ISOLATION AND IDENTIFICATION OF ENDOPHYTIC MICROORGANISMS FROM SILIBIUM MARIANUM PLANT

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Abstract. *In this study, endophytic Bacillus subtilis, Pseudomonas fluorescens, Bacillus vallismortis, Bacillus licheniformis, Malassezia japonica, Bacillus atrophaeus bacteria and Fusarium proliferatum were isolated from the stem, flower and leaf of medicinal milk thistle (Silibium marianum) and their identification done.*

Keywords: *silibium marianum, silymarin, milk thistle, plantation, microorganisms, endosymbiont.*

Introduction. *Silibium marianum* (SM), commonly called milk thistle, is a medicinal plant belonging to the *Asteraceae* family [1]. It is distributed throughout the world in wild or cultivated forms, but mainly in the Mediterranean region, especially in the North African region, where it grows naturally [2]. The genus *Silibium marianum* consists of two species: *S. marianum* and *S. eburneum* [3]. Extracts from the seeds of *Silibium marianum* are of great industrial importance due to their use as phytopharmaceuticals and food [4]. Currently, it is among the best-selling herbal products in the United States, Italy, and other countries [5].

The main active functional component in *Silibium marianum* is silymarin, which is mainly concentrated in the fruit peel and its content usually varies from 1.5% to 5% of the dry weight of the fruit [6]. The seeds of *Silibium marianum* contain a large amount of oil, which is obtained as a by-product of the production of silymarin. These oils are rich in unsaturated fatty acids, antioxidants, vitamin E and sterols [7]. Flavonolignans in *S. marianum* are structurally diverse, with 23 constituents isolated from purple and white flowered variants [8]. Silymarin has isosilybin, silycristin, isosilycristin, silybin, silydianin and toxifolin as biologically active flavonolignans.

These functional flavonolignans have biologically active, antioxidant, anti-inflammatory, hepatoprotective, neuroprotective and antifibrotic activities [1]. In addition, *Silibium marianum* inhibits the proliferation of cancer cells in liver, lung, breast, colon and other organs [9]. Since ancient times, the resulting compound from the fruit of *S. marianum* has been considered a “liver tonic” and has played an important role in preventing or treating liver toxicity from complementary medicinal products or natural toxins [10].

Silymarin is an effective antiviral treatment for hepatitis C virus (HCV) [11]. It also protects the liver from various toxic substances such as heavy metals, pesticides, alcohol, acetaminophen and CCl4 [12].

The most common commercial drugs used in the treatment of liver diseases today are Legalon-70 and Dura-silymarin. Both of them contain silymarin (derived from the seeds of the *S. marianum* plant) and are expensive and imported drugs [6]. Silymarin is also of particular importance cosmetically and dermatologically. It was found that silymarin and milk thistle fruit extract inhibit enzymes involved in the breakdown of intercellular matrix components, and also have a strong antioxidant and UV protective effect on the skin [14].

The use of silymarin in aquatic organisms has several beneficial effects. For example, the addition of 0.1% silymarin to Nile tilapia (O. niloticus) feed resulted in hepatoprotective and immunomodulatory effects. In addition, the addition of silymarin (60 mg/kg) to the diet of white grass carp (Ctenopharyngodon idella) significantly improved growth performance. Also, lysozyme activity increases in various fish species fed diets enriched with silymarin extract [15].

According to the research of El-Sappa et al., the application of silymarin in wheat plants grown under Cd stress significantly improves all studied biochemical, morphological and physiological changes. In addition, Alharbi et al investigated the application of silymarin-enriched bio-stimulant to maize leaf and found that it minimized Cd toxicity by suppressing oxidative stress and increasing antioxidant gene expression [11].

Demand for silymarin varies between 18 and 20 tons per year, and average annual sales are about $8 billion. In 2018, milk thistle was among the eight best-selling plant-based dietary supplements in natural retailers in the United States, with total sales of $10.419 million, ranking 20th in total sales in the United States [16].

Since *Silibium marianum* is an annual plant, it takes several months to a year to produce dried fruits and extract silymarin. Hence, cultivation methods need to be developed to produce silymarin for commercial purposes and achieve high productivity. It is worth noting that milk thistle is a thorny plant. The spines are 1 to 5 cm long and cause some difficulties in fruit collection due to their location on stems, leaves and flowers [16].

The amount of each compound in *Silibium marianum* depends on several factors, such as environmental conditions, geographical, genetic, and time of planting and harvesting of the plant [2] . Also, the use of herbicides creates the problem of contamination of fruits (seeds) with toxins. In addition, the plant is planted in rows, so the use of a combine harvester can cause damage to the crop, leading to a loss of up to 40% of the total yield at harvest [6]. An alternative strategy for CM production is in vitro using cells and tissues. However, a high production system is still not available [17].

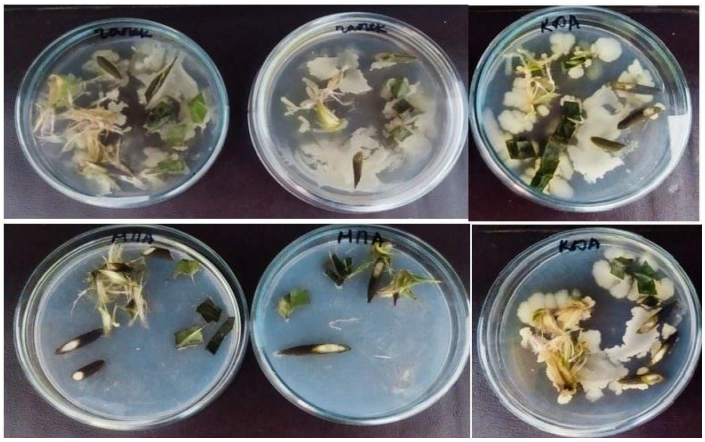
Today, it is important to isolate microorganisms synthesizing chemicals from medicinal plants from plant tissues, taking this into account, we aimed to isolate endophytic microorganisms from milk thistle (*Silibium marianum*) in this work.

Materials and research methods. The samples were first carefully washed in running water, then kept in 70% ethyl alcohol for 5 minutes and rinsed in distilled water. After that, it was treated with sodium hypochloride (NaClO) for 3 minutes and washed in sterile distilled water. The stems, leaves and flowers of the plant were crushed into 2 cm pieces and placed in petri dishes. Each cleaned plant piece was planted in nutrient media and incubated at 30°C for 2-7 days [18].

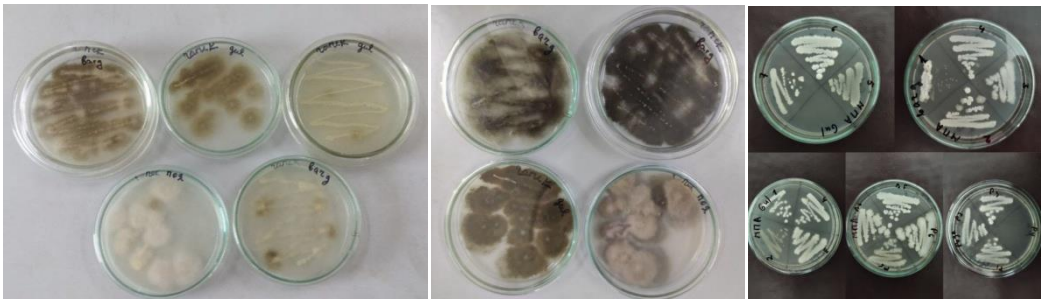
Research results. Medicinal milk thistle was planted in a small experimental field in medium saline soil conditions of Syrdarya region. Based on its natural and morphological characteristics, plant care was carried out.

When the plant reached the flowering phase, samples were taken from its underground parts: flower, leaf, stem and brought to the laboratory of the Institute of Microbiology. In order to get rid of microorganisms on the surface of the plant, sterilization was carried out using the above method, and it was planted in MPA, PDA and chapek media to isolate bacteria and fungi (pic 2). The grown bacterial and fungal isolates were purified and isolated (pic 3).

1- picture. Growth process of milk thistle plant.



2- picture. Initial appearance of the isolates grown after incubation.



3- picture. The process of cleaning fungal and bacterial isolates

MALDI-TOF analysis of isolated bacterial and fungal isolates was performed. According to it, it was determined that the isolates were *Bacillus subtilis, Pseudomonas fluorescens, Bacillus vallismortis, Bacillus licheniformis, Malassezia japonica, Bacillus atrophaeus bacteria* and *Fusarium proliferatum.*

Summary. In conclusion, bacterial and fungal isolates were isolated from the stems, leaves and flowers of medicinal milk thistle. As a result of MALDI-TOF analysis, it was determined that they are *Bacillus subtilis, Pseudomonas fluorescens, Bacillus vallismortis, Bacillus licheniformis, Malassezia japonica, Bacillus atrophaeus bacteria* and *Fusarium proliferatum fungus*. In the future, research in this direction will allow us to determine the priority of microorganisms living in plant organs and the separation of primary and secondary metabolites, and to make new discoveries in the field of agriculture and medicine.

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