



Influence of Fractional Composition of Dietary Supplements "Majmui Rahmoniy" on *Mycobacterium smegmatis*

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Authors' contributions

This work was carried out in collaboration among all authors. Author IM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors IM, IA and MM managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Despite the availability of anti-TB drugs, the proportion of cases of multidrug-resistant tuberculosis (MDR) is increasing. One of the important tasks to identify effective drugs in nature is the study of numerous experiments of traditional medicine. This work is devoted to the study of chemical, biochemical, medical evaluation of the new therapeutic food additive "Majmui Rahmoniy", and its effective effect on *Mycobacterium smegmatis* and MS Rv2349c gene which responsible for the phospholipase activity.

Keywords: Food addition; "Majmui Rahmoniy"; mycobacterium tuberculosis; MS_Rv2349c; Ms_Vec.

1. INTRODUCTION

To date, about 10 million cases of tuberculosis are recorded annually in the world.

Mycobacterium tuberculosis (Mtb) considered gram-positive sticks, dangerous for the body infectious diseases that affect the internal organs and tissues of the body, where mainly infected

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lungs. Lack of antibiotic adherence has led to an increase in multiple and totally drug-resistant strains of *Mycobacterium tuberculosis*, the causative agent of tuberculosis. *Mycobacterium tuberculosis* carry a virulent factor of pathogenicity, which helps to overcome the barriers of protection of the host, that is, they have the ability for a long time to remain in the host in a latent state and with the weakening of the host immunity to reactivate.

Despite the availability of anti-TB drugs, the proportion of cases of multidrug-resistant tuberculosis (MDR) is increasing. A factor in the development of these dangerous forms of the disease is the selection of strains carrying mutations in the target genes of anti-TB drugs [1]. The development of drug resistance in mycobacterium is mainly due to mutations in drug target genes or related genes. In a comparative study of pathogenic and non-pathogenic *Mycobacterium tuberculosis*, scientists found the presence of a gene responsible for phospholipase activity in pathogenic bacteria. An example is *Mycobacterium abscessus* as a major pathogen, among numerous non-pathogenic fast-growing bacteria [2-7] encoding a phospholipase gene that is involved in the intracellular survival of *M. abscessus* in amoebas [8]. Also, genes encoding phospholipase C (PLC) activity are present in many strains of slow-growing mycobacterium [9]. Among them, phospholipases C play a significant functional role. They generate diacylglycerin hydrolysis, which is involved in the activation of extracellular signaling kinases (Erk) via protein kinase C, resulting in macrophage activation [10,11].

These data provides the arguments to perform studies of suspected virulent genes and detection of molecules with properties of anti-TB drugs Identification of the chemical composition of biological additives can lead to find of many effective drugs and the production of antibiotics with a wide spectrum of action. On this regard, a previous an invented food additives based on petroleum products named "Majmui Rahmoniy", recommended by the Ministry of Health of the Republic of Uzbekistan, is successfully used in tuberculosis treatment by traditional medicine.

Food additive "Majmui Rahmoniy" is supplement which are preparing on the basis of hydrocarbon fraction obtained at a temperature limit of 190-200C from the kerosene base of oil. This supplement is widely used in folk medicine of Uzbekistan for the treatment of many diseases

such as tuberculosis, cancer, gastrointestinal diseases, diabetes, psoriasis, etc.

Different methods of treatment with kerosene are quite actively practiced in traditional medicine. People suffering from various diseases use it for external and internal use, since kerosene has a beneficial, healing effect on the body in various diseases. This tool can be used for preventive purposes.

This drug has so proven itself as an effective, efficient means that some companies in the CIS are interested in it. In particular, the Technoekos Scientific and Technical Center for Ecological Problems began to manufacture and sell it to the public through pharmacies and drugstores. He holds a patent for the preparation and use of a medicinal product based on naphtel derivatives of oil and walnuts [12-17].

Based on the many positive reviews about the benefits of kerosene in the treatment of many incurable diseases, the study of its quantitative and qualitative composition is currently of great importance in medicine and in the pharmacology. A study of the chemical composition of kerosene can help identify new effective drugs for the treatment of many diseases.

The purpose of this experiment was to isolate fractions of the food additive "Majmui Rahmoniy" by boiling point, and to study the effect of individual components of this on *Mycobacterium smegmatis* MS_Rv2349c and MS_vector.

2. METHODS OF RESEARCH

2.1 Fractionation

Fractionation of the composition of the food component based on oil products was carried out according to GOST 2177-82 Petroleum products, methods for determining the fractional composition.

2.2 Bacterial Strains, Plasmids and Conditions of their Cultivation

Bacterial strains *Escherichia coli* DH5 α and *Mycobacterium smegmatis* mc2 155 were provided by the Southwest University of China.

Middlebrook liquid medium (MB) 7H9 was used to grow the bacterium. Briefly, it where added 0.05% Tween 80; 0.5%, glycerol and 0.2%, glucose. Also was used solid medium

Middlebrook (MB) with agar 7H10. The antibiotic hygromycin (25 mcg / ml for mycobacterium and 50 mcg / ml for *E. coli*) and ampicillin (50 mcg / ml) were added to both mediums. Cultures were incubated at 37°C during 1-3 days.

2.3 Construction of Recombinant *M. smegmatis* Rv2349c

The Rv2349c gene has been successfully cloned (DNA sequence obtained from <https://mycobrowser.epfl.ch/>) using *M. tuberculosis* H37Rv genomic DNA and special primers using pALACE plasmid. Strains and plasmids of bacteria and their primers used in the recombination of strains are listed in Table 1.

Successfully recombinant Ms-Rv2349c was further confirmed by PCR amplification. Samples were subjected to SDS-PAGE and further detected through Western blotting with antibody against Myc (TIANGEN, China).

2.4 Determination of the Effect of Food Additive Fractions on Ms_Vec and Ms_Rv2349c

Determination of the effect of food additive fractions was carried out by measuring the differences in sensitivity between Ms_Vec and Ms_Rv2788 using the disk diffusion method. The

plate was incubated at 37°C for 12 hours without shaking and measuring the diameter of the zone of complete inhibition.

3. RESULTS

3.1 Recombinant *M. tuberculosis* Rv2349c and *M. smegmatis*

The *M. tuberculosis* Rv2349c gene encodes a putative 55 KDA transmembrane protein.

Since the Rv2349c gene can be a virulent factor, we used the avirulent and fast-growing *M. smegmatis* as a model to study the function of this Rv2349c gene. The Rv2349c gene of Mycobacterium tuberculosis was recombinant using primers listed in Table 1.

The successful recombination of Ms_ Rv2349c and Ms_Vec was verified by PCR and Western Blot (Fig. 1A).

3.2 Separation of Food Additive into Fractional Composition

The separation into fractions was carried out by dividing the boiling point of the food additive. Samples and boiling point during fractionation are shown in Table 2.

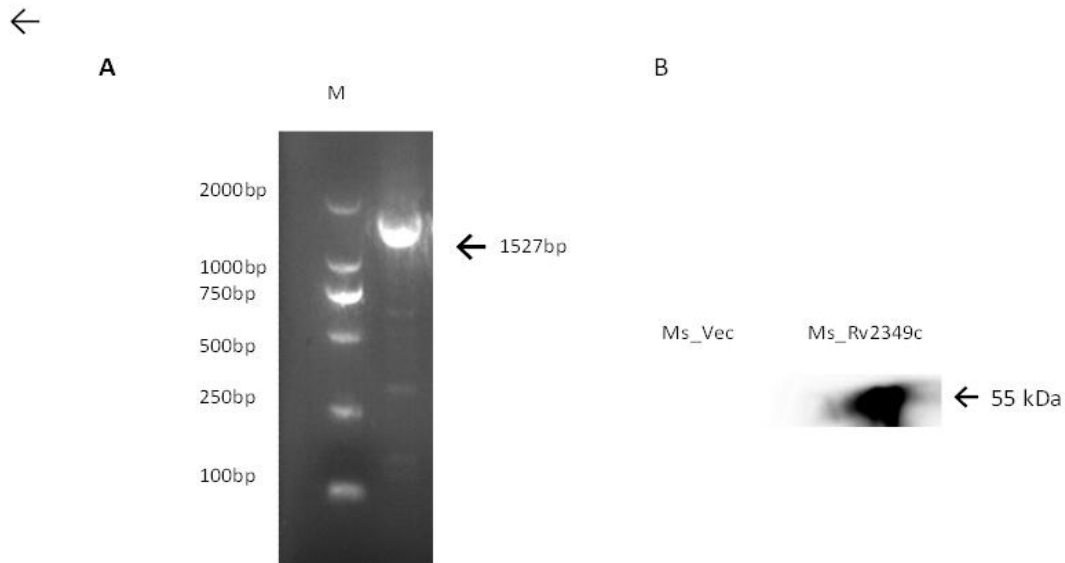


Fig. 1. Over expression of Rv2349c in recombinant *M. smegmatis*
 (A) Ms Vec and Ms Rv2349c were grown into an OD600 of 0.8 and then were subjected to PCR amplification to detect Rv2349c gene; (B) Ms Vec and Ms Rv2349c were subjected to Western blot to determine the expression of His-tagged Rv2349c protein in *M. smegmatis* by mouse anti-His antibody

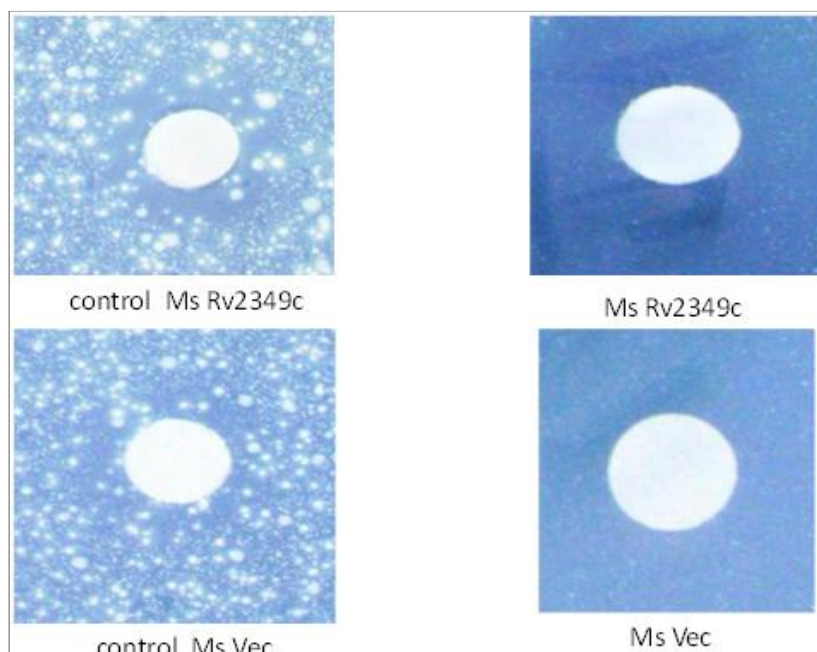


Fig. 2. Effect of food additives “Majmui Rahmoniy” fraction on Rv2349c and Ms Vec

Table 1. Primer, strains and plasmids used in the experiment

Strains	Descriptions
WT	Wild type <i>M. smegmatis</i> mc2 155 strain
MsVec	<i>M. smegmatis</i> with transformed with vector pALACE
Ms Rv2349c	<i>M. smegmatis</i> with transformed with vector pALACE_Rv2349c
<i>E. coli</i> DH5	Strain used in vector proliferation
Plasmids	Descriptions
pALACE	A replicative plasmid with Myc-tag used for gene expression in <i>M. smegmatis</i> and conferring hygromycin(kan) resistance
Primer	Sequence (5-3)
pALACE_Rv2349c F	TCGGGATCCATGTACGCGGAGCAT(BamHI)
pALACE_Rv2349c R	ACCATCGATGGCTAGCAGATGCCGC (ClaI)
MSMEG_0676-R	CAGCTCGCTCAGATCACGG

Table 2. Fractionation of food additives

Sample no	Boiling point
1	250°C
2	280°C
3	310°C
4	330°C
5	350°C
6	380°C
7	450°C
8	500°C

3.3 Mycobacterium Resistance for Fractions of Food Additives

When each fraction was exposed to 100%, 50%,25% Ms_Vec and Ms_Rv2788, the data

showed that the mycobacterium Ms_Vec and Ms_Rv2788 on all fractions were not stable compared to the control (Fig. 2).

4. DISCUSSION AND CONCLUSION

Home treatment was “universal health care” until after World War II, abundance made professional health care the norm.

The most popular "medicine" (back in those days) was kerosene. Kerosene (from the English. Kerosene, from the Greek. Kerys - wax) is used by a person in alternative medicine for the treatment of various diseases, and still remains quite popular and in demand. Such treatment

was a simple action, like a smear of the wound first that comes under the arm. Later, the bactericidal properties of kerosene were revealed. When stepping on a nail, they soaked a foot in kerosene, tensed the muscle, rubbed kerosene into the skin around the muscle. If someone had a cold, then put a drop of kerosene in a spoonful of sugar and swallowed. Cuts and abrasions rubbed with kerosene. People often sutured deeper cuts themselves. Vaseline (or other jellied petroleum products) was applied to burns.

Kerosene is a mixture of saturated hydrocarbons, which is a product of fractional distillation of oil. Boils at a temperature of 110 to 320°C (which depends on the degree of fractional purification). Specific gravity - 0.80–0.85 g / cm³. This liquid is lighter than water, with a specific mild odor and taste. The chemical composition, as well as many properties of kerosene, can vary quite a lot depending on which oil the product was obtained from, as well as on the methods of subsequent processing and purification[18].

Fans of traditional medicine believe that the healing of superficial damage to the skin occurs due to the mineral oils of kerosene. It is also believed that kerosene has a number of positive effects: strengthening the body, activating immunity, stabilizing the cell membrane, eliminating toxins, and anesthetizing. It is also used for treatment of various diseases, such as sinusitis, tuberculosis, injuries of various kinds, swelling of the skin, purulent tonsillitis, headaches, joint pains, skin problems and anti-cancer therapy [19-23].

One of the important directions of identification of effective drugs in nature is the study of numerous experiments of traditional medicine. The creation of a new drug by chemical synthesis or by searching from biologically active compounds or additives is considered very relevant. Identification of the chemical composition of biological additives can lead to the identification of many effective drugs and the production of antibiotics with a wide spectrum of action.

Based on the results, we can say that, each fraction, contain chemical components that have negatively affected on mycobacterium.

At this time, the mechanisms and pathways leading to the emergence and subsequent fixation of resistant strains of *Mycobacterium tuberculosis* are still not fully understood. From

research scientists it turned out that they may be more complex than expected. Some scientists have used and are using many ways to determine the molecular mechanisms that bacteria use to survive antibiotic treatment [24].

The obtained data encourage further study of the chemical composition of each fraction separately. The determination and study of the chemical properties of the components in the food Supplement "Majmui Rahmoniy" is essential for further determining their effectiveness in the treatment of drug-resistant tuberculosis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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