mRNA vaccines are relatively expensive and require sophisticated equipment for manufacturing, storage, and distribution that impair their applicability in many countries. There will be no end to this pandemic unless vaccines are available and affordable globally because non-vaccinated populations will continuously bring forth new virus variants that may eventually escape current vaccines.

We are establishing a new vaccination platform based on the display of antigens fused to a biofilm matrix protein of *B. subtilis*, an approved probiotic for humans. The oral administration, high stability, and inexpensive production combined with an efficient elicitation of humoral and cellular immune responses would make this vaccine practicable and affordable worldwide.

Race for a SARS-CoV-2 vaccine

In December 2019, an outbreak of severe acute respiratory syndrome caused by a coronavirus was reported in the Chinese city of Wuhan. The virus, named SARS-CoV-2, disseminated worldwide at an alarmingly rapid speed. In February 2020, it was declared by WHO as the pathogen responsible for the global pandemic, producing a new disease named COVID-19. Since then, COVID-19 has devastated health, education, and economic systems worldwide. Until now (end of April 2021), COVID-19 accounts for over 3.1 million deaths and over 148 million confirmed cases worldwide.

The SARS-CoV-2 is transmitted by air droplets and induces a severe pathological state, including lung thrombosis, frequent diarrhea, abnormal activation of the inflammatory response, and rapid deterioration of lung function by alveolar edema. Multiorgan symptoms after COVID-19 have been reported in an increasing number of patients ranging from cough and shortness of breath to fatigue, headache, palpitations, chest pain, joint pain, physical limitations, depression, and insomnia that persist for at least six months. These long-term consequences of the infection are so-called 'long COVID'.

Vaccines are an obvious route to defeat SARS-CoV-2, and a vaccine race has been taking place since the beginning of the pandemic, with more than 186 vaccines in pre-clinical and 86 vaccines in clinical development. These vaccines are of different categories such as protein subunit vaccines, non-replicating and replicating viral vectors that encode SARS-CoV-2 antigens, nucleic acids, live attenuated, and inactivated virus, and virus-like particles. The vast majority of these vaccines require an intramuscular injection of 1-2 doses administered within 14, 21, or 28 days. Of all these vaccines, only a few have passed clinical trial phases II/III and have been commercialized and distributed for immunization worldwide **(Tab. 1)**.

The main target of all these vaccines is the SARS-CoV-2 spike (S) protein, which mediates the binding of the virus particle to the cellular angiotensin-converting enzyme 2 receptors and facilitates viral entry into the host cells. For example, the vaccines developed by BioNTech/Pfizer and Moderna/NIH consist of lipid nanoparticles that encapsidate mRNA molecules of the SARS-CoV-2 S protein which, when delivered into the cytoplasm of the host, translate into prefusion stabilized full-length S protein. The protein is processed and presented at the cell surface to elicit humoral and cellular immune responses against the virus. This strategy proved to be highly efficient, with a 95% of protection. However, because of their high price and low storage temperature, mRNA vaccines are distributed mainly in developed countries **(Fig. 1)**.

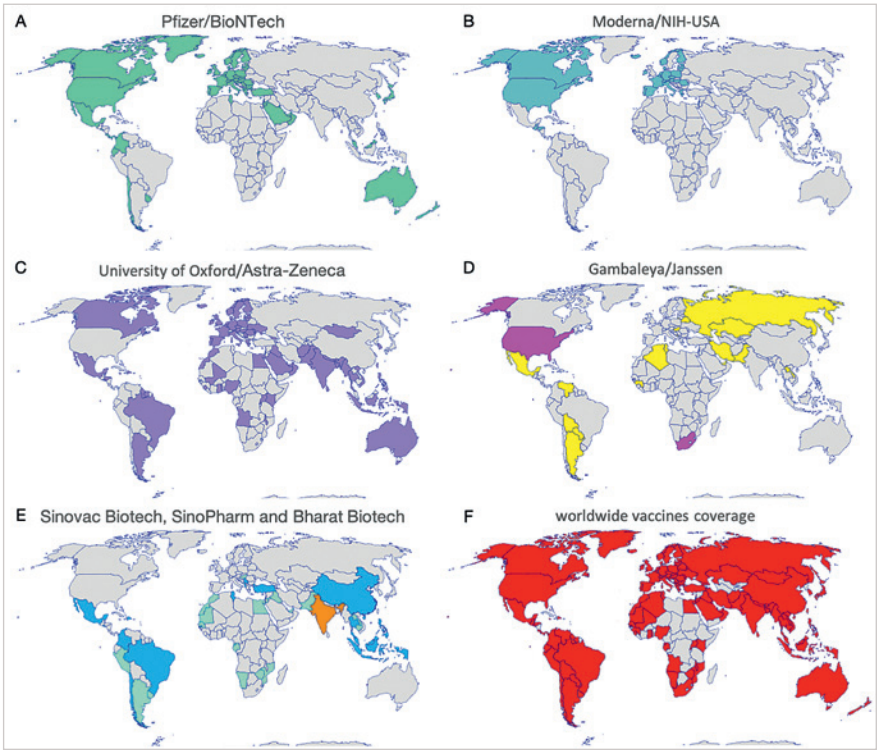

Fig. 1: Worldwide development/application of SARS-CoV-2 vaccines.

SARS-CoV-2 mRNA coated with liposomes from (A) Pfizer/BioNTech and (B) Moderna/NIH-USA.

Adenovirus vectors encoding SARS-CoV-2 proteins from (C) University of Oxford/Astra-Zeneca and (D) Gambaleya (yellow) or Janssen (pink).

(E) Inactivated SARS-CoV-2 from Sinovac Biotech (blue), SinoPharm (turquoise), or Bharat Biotech (orange).

(F) Worldwide use of all vaccines combined.

Data from https://ourworldindata.org/covid-vaccinations

Alternative vaccines include those based on adenovirus (AdV) vectors developed by AstraZeneca/Oxford, Gambaleya, Janssen, and CanSino **(Tab. 1)**, which have an affordable price per dose and do not require extremely low storage temperatures. These vaccines harbor the DNA encoding the S protein encapsidated in an AdV particle. For example, AstraZeneca uses a chimpanzee AdV, while Gambaleya uses the human AdV serotype 26 for the first dose and the AdV serotype 5 for the second dose. Janssen and CanSino both use AdV serotype 5. While these recombinant AdV vectors can enter host cells and deliver the S protein gene to be expressed into the cell nucleus, virus replication and formation of progeny virus are blocked.

Another type of vaccine is based on β-propiolactone-inactivated, replication-defective SARS-CoV-2. This strategy has been used by the Chinese companies SinoVac and Sinopharm, and also by Bharat Biotech from India. Interestingly, vaccines based on partial or full-length S proteins such as NVX-CoV2373 from Novavax or Soberana 02 from BioCubaFarma **(Tab. 1)** show to be highly protective in clinical phase III trials and are promising alternatives.

Coronaviruses RNA polymerases are prone to generate mutations and progeny virus in each replicative cycle differs genetically from the parent viruses. The new variants of SARS-CoV-2 such as UK B.1.1.7, South Africa B.1.351, and Brazil P.1. show mutations particularly in the S protein that improve the binding to the human ACE2 receptor. The Pfizer, Moderna, and Janssen vaccines appear to still be effective for at least the UK and Brazil variants. However, the South African variant escaped the AstraZeneca and Novavax vaccines and with the Pfizer, Moderna, Janssen and SinoPharm vaccines, the efficacy was substantially reduced.

Use of *B. subtilis* as vaccine agent

In addition to their exceptional longevity and resistance, spores of *B. subtilis* are ubiquitous. They can be found in diverse locations like soil, aquatic environments, arctic sediments as well as in the gut of insects and mammalian species, including humans. The robustness of the spores and the safety of spore-formers like *B. subtilis* is what makes this organism an excellent and powerful candidate for a vaccination agent.

An oral vaccine has several advantages over an injected vaccine. For example, there is no need for needles and special training for the administration. More importantly, oral vaccines can elicit mucosal humoral and cellular immunity, which is relevant when pathogens directly infect the intestine and airway mucosa.

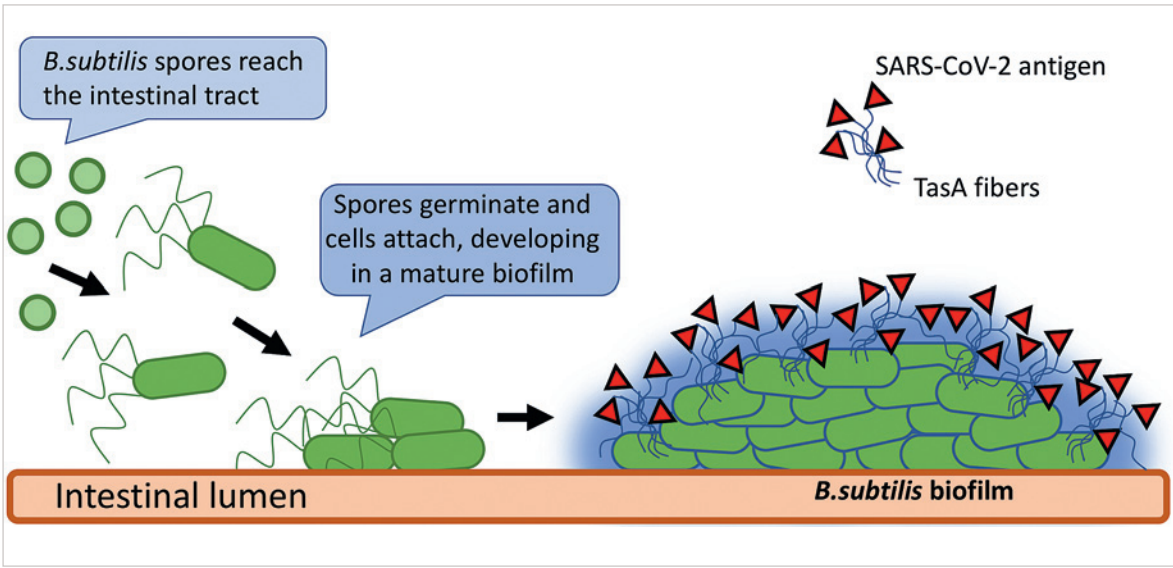

Fig. 2: An oral *B. subtilis* vaccine against SARS-CoV-2. The envisioned vaccine will be administered via the oral route using spores generated from a recombinant *B. subtilis* strain expressing the bacterial TasA protein fused with a SARS-CoV-2 antigen. The spores are able to traverse the stomach barrier, reach the gastrointestinal tract, germinate and develop into a biofilm. Upon biofilm development, expression of TasA (shown as fibers) fused to the antigen (red triangles) begins. The immune system is able to detect the antigen and mount an immune response.

Several studies have been published describing the use of *B. subtilis* spores as an antigen-delivery system to elicit an immune response by expressing antigens from various pathogens (1). The main strategies described include the use of *B. subtilis* expressing antigens from a plasmid or by genetically fusing antigens to outer coat layer proteins of the spore, like CotB, CotC or CotG.

Recently, we developed a novel strategy that takes advantage of the spore's resistance to harsh conditions, combined with the ability of *B. subtilis* to form biofilms (2-4). In this strategy, one of the proteins essential for *B. subtilis* biofilm development, TasA, was fused to antigens of the tapeworm *E. granulosus*. TasA is an abundant component of the extracellular matrix of the biofilm, conferring structural support to the bacterial community. In this vaccination model, the recombinant *B. subtilis* spores are administered via oral route. The spores bypass the stomach barrier, reach the small intestine, germinate and develop into functional biofilms that express the antigens of interest as a fusion protein with TasA. Using dog and mouse models, two antigens from *E. granulosus* fused to TasA were tested in independent experiments, resulting in the elicitation of a humoral response after the application of the recombinant spores (2). Importantly, no detrimental side-effects were observed after the application of the vaccine.

A *B. subtilis* vaccine platform to defeat the COVID-19 pandemic.

Among the hurdles that pharmaceutical companies face is their production capacity, since many of the current vaccines require complicated, delicate, and expensive procedures to get the final product. The high costs per vaccine dose (Tab. 1) and extreme cold chain are additional complications for distribution. There is scientific consensus that SARS-CoV-2 will become endemic, probably requiring vaccinations for years to come. For this reason, we need safe, efficient, and affordable vaccines that can be produced, distributed worldwide and be adapted to new emerging variants of the virus. We envision that an orally applied *B. subtilis*-based vaccine can be part of a global immunization program since it has the prerequisites and advantages of a suitable vaccine:

- It has been efficiently showing to elicit an immune response with no detectable side effects. *B. subtilis* is also a probiotic.
- The genetic manipulation of *B. subtilis* is well-described, facilitating the rapid modification of displayed epitopes, a recurrent topic due to the emergence of virus variants.
- The production of the spores is inexpensive and does not require a cold chain. These aspects will be of particular importance for countries with poor infrastructure.

Name *Pharmaceutical company*	Vaccine type	Efficacy	Immune response	Market price
BNT162b2 *Pfizer/BioNTech*	mRNA	95 % >16 yo; 2 doses 21 days apart; -80 °C storage; 30µg mRNA/dose	NAb; S-specific CD8+ T cells and Th1CD4+ T cells	19.50 USD p. dose
mRNA-1273 *Moderna/NIH-USA*	mRNA	94.1 % >18 yo; 2 doses 28 days apart; 100 µg mRNA/ dose	NAb	25-37 USD p. dose
AZD1222/Vaxzevria *AstraZeneca/University of Oxford*	chimpanzee AdV (ChADOx1/ AXD1222)	63.09 % >18 yo against symptomatic infection; 2 doses 28 days apart	NAb and Ag-specific T cells; Th1 response with IgG1/IgG3 antibodies; mono-, polyfunctional and cytotoxic T CD8+ cells	2.15-5.25 USD p. dose
Sputnik V *The Gambaleya National Research Centre for epidemiology and Microbiology; Academia of military Medical Sciences*	AdV vector serotypes 26 and 5	91.6 %; 2 doses 21 days apart	NAb and high IFN-γ-- CD4+T cells	10 USD p. dose
Ad26, COV2.S *The Janssen Pharmaceutical Companies of Johnson & Johnson*	AdV vector serotype 26	66 % 18-<55 yo, 82.4 % >65 yo; 1 dose	NAb	10 USD p. dose
Ad5-vectored COVID-19 *CanSino Biologics / Chinese military*	AdV5 vector	65.7% preventing symptomatic case, 90.98% preventing severe disease; 1 dose	NAb and T cells (CD4+ cells)	Unknown
CoronaVac *Sinovac Biotech*	Inactivated SARS-CoV-2	50.65-83.3 % 18-59 yo; 2 doses 14 days apart	NAb	29.75-60 USD p. dose
BBIBP-CorV *SinoPharm*	Inactivated SARS-CoV-2	79.34 %; 2 doses 21 days apart	NAb	36-72.50 USD p. dose
Covaxin (BBV152) *Bharat Biotech*	Inactivated SARS-CoV-2	81 % 12-65 yo; 2 doses 28 days apart	NAb and cell-mediated responses	3.35 USD p. dose
NVX-CoV2373 *Novavax*	Subunit vaccine	89.3 % 18-84 yo; 2 doses	NAb	16 USD p. dose
Soberana 02 *BioCubaFarma*	Subunit vaccine	80-95 % clinical stage I & II; 2 doses 14 days apart	NAb	Unknown

Tab. 1: Current vaccines against SARS-CoV-2 available in the market. NAb: neutralizing antibodies; Ag: antigen; yo: years old

This vaccine will consist of a *B. subtilis* strain expressing TasA fused to a selected epitope of SARS-CoV-2 derived from the S protein or the envelope protein E (Fig. 2). Research in the *B. subtilis* biofilm field from recent years has shown that, in addition to TasA, other proteins like BslA are also crucial for biofilm development. Work in progress is currently exploring the possibility of using BslA as an additional fusion protein partner, allowing for a simultaneous expression of at least two SARS-CoV-2 antigens, which in this way can diversify and improve the immune response against the virus.

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