



## Research Article

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# Isolation and Characterization of Endophytes From the Root of Medicinal Plant *Chlorophytum borivilianum* (Safed musli)

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## Abstract

Bacterial endophytes were isolated from the root of *Chlorophytum borivilianum* (safed musli) a medicinal plant of North India and screened for enzymes of biotechnological importance. Five bacterial species were isolated. Four isolates were gram positive while one isolate was found to be gram negative. All the isolates showed different enzymatic activity like amylase, protease, esterase and pectinase. These endophytes were identified by 16s rDNA amplification and sequencing as *Bacillus pumilus*, *Bacillus subtilis*, *Bacillus megaterium*, *Pseudomonas mendocina* and *Staphylococcus pasteurii*.

**Keywords:** endophytes, medicinal plants, *Chlorophytum borivilianum*, bacteria.

## Introduction

Endophytic bacteria are defined as those bacteria that colonize the internal tissue of the plant showing no external sign of infection or negative effect on their host<sup>1,2</sup>. Bacterial endophytes colonize an ecological niche similar to that of phytopathogens, which makes them suitable as biocontrol agents<sup>3</sup>. The endophytic niche offers protection from the environment for those bacteria that can colonize and establish in plant. These bacteria generally colonize the intercellular spaces, and they have been isolated from all plant compartments including seeds<sup>4</sup>. Symptom less internal colonization of healthy plant tissues by microorganisms is a widespread and well-documented phenomenon. Endophyte is an all-encompassing topographical term that includes all organisms which during a variable period of their life-cycle colonize the living internal tissues of their hosts without producing symptoms of disease<sup>5</sup>. Common endophytes include a variety of bacteria, fungi and actinomycetes, and they can be isolated from wild<sup>6</sup> or cultivated crops<sup>7</sup> of either the

monocotyledonous<sup>8</sup> or dicotyledonous plant groups<sup>9</sup>. Endophytic bacteria in a single plant host are not restricted to a single species but comprise several genera and species. No one knows if communities inside plants interact, and it has been speculated that beneficial effects are the combined effect of their activities. Endophytes from medicinal plants have become a hot topic for metabolite discovery because of their high biodiversity and predicted potential to produce novel compounds. Endophytic fungi produce bioactive metabolites which play an essential role to provide protection to their host against attack by other pathogen and environmental factors. Endophytes could lead to development of novel pharmaceutical agent against human disease. In the present investigation endophytes were isolated from the medicinal plant *Chlorophytum borivilianum* (Safed musli). Safed musli is a healthy plant with the ability to withstand severe fluctuations in climatic conditions. It is propagated by tubers and it is used in Ayurvedic medicines in India. It is well known for its aphrodisiac properties. *Chlorophytum borivilianum* belongs to the family Liliaceae. It is an annual herb of 1.5 feet high with sub-erect lanceolate leaves

erect dense flowered racemes of white colors and tuberous root system. Safed Musli is used for the preparation of health tonic that is used in general weakness and debility.

## Experimental

### Isolation of Endophytes

Fresh healthy root of plant were used for the isolation procedure. The samples were washed with sterile distilled water to remove the soil particles. For surface sterilization they were treated with ethanol 70% for 30 seconds and then treated with sodium hypochlorite (3-5% available chlorine) for 3 minutes. Samples were exhaustively rinsed with sterile water so that all the epiphytic microorganisms can be removed. Roots were dissected into pieces of 1 cm and aseptically transferred to Petri dishes containing 3% water-agar. Plates were incubated at room temperature for 3-4 days. The confirmation of isolates was done by streaking the same isolates on the plate containing 3% agar and sterile root extract as the only source of nutrients.

### Identification of isolates

The morphological identification of isolate was carried out by performing gram staining, spore staining and capsule staining. The isolates were identified by biochemical tests.

### Determination of the enzymatic activity

The activities of the following enzymes were detected on solid media; amylase, esterase, protease and pectinase. The enzymatic activities were performed by initially growing the isolates in Nutrient broth for 24 h at 30°C.

### Determination of amylolytic activity

The isolates were streaked on the plate containing nutrient agar (NA) with 0.2% soluble starch as substrate, pH 6.0 which is previously sterilized. After incubation the culture was treated with Lugol's iodine, which allows the formation of clear halos around the colony<sup>10</sup>.

### Determination of the proteolytic activity

For determination of casein hydrolysis the test organisms were spot inoculated on casein agar plate, which contains (g/l) nutrient broth 8.0, glucose 1.0 and agar 18.0, pH 7.8 with separately autoclaved 15.0ml skimmed milk. After incubation, 2.0ml of HCl 0.1 mol/l was added on the plate and formation of clear halos around the colony were observed<sup>11</sup>.

### Determination of the pectinolytic activity

For determination of pectin degradation capacity the test isolates were spot inoculated on pectin agar plate. The pectin agar plate contains (g/L) nutrient broth 13, pectin 5, agar 18 and cetyl pyrimidium chloride 1% as developer. After incubation the plates were covered with developer, and the presence of clear halos around the colony indicate pectin degradation<sup>12</sup>.

### Determination of the esterase activity

The media contain following components to determine the ester hydrolysis ability of test isolates (g/l<sup>-1</sup>) peptone 10.0, NaCl 5.0, CaCl<sub>2</sub> 2H<sub>2</sub>O, 0.1, agar 18.0, pH 7.4. To the sterilized culture media, previously sterilized Tween 80 was added in a final concentration of 1% (v/v). The precipitation of ester compound around the colony indicates the presence of esterase enzyme<sup>13</sup>.

### 16s rDNA sequencing:

Isolates were subjected to 16S rDNA amplification and sequencing for identification. Colony PCR was used to amplify the 16S rDNA by using universal primers. The amplicons were subjected to sequencing. Then the sequence was searched in the database using BLAST at NCBI<sup>14</sup>.

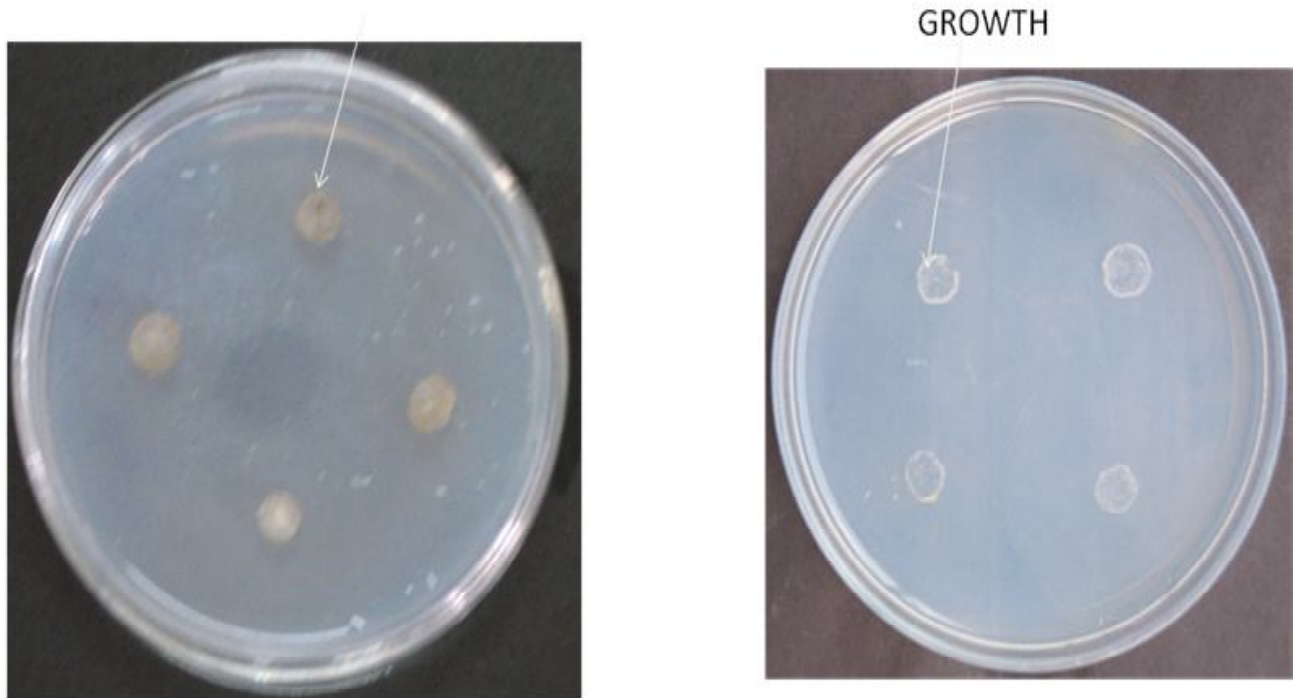
## Results

### Isolation of endophytes

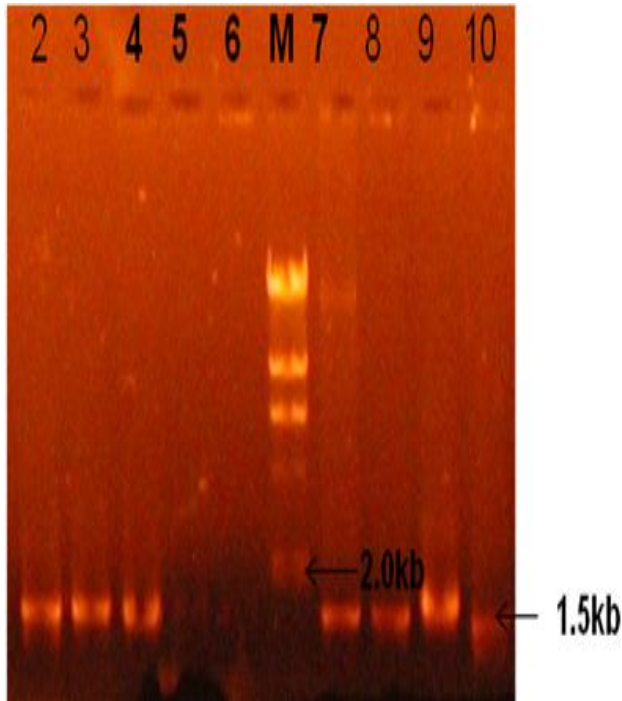
After 3-4 days of incubation the growth of organism was observed below the root samples, as shown in Figure 1. The microbial growth surrounding the root on water agar indicated that the organism gained their nutrients from the root only. Portion of this growth was then streaked on the Luria agar plates and 22 different colonies were obtained. Out of 22 different isolates only 12 isolates grew on root extract water agar plates which were selected for further study. Out of 12 isolates 11 isolated were gram positive while one isolate was gram negative in nature.

### Determination of the enzymatic activity

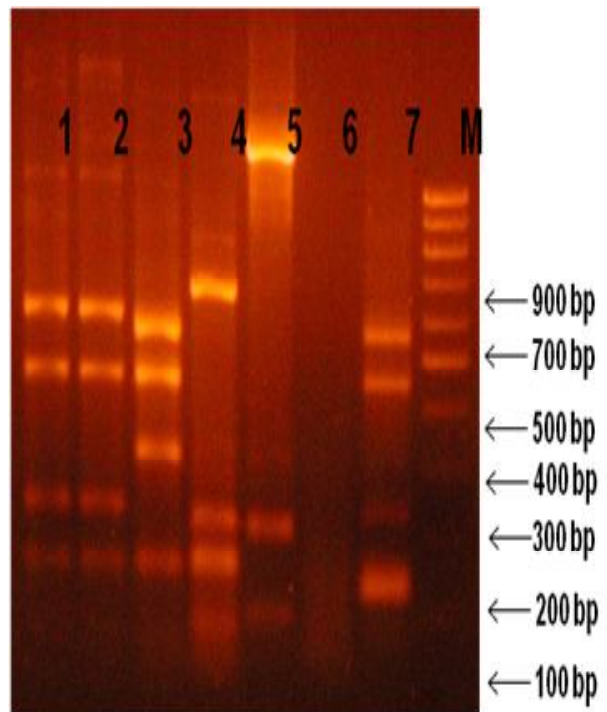
It was observed that all the isolates possessed at least one enzymatic activity tested (Table 1). Of the total isolates obtained 80% were amylase and protease producers, 60% were esterase producers while 20% were pectinase producers. All the isolates showed IAA production. Amylase positive isolates indicated starch degradation on starch agar plates. Plant tissue stores starch as a food source and the endophytes can consume the starch before other new colonizers appear<sup>15</sup>.



**Figure 1:** Isolated colonies of bacteria on water agar plates A) root samples were placed on the Agar plate showing root samples (B) Agar plate showing bacterial colonies



**Figure 2:** Amplification of 16s r DNA from the genomic DNA of endophytic isolates of Chlorophytum  
 M=  $\lambda$  HindIII  
 Lane 2-10, bacterial isolates of Chlorophytum



**Figure 3:** ARDRA pattern of the amplified 16s rDNA of bacterial isolates  
 Lane 1-7, bacterial isolates of *Chlorophytum*

**Table 1.** Enzymatic activity of endophytes from *Chlorophytum borivilianum*\*

Endophyte/Isolates	Enzyme activity (EI)** (µg/ml)				
	Amylase	Protease	Pectinase	Esterase	IAA
<i>B. pumilus</i>	0.82	0.80	0.72	0.36	11.66
<i>B. megaterium</i>	0.80	1.0	-	-	30.00
<i>B. subtilis</i>	0.79	0.56	-	-	48.33
<i>S. pasteuri</i>	-	0.75	-	0.26	1.60
<i>P. mendocina</i>	0.61	-	-	0.36	21.66

\* An average of three repetitions

\*\* The enzymatic index represent the halo diameter of degradation/diameter of colony in cm

Amylase producer were *B. pumilus*, *B. megaterium*, *B. subtilis* and *Pseudomonas mendocina*. Clear halo on Tween 80 plates indicated esterase activity of the endophytes. Esterase breaks down fats into fatty acid and glycerin, but it also brings the reversible reaction. In reversible reaction, esterase enzyme helps the plant in production of saponine which gives a significant medicinal property to the plant. Esterase producers were identified as *Ps. stutzeri*<sup>16,17</sup>. In the present study *B. pumilus*, *Staphylococcus pasteuri* and *Pseudomonas mendocina* showed esterase activity. *Bacillus* and *Pseudomonas* species are known to produce esterase. Protease produces as identified by casein hydrolysis were *B. pumilus*, *B. subtilis* and *Staphylococcus pasteuri*. Few of the isolates showed positive activity towards pectin degradation.

### 16SrDNA gene sequencing and identification of isolates

Isolates were selected based on the basis of ARDRA (amplified rDNA restriction analysis) and subjected to sequencing. On the basis of preliminary characteristic and ARDRA pattern six isolates were subjected to 16SrDNA sequencing for identification (Fig.2 and 3). Hence total five isolates were selected for sequencing and blast search.

### Discussion

It was reported that *Ps. stutzeri*, *B. megaterium* and *B. licheniformis* produce extracellular amylase<sup>18,19</sup>. Pectic substances are present in primary wall and cambial tissue, where it forms the membrane that separate the young daughter cells produce by cambium<sup>20</sup>, so pectinase positive isolates may be the latent pathogen to the plant. *B. pumilus* showed pectinase activity. *Pseudomonas spp.* are known to be beneficial to plants because of their ability to promote plant-growth and/or act as BCAs against a number of plant diseases and pests<sup>21</sup>, and some *Pseudomonas*

*spp.* can also be endophytically established<sup>21,22</sup>. *Bacillus sp.* and *Staphylococcus sp.* are present in soybean (*Glycine sp.*) and Arabidopsis plant<sup>23</sup>. Endophytic bacteria live in the interior of plant without affecting the host<sup>24</sup>. Endophytes have been isolated from grass<sup>25</sup>, Corn<sup>26</sup>, Cotton<sup>27</sup>, Bryophytes<sup>28</sup> and *Solanum lycocarpu*<sup>29</sup>. Different genera and species of bacteria were isolated during the present study. Earlier isolation of endophytic *Staphylococcus* was reported<sup>30,31</sup>. This is the first report on the isolation of endophytic microorganisms from *Chlorophytum borivilianum* with biotechnologically useful enzymatic activity.

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