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Research Article

Isolation of Endophytic Bacteria from Medicinal Plants and Screening for Their Enzyme Production Ability

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ABSTRACT

A total of 45 isolates were isolated from the roots, stems, and leaves of medicinal plants found in fields and yards such as *Plantago major* L., *Hypericum perforatum* L., *Kalanchoe daigremontiana*, *Cichorium intybus* L., *Melissa officinalis* L., *Mentha piperita* L., *Matricaria recutita* L. Among them, it was observed that endophytic bacteria were found relatively more in *Kalanchoe daigremontiana* and *Cichorium intybus* L. Isolate KD-L7 extracted from the leaves of the medicinal plant *Kalanchoe daigremontiana* has a high affinity for 1% casein and 1% starch, the size of its hydrolysis zone was 6-10 mm, its α-amylose activity was 14.2 units/ml, whereas protease activity was 28.6 units/ml.

Keywords: Endophyte; Phytohormone; Biologically active substances; Plant-endophyte symbiosis; Microorganisms; *Bacillus amyloliquefaciens*; *Kalanchoe daigremontiana*; Bacteria

INTRODUCTION

Currently, the use of endophytic bacterial isolates isolated from medicinal plants in processes of solving various problems in the fields of agriculture and medicine, *i.e.* improving plant nutrition, their protection from different phytopathogens, inhibition of root rotting pathogens, increase soil fertility and agricultural crop productivity, accelerating the germination and growth of seeds, ensuring the resistance of plants to various stresses, occupies an important place in the national economy. In addition, they are considered useful in biological protection of mankind and have high enzyme production abilities, and obtaining and using probiotics or creating and using biological preparations, based on living culture associations of endophytic bacteria synthesizing biologically active secondary metabolites, is also essential part of the national economy.

Today, the intensive development of applied microbiology makes it possible to isolate new generation endophytic microorganisms synthesizing many biologically active secondary metabolites (e.g. proteins, enzymes, vitamins, phytohormones, etc.) [1-2] and their use in agriculture and medicine open wide opportunities in the national economy [3]. Medicinal plants can be used to create a new generation of biological preparations in the form of a mono- or mixed culture association, to produce probiotics by breaking down milk protein according to their ability to produce enzymes, to obtain soft ketchup and mayonnaise from starchy products, as well as to improve the properties of the intestinal microflora in humans as probiotic microorganisms [4-5].

Purpose of the work: Screening of medicinal plant isolates for enzyme producing abilities.

MATERIALS AND METHODS

Endophytic bacterial isolates were isolated from stems, leaves and roots of some medicinal plants growing in fields and yards in Uzbekistan. During the isolation of endophytic bacteria, the roots, stems and leaves of the plants were washed in distilled water, and then sterilized in 96% alcohol, and the internal tissues crushed and the resulting liquid part was taken for research. The obtained plant suspensions were inoculated into meat Peptone Agar (GPA) medium and incubated at 37°C for 48 hours. As a result, a total of 45 isolates were isolated from stems, leaves and roots of different medicinal plants. The 45 isolated endophytic bacteria were screened according to their enzyme production abilities and selected for further studies. Enzyme activities were determined according to GOST [2].

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RESULTS AND DISCUSSION

It is known that as a result of the intensive development of applied microbiology and biotechnology, a new generation of endophytic microorganisms has been isolated and their wide use in the national economy is realized [6]. According to the analysis of the data presented in the literature, endophytic microorganisms are found to be more common in the roots, stems and leaves of plants. Because roots, stems and leaves are rich in nutrients necessary for microorganisms and living condition is favourable for them, the endophytic microorganisms found in plant leaves and roots can move up and down the plant while dissolved in water [7-9].

Taking into account the above-mentioned information, in order to isolate endophytic bacteria isolates from some medicinal plants found in fields and yards of our country, bacterial isolates were isolated from the tissues of the roots, stems and leaves of medicinal plants included in the list of the pharmacopoeia, such as *Plantago major* L., *Hypericum perforatum* L., *Kalanchoe daigremontiana*, *Cichorium intybus* L., *Melissa officinalis* L., *Mentha piperita* L., *Matricaria recutita* L. etc. (Table 1).

Table 1: Endophytic bacteria isolates of selected medicinal plants.

The name of medicinal plants	Root (R)	Stem (S)	Leaf (L)	Total
Plantago major L.	2	4	1	7
Hypericum perforatum L.	2	2	1	5
Kalanchoe daigremontiana	2	3	3	8
Cichorium intybus L.	2	3	3	8
Melissa officinalis L.	1	3	1	5
Mentha piperita L.	3	3	1	7
Matricaria recutita L.	2	2	1	5

According to the results of the research, a total of 45 isolates were isolated from stems, leaves and roots of the selected medicinal plants. Some isolated endophytic bacterial isolates were studied for their affinity levels for 1% casein and 1% starch and parameters such as hydrolysis zones, color, transparency, colony edges and surface parts (Table 2).

Based on the obtained results, it is determined that *Plantago major* L. (PmL.) isolates PmL-R1 and PmL-R2 extracted from the root, PmL-S4, PmL-S5 and PmL-S6 isolates extracted from the stem and leaf had affinity for 1% amylose, and PmL-S3 and PmL-L7 isolates extracted from the stem had affinity for 1% casein; *Hypericum perforatum* L. isolates HpL-R1 and HpL-R2 extracted from the root had affinity for 1% amylose, HpL-S3, HpL-S4 and HpL-L5 isolates extracted from the stem and leaf had affinity for 1% casein; All isolates extracted from the root, stem and leaves of *Kalanchoe Daigremontiana* (KD) had affinity for both 1% casein and 1% amylose; *Cichorium intybus* L. (CiL.) isolates-CiL-R1 and CiL-R2 extracted from the root had affinity for both 1% casein and 1% amylose, CiL-S3 and CiL-S4 isolates extracted from stem had affinity for 1% amylose, CiL-S5 isolate had affinity for 1% casein, all CiL-L6, CiL-L7 and CiL-L8 isolates extracted from the leaf had affinity for 1% amylose; *Melissa officinalis* L. (MoL.) isolates all MoL-R1, MoL-S2, MoL-S3 and MoL-S4 separated from the stem and leaves had affinity for 1% casein, MoL-L5 isolate had affinity for both 1% casein and 1% amylose; all *Mentha piperita* L. isolates-MpL-R1, MpL-R2, MpL-R3, MpL-S4, MpL-S5, MpL-S6 and MpL-L7 extracted from the root, stem and leaf had affinity for 1% casein; *Matricaria recutita* L. isolates MrL-R1 extracted from the roots had affinity for 1% amylose, MrL-R2 isolate had affinity for 1% casein and 1% amylose, MrL-S3, MrL-S4 and MrL-L5 isolates extracted from the stem and leaf had affinity for 1% casein [10].

Among these isolates, it was observed that the size of the hydrolysis zone of the isolate extracted from the leaf of *Kalanchoe daigremontiana* KD-L7 was 6 mm-10 mm, it possessed clear silk color, flat edge, clear transparency, dense and shiny surface, and it had high affinity for 1% casein, where its affinity level for 1% amylose was relatively less.

Table 2: Cultural and morphological characteristics of selected endophytic bacterial isolates.

Colonies										
No.	**Naming**	**Forms**	**Size, mm**	**Hydrolysis zone size, mm**	**Color**	**Edge**	**Transparency**	**Surface part**	**Affinity for 1% casein**	**Affinity for 1% starch**
1	PmL is R1	Spherical	2-3	1-4	Pure white	Flat	Transparent	Shiny smooth	-	-
2	PmL is R2	Spherical	1-2	2-3	Brown	Not flat	Opaque	Dense	+	+
3	PmL-S3	Spiral	0.5-2	1-2	Whitish	Not flat	Opaque	Dense	+	+
4	PmL-S4	Spiral	3-4	2-4	Whitish	Flat	Translucent	Smooth	-	-
5	PmL-S5	Rod shaped	2-3	3-4	Pure white	Dentate	Translucent	Embossed	-	-
6	PmL-S6	Rod shaped	2-4	3-6	Pure white	Dentate	Transparent	Embossed	-	+
7	PmL-L7	Spiral	1-2	4-6	Dull milk-white	Not flat	Water-clear	Dense, shiny	++	+
8	HpL-R1	Striated	4-5	4-5	Brown	Gnarled	Translucent	Embossed	+	-
9	HpL-R2	Striated	3-6	2-5	Brown	Flat	Translucent	Dense, shiny	-	-
10	HpL-S3	Spiral	1-2	3-4	Milk-white	Flat	Opaque	Dense, shiny	+	-
11	HpL-S4	Spiral	2-3	3-4	Dull milk-white	Flat	Opaque	Shiny	+	+
12	HpL-L5	Rod shaped	2-6	1-4	Dull milk-white	Flat	Translucent	Dense, shiny	-	-
13	KD-R1	A small stick	2-3	2-3	Milk-white	Dentate	Water-clear	Embossed	+	+
14	KD-R2	A large stick	1-2	2-5	Whitish	Flat	Water-clear	Shiny smooth	+	+
15	KD-R3	Striated	2-3	4-5	Brown	Dentate	Translucent	Embossed	+	+
16	KD-S4	A small stick	2-3	1-2	Milk-white	Flat	Water-clear	Shiny	++	+
17	KD-S5	Striated	1-3	3-4	Pure milk-white	Flat	Water-clear	Shiny	++	+
18	KD-S6	Rod shaped	3-4	3-4	Whitish	Flat	Transparent	Shiny	+	+
19	KD-L7	Rod shaped	4-6	6-10	Pure milk-white	Flat	Water-clear	Shiny smooth	+++	++
20	KD–L8	Spiral rod	2-4	4-6	Pure milk-white	Flat	Water-clear	Shiny smooth	++	+
21	CiL is R1	Spherical	3-6	2-6	Milk-white	Flat	Transparent	Dense, shiny	+	+
22	CiL is R2	Spherical	3-4	3-5	Milk-white	Flat	Transparent	Dense, shiny	+	+
23	CiL-S3	A small stick	4-5	1-3	Dull milk-white	Dentate	Translucent	Embossed	+	+
24	CiL-S4	Spiral rod	2-5	3-4	Dull blackish	Dentate	Opaque	Embossed	+	+
25	CiL-S5	Striated	4-5	3-5	Dull blackish	Dentate	Opaque	Embossed	+	+
26	CiL-L6	A small stick	1-2	3-4	Whitish	Gnarled	Translucent	Embossed	+	+
27	CiL-L7	A large stick	1-3	4-5	Brown	Gnarled	Translucent	Shiny	+	+
28	CiL-L8	Rod shaped	2-4	2-7	It's dark	Flat	Translucent	Shiny smooth	+	+

29	MoL-R1	A large stick	3-5	3-6	Dull milk-white	Flat	Translucent	Shiny smooth	+	+
30	MoL-S2	Striated	2-4	1-4	Brown	Gnarled	Translucent	Embossed	+	-
31	MoL-S3	A large stick	1-4	3-5	Brown	Flat	Translucent	Dense, shiny	+	+
32	MoL-S4	Spiral	2-5	6-10	Brown	Flat	Translucent	Shiny	+	+
33	MoL-L5	Rod shaped	2-3	5-8	Brown	Flat	Translucent	Embossed	+	++
34	MPL-R1	Rod shaped	3-4	4-6	Whitish	Gnarled	Transparent	Dense, shiny	+	+
35	MPL-R2	Spiral	2-4	2-6	Whitish	Dentate	Water-clear	Shiny	+	+
36	MPL-R3	Spiral	3-6	3-5	Pure milk-white	Flat	Water-clear	Shiny	+	+
37	MPL-S4	Rod shaped	3-4	3-4	Whitish	Flat	Transparent	Dense, shiny	-	+
38	MPL-S5	Rod shaped	2-5	4-5	Whitish	Flat	Water-clear	Shiny	-	+
39	MPL-S6	Rod shaped	4-5	3-6	Whitish	Gnarled	Transparent	Dense, shiny	-	+
40	MPL-L7	Rod shaped	3-4	3-5	Dull milk-white	Gnarled	Opaque	Embossed	+	+
41	MrL-R1	Rod shaped	1-2	4-5	Whitish	Flat	Transparent	Dense, shiny	-	+
42	MrL-R2	Rod-shaped	1-4	4-7	Pure milk-white	Gnarled	Transparent	Shiny embossed	++	++
43	MrL-S3	Spherical	3-4	2-6	Brown	Not flat	Translucent	Dense, shiny	+	+
44	MrL-S4	Spherical	3-5	2-4	Brown	Gnarled	Translucent	Embossed	+	+
45	MrL-L5	Spherical	1-5	3-5	Blackish	Dentate	Opaque	Embossed	-	-

It is observed that while hydrolysis zone size of MoL-L5 isolate isolated from leaves of one of the medicinal plants, *Melissa officinalis* L., was 5 mm-8 mm, the color was brown, the edge was flat and its transparency was translucent and it had embossed surface, the isolate MrL-R2 extracted from the root of *Matricaria recutita* L. had a hydrolysis zone size of 4 mm-7 mm and its color was clear, smooth, rough, transparent, and shiny.

Accordingly, KD-L7 isolate extracted from the leaf of *Kalanchoe daigremontiana* L. was selected as an object in our further studies because of its higher affinity for 1% casein and 1% amylose and larger hydrolysis zones comparing to isolates MoL-L5 extracted from the leaf of *Melissa officinalis* L. and MrL-R2 extracted from the root of *Matricaria recutita* L.

In the industry, the demand for proteolytic enzymes that break down milk protein is increasing [2,10]. Therefore, isolation of highly active producers from medicinal plants, and their use in production is critical as they are considered safe and environmentally friendly. In particular, it is necessary to extract microorganism isolates from medicinal plants that can be grown in fields and yards in our country and to study them thoroughly.

It is known that active hydrolytic enzymes are mainly secreted from bacterial cultures; therefore, selection of active strains in pharmaceutical enzyme production processes and their use in production industries is one of the important factors.

Therefore, in our next study, bacterial isolate-KD-L7, which have been chosen because of its high affinity for 1% casein and 1% amylose and large hydrolysis zone, was grown in meat peptone liquid nutrient medium in 37°C for 60 hours and after the incubation period it was screened according to its ability to produce enzymes in the resulting Culture Fluid (CF) (Table 3).

Table 3: Screening of KD-L7, endophytic bacterial isolate extracted from medicinal plants, according to the ability to produce enzymes.

No.	Enzymes	Activity in CF, units/ml	Protease activity, units/mg
1	α-amylose	14.2 ± 1.1*	1.2 ± 0.08**
2	Glucoamylose	3.4 ± 0.2	0.92 ± 0.02

3	Protease	28.6 ± 1.3	1.4 ± 0.06
4	Cellulase	1.4 ± 0.024	0.35 ± 0.012
5	Xylanase	0.4 ± 0.02	0.24 ± 0.03

Note: P ≥ 0.05 *underlined numbers are reliably different from the control variant.

As can be seen from the table, according to the ability of the KD-L7 endophytic bacterium isolated from the *Kalanchoe daigremontiana* to produce enzymes, α-amylose activity was 14.2 units/ml, and protease activity was 28.6 units/ml. Therefore, it was found that the selected bacterial isolate KD-L7 synthesizes mainly amylose and protease enzymes more than other enzymes.

Thus, isolate KD-L7 was selected for further studies due to its protease and α-amylose activities. The KD-L7 isolate, selected as a result of the screening, was identified using the MALDI TOF mass spectrometry method. The tested KD-L7 isolate was identified as *Bacillus amyloliquefaciens* [8-10].

CONCLUSION

In conclusion, among the medicinal plants such as *Plantago major* L., Hypericum perforatum L., *Kalanchoe daigremontiana*, *Cichorium intybus* L., *Melissa officinalis* L., *Mentha piperita* L., *Matricaria recutita* L. found in fields and yards, relatively more endophytic bacteria were found in *Kalanchoe daigremontiana* and *Cichorium intybus* L. The isolate KD-L7 isolated from the leaf of the medicinal plant *Kalanchoe daigremontiana* showed a higher affinity for 1% casein and 1% starch than other isolates, as well as had larger hydrolysis zone, and relatively high protease enzyme activity.

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