The Quarterly Scientific Journal of Applied Biology
Vol. 35, No 3, Autumn 2022, p: 22-24
Journal homepage: https://jab.alzahra.ac.ir
10.22051/JAB.2021.34043.1392

Isolation and Identification of Endophytic Bacteria from Safflower (*Carthamus tinctorius* L.) Root and Investigating their Effect on Seed Germination and Plant Growth

S. Nazarpour[1], M. Mohammadi-Sichani[2*], M. Ranjbar[3]

1- Msc student in Microbiology, Falavarjan Branch, Islamic Azad University, Esfahan, Iran.
2- Assistant Proffessor of Biotechnology, Falavarjan Branch, Islamic Azad University, Esfahan, Iran.
*(Corressponding author: Ma.Mohammadi1347@iau.ac.ir)
3- Assistant Proffessor of Biology, Falavarjan Branch, Islamic Azad University, Esfahan, Iran.

Received:2021.9.18 **Accepted: 2022.07.02**

Introduction

The safflower (*Carthamus tinctorius* L.) belongs to the Asteraceae family. Safflower seeds have 25 to 40% oil and about 20% protein. Safflower oil is of high quality compared to other vegetable oils. Safflower is an oil plant and has industrial, medicinal and food uses (Turgumbayeva *et al*., 2018, Chakradhari *et al*., 2020). The Plant growth stimulating bacteria are a large group of bacteria that are present in the rhizosphere of plants and increase and stimulate plant growth in different ways (Santoyo *et al*., 2016). A group of these bacteria called endophytes enter the internal tissues of the plant. These microorganisms grow outside or inside the plant cells without causing obvious damage. In this type of relationship, a balanced life is provided between the plant and the endophytic bacteria. The Endophytic bacteria help the growth of the host by producing growth stimulating hormones such as indole acetic acid (IAA) or preventing the production of growth inhibiting hormones such as ethylene (Fouda *et al*., 2021). Today, the use of endophytic bacteria as biological fertilizers has created a revolution in agriculture (Orozco-Mosqueda *et al*., 2021). Various studies on the effect of endophytic bacteria on the activities of the host plant through the production of auxin and gibberellin growth hormones, phosphate dissolution, nitrogen fixation, siderophore production, the ability of biological control of pathogenic microorganisms and increasing the resistance of plants to stress abiotic and biotic are being implemented (Gamalero *et al*., 2020). Studies have shown that most endophytic bacteria increase plant growth and tolerance against environmental stresses. The aim of this study was to identify safflower endophytic bacteria and to detect their effects on seed germination and plant growth.

Methods and Materials

The safflower plant of Isfahan variety was collected from agricultural fields. After washing and disinfecting, the roots were completely crushed in a sterile mortar. The secreted liquid and crushed root tissue were cultured in nutrient broth medium. Then the samples were cultured on Nutrient Agar by sreak plate method and incubated at 23°C for 24-48 hours. (Duan *et al*., 2013, Khan *et al*., 2020). The macroscopic and microscopic characteristics of the isolates were investigated on the nutrient agar medium. After preparing the direct slide, according to the result of the warm reaction of each isolate, biochemical tests such as catalase, oxidase, VP, MR, SIM, OF, TSI and urease were used to identify the isolates (Nahon et al. 2015). The isolates were identified by polymerase chain reaction (PCR). The amplified fragment was sent to Royan-Biogene Company for sequencing. The results were checked by Chromas software and evaluated with the help of BLAST server in NCBI database (Suhandono *et al*., 2016).

Auxin activity and phosphate solubilization ability of isolates were measured by Salkowski and ammonium molybdate method, respectively. The effects of isolates were evaluated on the safflower seed germination and growth. The activities of pectinase, xylanase, amylase and protease enzymes were investigated in the isolates (Bhange *et al*., 2016). Antifungal activity of safflower endophytic isolates was investigated by toxic food method against *Aspergillus niger* (ATCC: 9142) and *Alternaria alternata* (PTCC: 5224).

The collected data were analyzed with SPSS version 17 software. Data variance analysis and mean comparison were done using Duncan's test.

Results and Discussion

Five endophytic bacteria including *Micrococcus luteus*, *Pseudomonas corrugata*, *Pseudomonas fluorescens*, *Pseudomonas brassicacearum*, and *Bacillus megatrium* were isolated from safflower roots. In treatment with *Bacillus megatrium* strain, the mean of germinated seeds by exposure to the concentration of 10^5 bacteria per ml was significantly higher than concentration of 10^8 bacteria per ml. Coleoptile length and root length in the control group were significantly shorter than the treatment group. There was a direct relationship between the amount of auxin produced by endophytic isolates and their ability for phosphate solubility with increasing safflower coleoptile and root length, and germinated seeds. In a study, four endophytic bacteria including *Micrococcus luteus*, *Bacillus megaterium*, *Pseudomonas chlororaphis* and *Pseudomonas fluorescence* were isolated from safflower. The amount of auxin production in each bacterium was equal to 15.3, 30.6, 22.48 and 19.28 µg/ml, respectively (Singh & Dubey, 2018). The findings of this project in the highest amount of auxin production are similar to the findings of the present project in Bacillus genus. *Pseudomonas brasicacearum* strain produced all the four enzymes, pectinase, amylase, protease and xylanase. The *Pseudomonas fluorescence* with the most effectiveness inhibited the growth of *Aspergillus niger* by 30.76%.

Conclusion

The isolated endophytic bacteria in the present study are suggested as stimulants of plant growth in the field due to their ability to produce auxin and to dissolve phosphate and their direct effect on safflower growth factors.

Key words: Antifungal effect, Growth-promoting bacteria, Plant root, Safflower

Declaration of conflict of interest: *The author declare that they have no conflicts of interest.*