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Cure of the Intestinal Disorders

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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.

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 scientist 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 the closed for general public employment institution Microb. Some contacts became very useful for Dr. Tyurin for making his scientific presentations at Saratov State Medical University during his course of studying, and for his future work as the Ph.D-student in Moscow, when Dr. Tyurin has gotten as a present over 5 kg of the Japanese Agar-Agar he later used in Moscow for his Ph.D-associated Research and Development (RnD). While studying at Saratov State Medical University and visiting the Microbiology and Immunology Department of said University early in 1981 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

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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 organization Microbe dealing with plague-cholera causative agents during their futile attempts to create the new biological weapons of mass destruction with the tremendous distructive power and difficulty to cure. That mentioned closed system required mandatory checking of the background by the KGB and the origination from families of the SPCU-related people working at the high levels of the Communist party organizations 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 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 coming to Moscow, where the 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 1st 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. Shederov got the promotion from the KGB for his work in Moscow since 1984, Gabrichevsky Research Institute for Epidemiology and Microbiology in 1990. 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 [1,2]. The Author had specialization during his Ph.D.-Studentship years on 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 [1,2].

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 face discovering other new planets similar to Earth by the temperature and the atmosphere content in the coming future.

Materials and methods

The isolation and investigation of intestinal bifidobacteria of the volunteer was performed as described [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 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 [7,9-17].

Total genomic DNA from B. breve 839 was isolated by the procedure [10]. 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 [18].

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 stipulated that before [2,3].

Electron microscopy of intestinal tissues

The Author has great connections in the local community and therefore was able to ask the local University of the donation. The University had rabbits and some of them had to be sacrificed for the whole blood removal for the antibodies generation process for their R & D. The freshly dead rabbit bodies are normally discarded using the standard procedure to ensure the proper dead tissue disposal. Knowing that process, the Author has approached the University staff and kindly asked if certain rabbit body part could be left for the Author further processing. The Author has selected the rabbit body parts from the rabbit intestine. The Electron microscopy of the intestinal cells was performed as described [21]. The blood samples shown in tubes were obtained from the same University employees, who granted the access of the Author to the rabbit intestinal cells. The images of the chilomicrons were obtained from the intestinal samples of the rabbits immediately disposed after their meals, as offered by the donation to the Author of this original article by the employees of said University (**Figure 1**). The sample with turbid blood serum was obtained just after the rabbit meal (**Figure 2**).

Results

The isolation of intestinal bifidobacterium and its species identification has happened as that was described before [1]. As the mandatory part of the genome tailoring procedure we have reduced the genome of B. breve 839 by 39048 bp and introduced only 2893 bp of the recombinant DNA corresponding to

the recombinant genes of pesticin and the hypothetical protein YPMT1.21c DNA sequences. That gave us the advantage of the shortening of the cell duplication time by 12 min. Said shortening of the cell duplication time resulted in the predominant multiplication of the recombinant strain of bifidobacteria in the intestinal content of our volunteer resulting in the expression of the recombinant proteins from Yerisinia pestis in the intestinal content of the volunteer, as we have confirmed by the checking of the total DNA of the resulting recombinant strain B. breve 839 YR for the presence of the nucleotide sequences of the Yersinia pestis pesticin and the recombinant hypothetical protein YPMT121c.

The recombinant DNA sequences we used are the following. The recombinant pesticin DNA sequence was aaaaattattttaacaatccactatcgatatctttttgcaccagagcgccctctcgtttacgtctgtcagacattccatcaacaatattattaaaagcatttacaaggccattccagtcttttgcgataactttattccatactgtgggagcagttctggataacttaaaccctttttgatatccaatagacaccagtgctgtacgggttctcaacggtaaatcgctgaaccctttttgatatccaatagacaccagtgctgtacgggttctcaacggtaaatcgctgaaccgaagaccgatattagcgtcattgaaaagaccttcaatcttatgtgagaatttatcaatataaatattagataagagatgagcttcattatcagaaagcgtcagaggtgctgttctcactttatcataagcctccttccctcgaagcatataatacccatcaagtctatctgcaatatactgagggacaccgtcattcaataaatcctgtttgcttcgctgaccaaggtcaaccccggaaccgaatgtaacaccggtactgttaaaataatcgctactaggattagacggaaaatgacttgtcggattaaaccccttcaaaaccattactggagaaaatatcgtggtcaacaatatttaccgaacgacgtaaaaattccttcagttgactaatattgtcaaagttatgacagtgttgtccgctaggacgatgcgatttcggttattattcagaatgtcttcgttctctttcttatcgagatgttcaatagattcggcaatcgttccctcaagaaccatgacacggtagactttcacaccgtctttttcctgacctgtttcaacagttattttctgttcgtaagacacggtcccttcagtttttgaaattttactttcctggcggatcttatttgaatattcactgtctttctccatctccgtatcaatcggaaaccccataatgtacatcgtttaaaattactccggccaggcagatccacataatgtggtaatgcaattgtaatcgaattagcttcaaaatttggtctgtaactgcttaatgtacttccggaaaagagaaaagccggaacaccacctgaaccattcactaccattgtatctgacataaaaa.The recombinant hypothetical protein YPMT121c DNA sequence was aaaaattacttgagtccgattttccccagaccggaggtcaacttatccatagtcgacttgctcttcggctggtttactttgtgggtcggtttgctattggaaccagcaccctgcttggcttcgctgcgcaacagtgcgtcagcttccggatctgcacgcagaatgcgttcaatcgcggattcgaacggtaacggtttgccttcaccgtcaaccagaactgcacgctctttctaccggctggcttatcaaagcccacaacactaccgtcttcacccacttcgaaatgagagccgtagataacgcgagccttagccggagtcatcagaactttgtcacgcaggaaggcagagccgctgaaggaagcgccaacagtcatttcgaccagttgagcctggtgcgcttcgatcatctgctttttcacagcatcgaactcaccacggcgttccagttcatggtgcgcttcgatcatctgctttttcacagcatcgaactcaccacggcgttccagttcagcctgctccgcctcccggcgtgcggtttctgcggcttgttcagcttcgagaagctggcgagcacgcgccgggtcaatatcaccgtactgagccagctgatcggccaatgtgcgctctttctccttgcgcttcatgttctctttcagcaggtcagcaccagctttcttggacttacgcagttctgccagcaactcttcctgagtcataccggcatactcgtcgtcgtcacccttcggttgctctgccagcaactcttcctgagtcataccggcatactcgtcgtcgtcacccttcggttgctctttttgctcgccctgtttacctgggtcttgattgccctgctcgttgtctccagcaggagcaccgccaccagcgcctccgcgctcatgcgcttcagctgcatccatcaggccaccgacgggccataagcatttgccacagattcataaaaa.

Said DNA sequence was inserted by the means of the electro transformation using the patented in the USSR the Author's electro transformation generator [19,20]. The crucial difference of the results obtained using said the Author's patented electroporator is the absence of the restrictions on the size of the DNA introduced using said electro transformation generator, for its stable expression [2,3,7-20]. To make sure the resulted recombinants were stably expressing said recombinant human pancreatic Lipase, their genome was substantially reduced as described [7-17], the intact genome of the bifidobacterial strain used to create the stable genetically engineered was substantially reduced by the eliminating of the genome segments as described herein.

The proof of the ingestion in the bloodstream of the recombinant proteins was shown indirectly by the taking images of the chilomicrons in the bloodstream of rabbits and the blood serum After the rabbit meals.

Figure 1 shows the hilomicrons, the fat particles, absorbed by rabbits from their meals (Figure1). That absorption was not possible if no recombinant proteins were absorbed by the blood capillaries of the small rabbit intestine.

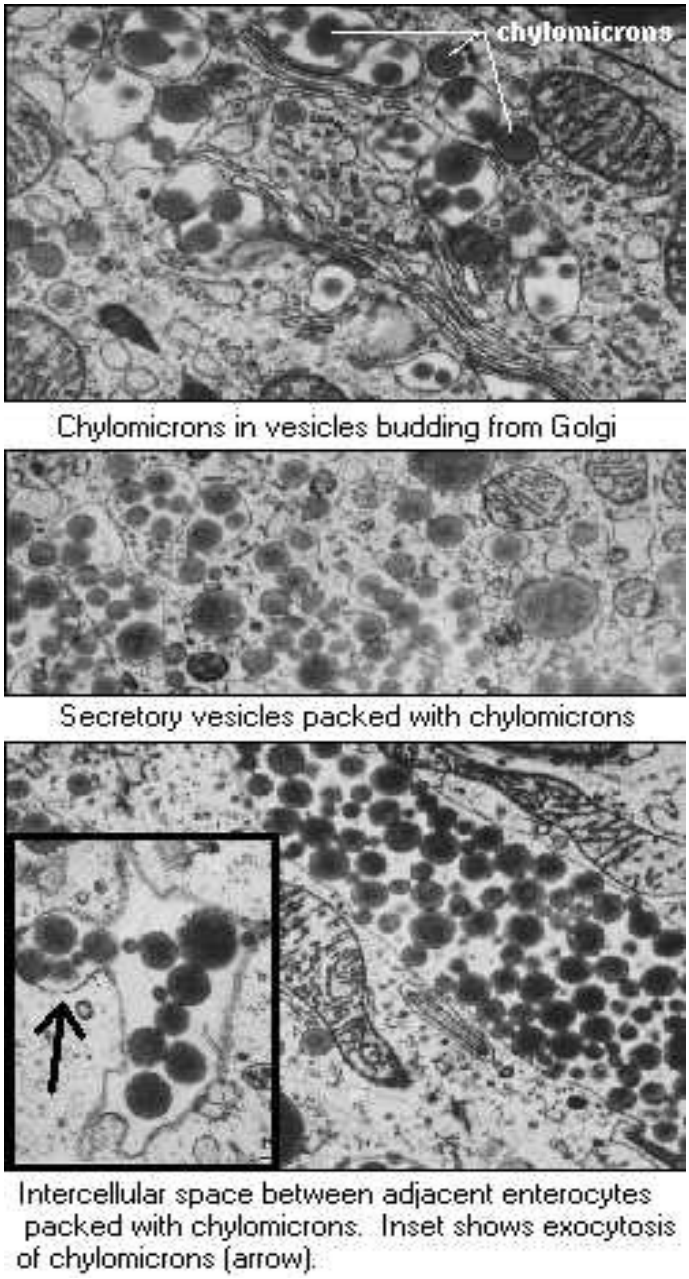

Figure 1: Chilomocrons of food fat absorbed by the small intestine blood capillars from the rabbit food.

Rabbit blood serum coming from the intestine had changes in its transparency, as the food fat created the turbidity in the blood stream coming from the small intestine as Figure 2 shows.

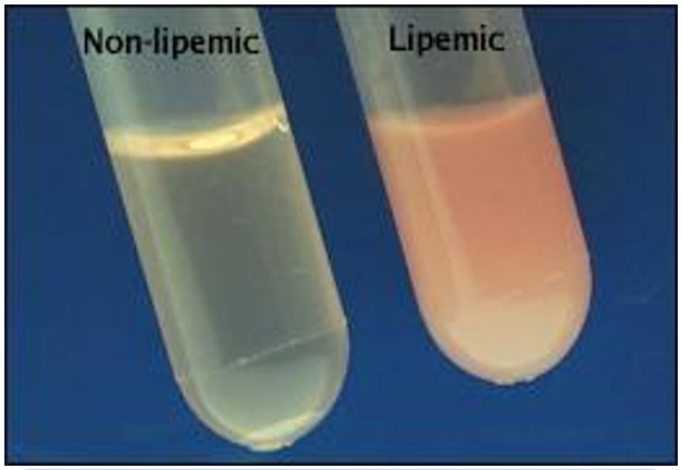

Figure 2: Blood plasma with the chilomicrons from the food fat (on the right pert of the image).

The PCR performed with the genomic DNA of the recombinant strain B. breve 939 YR showed the anticipated for the DNA encoding the recombinant pesticin 209 bp fragment. The anticipated fragment of 171 bp was shown for the PCR products per the PCR per the gene coding for the hypothetical protein YPMT121c.

Discussion

We have discussed the prospects of our planet in the future at our corporate web site, and noted the coming in 20-50 years from now the shortage of the fresh water. 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 °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 breathe and produce CO_2 as well. It is anticipated the 15 billion people on Earth by 2050 [5]. That increases more the air CO2 content, leading to the increased fresh water loss as discussed. 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 [2-17].

Now it is the role of the immunologists to find the antibodies for the recombinant proteins expressed inside of the volunteer's organism.

In this article, the Author has shown that the expression of the recombinant proteins 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.

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