Clinical Medicine Insights
Received 10 Feb 2022 | Revised 13 Feb 2022 | Accepted 18 Feb 2022 | Published Online 20 Feb 2022

DOI: https://doi.org/10.52845/CMI/2022-3-1-4
CMI 02 (02), 262–267 (2022) ISSN (O) 2694-4626 IF:1.6

RESEARCH ARTICLE

Vaccines against potential pathogens[Vaccine against Plague.]

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[1]CEO, Executive Chairman, President of Microbial Biocatalyst International, Inc. and Inorgcarbdiesel, Inc. Dr. Tyurin has gained two Doctoral Degrees in the former Soviet Union: M.D. in Internal Medicine (1986, Saratov State Medical University) and Ph.D. in Molecular Biology, Microbiology and Molecular Pharmacology (1990, The USSR Research Institute for Antibiotics). Dr. Tyurin additionally has B. Sci. in Biological Engineering (1984) and M. Sci. in Biology (1985) both from Saratov State Medical University.

Abstract
We proposed human intestine as the gate for the delivery of the therapeutic recombinant proteins expressed inside of the human body. The normal intestinal microflora was used to express the selected genes of the pathogenic organism *Yersinia pestis* the causative agent for plug in humans and animals. We have confirmed the production of the selected proteins by the PCR to their DNAs expressed in intestinal bifidobacteria chosen. Now it is the role of the immunologists to find the antibodies for the recombinant proteins expressed inside of the volunteer's organism.
Keywords: Expression of the recombinant proteins inside human's body, Bifidobacterium breve, use of genome tailoring technology to express the recombinant proteins inside of the volunteer's body.

1 | INTRODUCTION

The Author started his medical education at Saratov State Medical University after he spoke with his cousin Galina about becoming the Ph.D-Scientist working with the *Yesrinia pestis* the causative agent of plague and *Vibrio cholera* the causative agent of cholera at the closed for the employment of the general public Institution Microbe in Author's native city Saratov (now dissolved). Said scientists had salaries substantially exceeding that of the regular former Soviet society members, just like the Author. For instance, the Soviet Academician was getting his salary of 1,000 Russian rubles, while at the mentioned organization Microbe Senior Ph.D. Researcher was getting 2,600 rubles, etc. The Author has made multiple friends among said Ph.D-.level Scientists from that closed for general public employment institution Microbe. Some contacts became very useful for Dr. Tyurin for making his scientific presentations at Saratov State Medical University during his course of studying. The most remarkable was the gift of the Microbe Institute medical advisor when she gave to Dr. Tyurin 5 kg of Japanese Agar-Agar. The Author could use that agar during his already scheduled Ph.D. studies at the USSR Research Institute for Antibiotics in Moscow, the former USSR, and for his future work as the Ph.D-student in Moscow, when Dr. Tyurin has gotten as a present those 5 kg of

the Japanese Agar-Agar he later used in Moscow for his Ph.D-associated research and development (R & D). While studying at Saratov State Medical University and visiting the Microbiology and Immunology Department of said University from early in 1981 till his graduation date in 1986 the Author has learned that what he wanted to become after the graduation of Saratov State Medical University was not possible, complicated by his origin of the regular private person. He must note that his cousin Galina was the daughter of the SPCU (Soviet Communist Party) Second Secretary of the Saratov Region SPCU Committee Vladimir Rodionov. That gave her the protected path to become the Ph.D-Scientist in said closed for the general public employment former Soviet Union institution Microbe dealing with plague-cholera causative agents during the futile scientists attempts to create the new biological weapons of mass destruction with the tremendous destructive power and difficulty to cure. That mentioned closed system required mandatory thorough checking of the background by the KGB and the origination from families of the high ranked SPCU-related people in the former Soviet Union, which was quite opposite to what the Author had in his background. While learning about that in the course of study at Saratov State Medical University, the Author continued to want to become the professional Ph.D. level scientist working with microorganisms, and he was not any longer inspired by the levels of the respective salaries such noted secretly working on the weapons of the mass destruction Ph.D.-Scientists had in the former Soviet Union. On the second year of the Author's education (1982) at Saratov State Medical University the Author has met Professor Boris Shenderov, a person who just returned from Zambia where he worked as the Professor at the Lusaca University. Dr. Shenderov worked for the KGB and that was the reason he was in Zambia for his "work". Dr. Shenderov visited Saratov State Medical University he graduated from as well. He has visited Saratov State Medical University in 1984 before moving to Moscow, where the former Soviet KGB gave Dr. Shenderov the rank of the colonel and the new work of the Professor at the Laboratory of the Industrial Hygiene at the USSR Research Institute for Antibiotics, Moscow, the former USSR. So, Dr. Shenderov used data the Author has provided to him on the Author's studies of Non-Fermenting Glucose Gram Negative organisms predominantly Pseudomonas isolated from the hospital patients in the Saratov Region. Said data gave the opportunity to publish them in the Journal for Dr. Shenderov's future Moscow Institution "Antibiotics" in 1984 (the 1^{st} publication Dr, Tyurin had in the Former Soviet Union [1] while being a simple medical student). Dr. Shenderov invited Dr. Tyurin to continue the education in Moscow to become his Ph.D-Student upon the graduation of the Saratov State Medical University, which the Author did in 1986. Upon the graduation of his PhD.-Studentship the Author joined Dr. Shenderov at his another new work place, as Dr. Shenderov got the promotion from the KGB for his work in Moscow since 1984, at Gabrichevsky Research Institute for Epidemiology and Microbiology in 1990 where Dr Shenderov became the Director. In 1992 the Author has left Dr. Shenderov and his KGB-related work community and joined the Author's new work acquired at VNIIGENETIKA (the Adjinomoto-GNIIGENETIKA Research Institute), the Author's last place of work at the Russian Federation before moving permanently to the USA.

The Author has already described the known before place of entry to the human body bloodstream - human intestine [2,3]. The Author had specialization during his Ph.D.-Studentship years on the Molecular Biology, Microbiology and Molecular Pharmacology of the normal intestinal microflora of humans and animals. The Author has become the king of lactobacilli and substantially intensified his work with the human intestinal bifidobacteria at GNIIGENETIKA in 1992-1998, the predominant organisms in the intestine of many humans [4]. In this original research paper the Author describes his personal experience with the volunteer he has successfully vaccinated by creating the recombinant strain of bifidobacteria isolated from the intestinal content of said volunteer as described in [2,3] and making said

Supplementary information The online version of this article (10.52845/CMI/2022-3-1-4) contains supplementary material, which is available to authorized users.

volunteer to ingest the recombinant strain of his own intestinal bifidobacteria.

As the target for the expression in the recombinant *Bifidobacterium breve* 839 strain the Author has chosen the DNA with the known nucleic acid content originally isolated from the Yersinia pestis strain. Said strain was the causative agent for the human and animals plug [6]. The Author's choice for said proteins was dictated by the ethiological role of the *Yersinia pestis* selected as the possible ethiologic agent of the emergent diseases the outer Space travel crews might possibly face discovering other new planets similar to Earth by the temperature and the atmosphere content in the coming future, the immediate projects of the Environmental direction. .

2 | MATERIALS AND METHODS

The isolation and investigation of intestinal bifidobacteria of the volunteer was performed as described [2,3]. Using the described selective medium for the isolation of the intestinal bifidobacteria [2] the Author has isolated *Bifidobacterium breve* 839 strain from said volunteer's freshly collected intestinal content (fresh feces). Said strain was subjected to the reduction of its genome by removal of not essential for the vital functions of said strain natural genome genes at their positions 4346...4816 bp,10023...10574 bp, 16239...17477 bp, 19324...20316 bp, 20927...21592 bp,22486...23799 bp,237007...238836 bp, 24676...26007 bp, 31295...33502 bp,34410...36290 bp, 37707...39254 bp, 538583...541012 bp, 643441...645516 bp, 817368...820625 bp, 1476554...1481404 bp and 2258202...2261981 bp using the procedures described in [7-16].

Total genomic DNA from *B. breve 839* was isolated by the procedure [4,18]. The primers for the PCR to check the presence of the recombinant pesticin and the hypothetical protein YPMT1.21c DNA sequences were designed using the publically available tool [17].

The process of genetic modification of said *B. breve* 839 strain took less then 200 hours to ensure the strain regained the capability to adhere back to the intestinal wall of the volunteer as we have discussed before [2,3].

Bifidobactrium breve 839 pesticin Submission ID 2526713.

Bifidobacterium breve 839 hypotetical protein YPMT1.21c submission ID 2532183.

3 | RESULTS

Development of the recombinant strain of *B. breve 839* PLUG was performed as already described in [2,3]. Said strain of intestinal human bifidobacteria of the volunteer has expressed both recombinant genes of pesticin and the hypothetical protein YPMT1.21c as that was shown by the PCR the Author has performed with the total genomic DNA isolated from *B. breve* 839 PIUG by the method described in [18] with the proper modifications of the procedure to isolate the total genomic DNA.

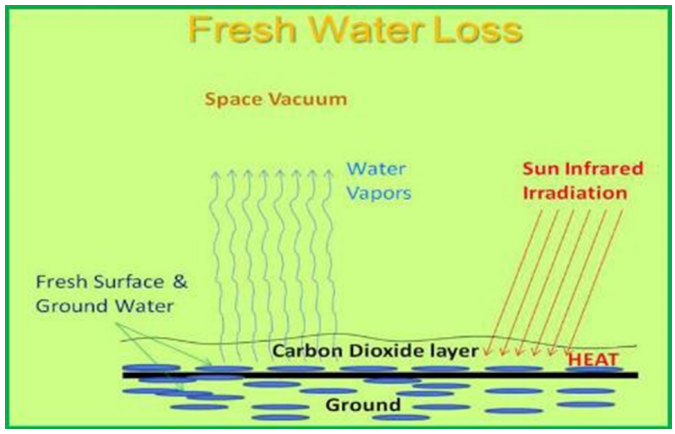
The PCR for the presence of the both recombinant genes of pesticin and the hypothetical protein YPMT1.21c ended up with the revealing of the proper DNA fragments on the agarose gel for the PCR products [17]. Upon said testing the 25 ml of the 72-h broth culture of *B. breve* 839 PLUG grown in the liquid m3edium for the isolation of bifidobacteria with no antibiotics added were. ingested by the volunteer. In one hour he came back to his work and has ever worked as before the ingestion. Now, it is the task for the immunologists to investigate the blood stream of said volunteer to recover the antibodies to the *Yersinia pestis* pesticin and the hypotetical protein YPMT1.21c.

4 | DISCUSSION

We have discussed the prospects of our planet in the future at our corporate web site, and noted the coming in 10-20 years from now the shortage of the fresh water, leaving the Earth to the outer Space as described [2,3]. Indeed, accumulated in the air CO_2 is one of the heaviest gasses in the air blend, reaching its density 1.97 g/cubic meter [3]. The CO_2 in the air gas mixture under the no wind environmental

conditions spreads on the ground surface and selectively absorbs all the infrared energy of the Sun light, thus heating the ground significantly. That causes the extra evaporation of the fresh water from soil to the air. As you know, Global Warming presents itself in various forms, specifically with increased frequency of rainy weather, long rainy days, tornadoes, etc. But the Earth gravity has been stable for the last few million years from now. Therefore, under the constant gravity force applied, more fresh water vapors are in the air. The space, surrounding Earth, as any Space anywhere, has vacuum. That vacuum sucks fresh water vapors from Earth air, and such fresh water vapors travel in the Space in the unknown direction away from the Earth. In 2010 NASA has bombarded the Moon and found plenty of ice on its dark and very cold surface. The Earth satellite Moon is located 220,000 miles away from Earth. One Moon's side is always dark and cold as it never gets Sun light irradiation. It is very cold, as cold as the Space vacuum, -273 ^{o} C. So NASA were guessing where said ice came from? Moon worked as the cold trap for the fresh water vapors coming from Earth in the Space vacuum [http://syngasbiofuelsenergy.com]. What will happen next and the most important, when? We might give the time frame for 10-20 or 10-50 years from now, based on the 2010 HASA discovery of ice on the Moon and the NASA conclusion of their findings: Earth as planet has passed the "point of no return" to the normal life. It is impossible to anticipate, that the fresh water loss to the outer Space may be stopped at any time even if the Earth population is suddenly decreased in its amount. The extra air CO_2 comes from the intensified petroleum use, and the use of its products for combustion, producing CO_2. People breath and produce CO_2 as well. It is anticipated the 14 billion people on Earth by 2050. That increases more the air CO_2 content, leading to the increased fresh water loss as discussed.

Fig 1 shows our understanding of the fresh water loss process.



Fig, 1. Fresh water loss to the Space vacuum

We have no any idea, what will happen soon, if no new planets, similar to Earth, will be discovered and the overcrowded Earth population will not start to move there. We do anticipate, that the reduction of the air CO_2 content is absolutely necessary and possible by the replacing of the existing economy based on the power generation suing the products of petroleum distillation on the petroleum refineries by the economy based on the energy generation using the discovered by the Author carbon negative technologies of the fuels and chemicals production [2-16].

In this article, the Author has shown that the expression of the recombinant proteins originated from *Yersinia pestis* and expressed in the human intestinal bifidobacteria happens efficiently right in the body of the volunteer by the engineered strain of the intestinal bifidobacterium isolated and then returned back to the intestine of said volunteer. Based on the described expression of the recombinant proteins, the Author has concluded, that that will be possible to perform the immunization of the proposed coming soon manned crews of the outer Space flights intended to discover new planets in the Universe suitable for the relocation of the overcrowded Earth. Said immunization does require certain genetic manipulations which are possible on the board of said outer Space travel vehicle(s) by the suing the described technologies of genome replacement in the intestinal microflora of the potential crew members of said vehicle. This circumstance closes the need for

the special medical personnel on board of said outer Space travel vehicle(s) to perform the immunization of the crew from the emergent infections reasonably anticipated for the existence in the new outer Space locations. This approach may have crucial importance for the manned crews life during said long term outer space travel missions proposed.

5 | DECLARATIONS

Ethical Approval and Consent to Participate. This article does not contain any section, requiring Ethical Approval. The only Author is complied with the Consent to Participate. Consent to Participate. The Author complied with the consent to publish this article.

Consent to Publish. This original article has not been published anywhere or is under the consideration to publish anywhere else beside this Journal. The Author complied with the Consent to Publish this original article. Authors Contribution. Dr. Michael V. Tyurin has planned all the experimental work, conducted all the experiments, analyzed the experimental data, wrote this original manuscript, edited ot as appropriate and submitted for this publication the edited original manuscript. FUNDING was done by the private investors, who declined to provide their names and their business affiliations. The investors noted, the author should decline any source of funding. Competing Interests. The author declares his personal conflict of interests with the law firm in Houston, TX Hirsch and Westheimer, which has destroyed his corporate website http:syngasbiofuelsenergy.com, and with the major petroleum and gasoline/diesel fuel companies in Houston (TX), with the Houston Police (City of Houston) and with the Houston FBI ignoring the Author's concern about his attempted murder committed by SHELL after the Author has presented to SHELL his proprietary technology for gasoline manufacture from the air CO2, not from petroleum (Tyurin MV, et al. 2019) The attempted murder is of no interest for the corrupt by the major petroleum corporations in Houston, TX Houston FBI. The Author has no intent to file the lawsuit against the Houston FBI at any point, but he is inclined to make this case the public domain. Houston FBI does not follow the established in the USA Federal Laws. Availability of data and materials, All the data and materials are available if necessary from the Author of this manuscript. The Authors' information. The Author is the owner of his mentioned TEXAS businesses Microbial Biocatalyst International, Inc. and Inorgcarbdiesel, Inc. The work has been done at the corporate site with the USPS address P. O. Box 300230, Houston, TX 77230.

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How to cite this article: M.D., Ph.D D.R.M.V.T. Vaccines against potential pathogens[Vaccine against Plague.]. Clinical Medicine Insights. 2022;262−267. https://doi.org/10.52845/CMI/2022-3-1-4