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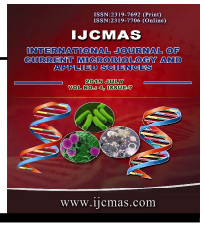

Original Research Article

Molecular Profiling and Bacteriocin Production of Endophytic Bacteria Isolated from *Solanum trilobatum* L. Leaves

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K e y w o r d s

Bacterial endophyte, *Bacillus subtilis*, Tryptone, *Solanum trilobatum*

A B S T R A C T

Bacteriocins are bacterial ribosomal synthesized antimicrobial peptides lethal to bacteria other than the producing strain. In the present investigation, a bacterial endophyte was isolated from *Solanum trilobatum* L. (Solanaceae) leaves. The isolated endophyte was identified as *Bacillus subtilis* (BMP01) by 16S rRNA gene sequence analysis. The production of bacteriocins of *Bacillus subtilis* (BMP01) was evaluated by using well agar method. Different concentrations of NaCl, yeast and tryptone extracts were selected as variables for maximum production of bacteriocin and significant effects of variables were observed on the production of bacteriocin.

Introduction

Bacteriocins are bacterial ribosomal synthesized antimicrobial peptides lethal to bacteria other than the producing strain (Nissen-Meyer and Nes, 1997). Members of the *Bacillus* group are considered good producers of antimicrobial substances, including peptide and lipopeptide antibiotics, and bacteriocins (Stein *et al.*, 2005). Bacteriocins from *Bacillus* species offer a much broader spectrum of potential applications compared with lactic acid bacteria bacteriocins. Almost every plant on the earth hosts endophytic bacteria that could serve as potential source of novel natural products, which are of a great potential not only in medicine but also in various other sectors of the biotechnology industry (Strobel, 2002; Guo *et al.*, 2008). However, endophytes from medicinally important plants are of a great interest, especially in understanding their potential medicinal properties and to explore their potential applications (Mehanni and Safwat, 2010).

Solanum trilobatum (Solanaceae), a thorny creeper with bluish violet flowers, more commonly available in Southern India has

been used traditionally in Siddha system of medicines, to treat various diseases. Popularly called 'Thoothuvalai' by the local tribes, villagers and herbalists in Tamil Nadu, this ethnobotanical herb is known to have unique medicinal properties (Doss *et al.,* 2009). The extensive survey of literature revealed that *S. trilobatum* is an important source of many pharmacologically and medicinally important chemicals, especially steroidal hormone solasodine and other chemicals like solasonine, diosgenin and various useful alkaloids. The plant is extensively studied for the various pharmacological activities like hepatoprotective, anti-inflammatory, anti-microbial and haemolytic activity, immunomodulation, antibacterial etc. Accordingly, the present investigation was deliberated to molecular profiling and bacteriocin production of endophytic bacteria isolated from *Solanum trilobatum* L.

Materials and Methods

Isolation and identification of endophytic bacteria

The endophytic bacteria was isolated from leaf of *Solanum trilobatum* L. leaves and identified on the basis of 16S rRNA gene sequence analysis. (Who method}

Identification of the isolate by 16S rRNA gene sequencing

DNA extraction, polymerase chain reaction (PCR) and DNA sequencing Genomic DNA was extracted by using DNA purification kit (Genei, Bangalore, India). The PCR analysis was carried out with a volume of 20 mL of mixture in a DNA gradient thermocycler (Model no. P 818070424, Astec, Fukuoka, Japan). The procedure comprised 35 cycles at 92°C for 1 min, 45°C for 1 min and 72°C for 1 min. The primer used for amplification was 5' GCAAGTCGAGCGGACAGATGG GAGC 3' and 5' ACTCTCGTGGTG TGA CGGGCGGTG 3'. The amplified PCR product was harvested from 1 % agarose gel and purified with gel extraction and PCR purification kit (Sigma-Aldrich, St. Louis, MO, USA). The purified product was sequenced with an automated sequencer (ABI PRISM 310, Applied Biosystems, Foster City,
CA, USA).

BLASTN analysis

The 16S rRNA sequence was then analyzed for similarities by using BLASTN tool (BLASTN, National Center for Biotechnology Information, Bethesda, MD, USA www.ncbi.nlm.nih.gov:80/BLAST/).

Phylogenetic tree analysis

The phylogenetic tree analysis was done by the Molecular Evolutionary Genetics Analysis MEGA 4.1(Tamura *et al.,* 2007) software and the tree was constructed by using the neighbor-joining method (Saitou and Nei, 1987).

Bacteriocin production (Ansari *et al.,* 2012)

Bacteriocin production by *Bacillus subtilis* (BMP01) was carried out in modified TY medium (Tryptone - 10.0, Yeast extract 5.0, NaCl 5.0g/L) having initial pH 7.0 and sterilized at 121ºC for 15 minutes. Inoculums (100 ml) were grown in the medium at 37º C for 24 hours. One of the samples was used for measuring optical density (O.D.) at 600 nm. After that, the broth were transferred to 500 ml conical flask and placed in shaking incubator with the agitation of 150 rpm for 10 minutes. After agitation, the cells were harvested by centrifugation at 10, 000 rpm for 10 minutes at 4ºC and cell free supernatant was filtered

through 0.22µm filter membrane under sterile conditions and stored at -20ºC for further studies.

Antibacterial assay

The antibacterial activity of bacteriocin (cell free supernatant) was detected against *Escherichia coli* and *Staphylococcus aureus* by agar well diffusion method (Tagg and McGiven, 1971). Nutrient agar plates were spread with 100 µl suspension of each indicator strain containing 2 x 10^8 cfu/ml (Iqbal, 1998). Cell free supernatants (25µl) were added to 5 mm wells on nutrient agar plates. The plates were incubated for 24 hours at specific temperature according to indicator strains used. All the experiments were performed in triplicate and the results were the mean of the observations. The antagonistic activity in arbitrary unit/ml (AU/ml) was calculated (Bhaskar *et al.*, 2007) as a measure of bacteriocin production.

$$AU/ml = \frac{\text{Diameter of the zone of clearance (mm)} \times 1000}{\text{Volume taken in the well (µl)}}$$

Effect of NaCl, tryptone and yeast extract concentration on bacteriocin production

Different concentrations of NaCl were used in the modified TY medium ranging from 0.2 to 1%. Similarly, tryptone and yeast extract concentrations were also varied from 0.2 to 1%. All the experiments were performed in triplicate and the results were the mean of the observations.

Results and Discussion

16S rRNA gene sequence based bacterial identification has been used for the rapid and accurate identification of endophytic bacterial isolates (Clarridge, 2004). Thus, we amplified the 16S rRNA gene for rapid and precise identification of the isolated endophytic bacteria.

The 16S rRNA gene sequence of isolated endophyte obtained in this study were deposited in GenBank under the accession number KJ816348.1. BLAST homology analysis was also carried out to compare with other 16S rRNA, partial and complete sequences available in the GenBank of NCBI and it revealed that the sequence of isolated endophyte showed the maximum homology (99%) with *Bacillus subtilis* DSM 10 (GenBank Accession Number KJ812207.1). Therefore, the isolate from *Solanum trilobatum* L. leaves was identified as *Bacillus subtilis* (BMP01). Evidently, the prevalence of genus *Bacillus* commonly associated with plants as endophyte as reported in similar works (Krid, 2010; Borriss and Maheshwari, 2012; Ma *et al.*, 2013; Jagadesan and Bhore, 2014).

The evolutionary history was inferred using the Neighbor-Joining method. The overall tree topology suggested that the tree is divided into 2 main clades namely A, B. The clade A with two taxa and the clade B had totally 16 taxa including test organism. The isolated endophyte *Bacillus subtilis* (BMP01) shared with *Bacillus subtilis* (KJ528401) (Fig. 1).

The antibacterial activity of bacteriocins was observed against two indicator strains such as *Escherichia coli* and *Staphylococcus aureus.* The optical density value of different concentration of NaCl, yeast and tryptone on bacteriocin production was observed at 600 nm (Table 1). NaCl concentration was varied for bacteriocin production it was observed that at 0.6% maximum production was achieved and maximum zone of inhibition was observed (Fig. 2 & 3). Similarly maximum bacteriocin production was observed at 0.6 % of yeast concentration (Fig. 4 & 5).

Table.1 Optical density (OD) values (600 nm) at different NaCl, yeast and tryptone concentration

S. No.	Different concentrations (%)	OD values at 600 nm		
		NaCl	Yeast	Tryptone
1.	0.2	0.150	0.137	0.233
2.	0.4	0.194	0.213	0.348
3.	0.6	0.222	0.235	0.471
4.	0.8	0.212	0.225	0.503
5.	1	0.186	0.221	0.580

Fig.1 Evolutionary relationships of 18 taxa

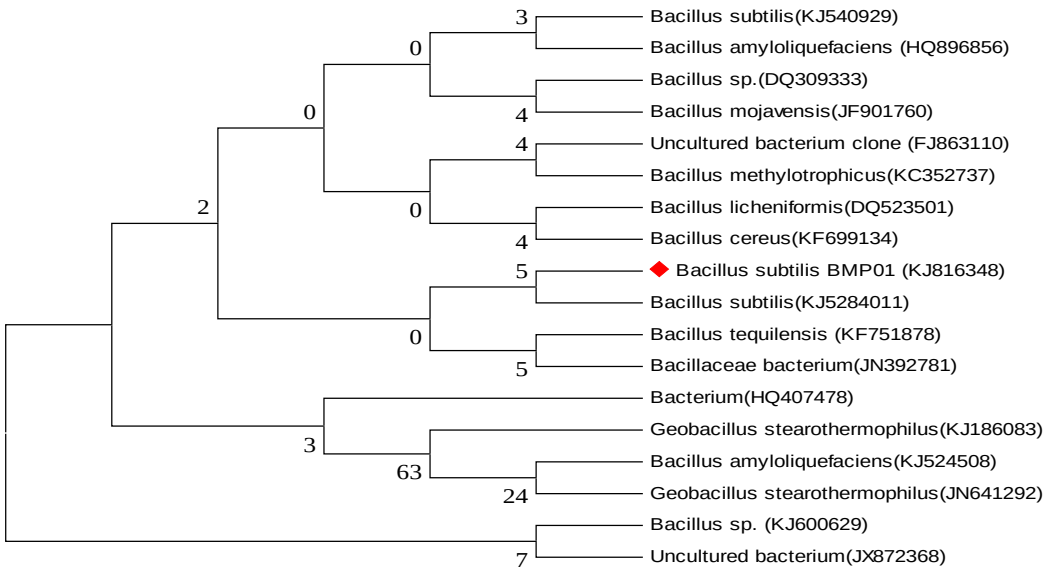


Fig.2 Production of bacteriocin at different NaCl concentration (Indicator strain - *Escherichia coli*)

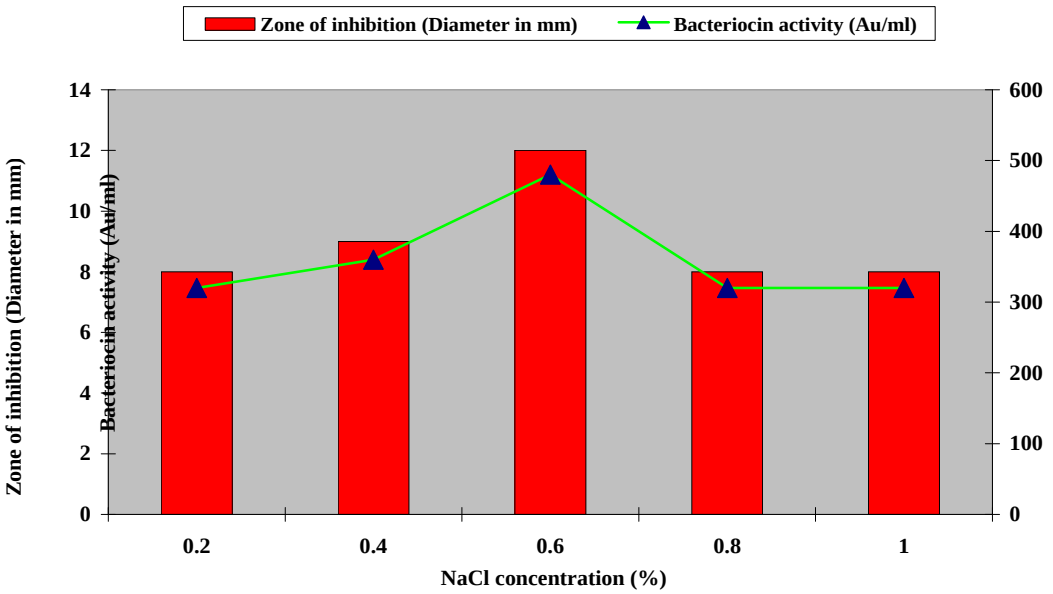


Fig.3 Production of bacteriocin at different NaCl concentration (Indicator strain - *Staphylococcus aureus*)

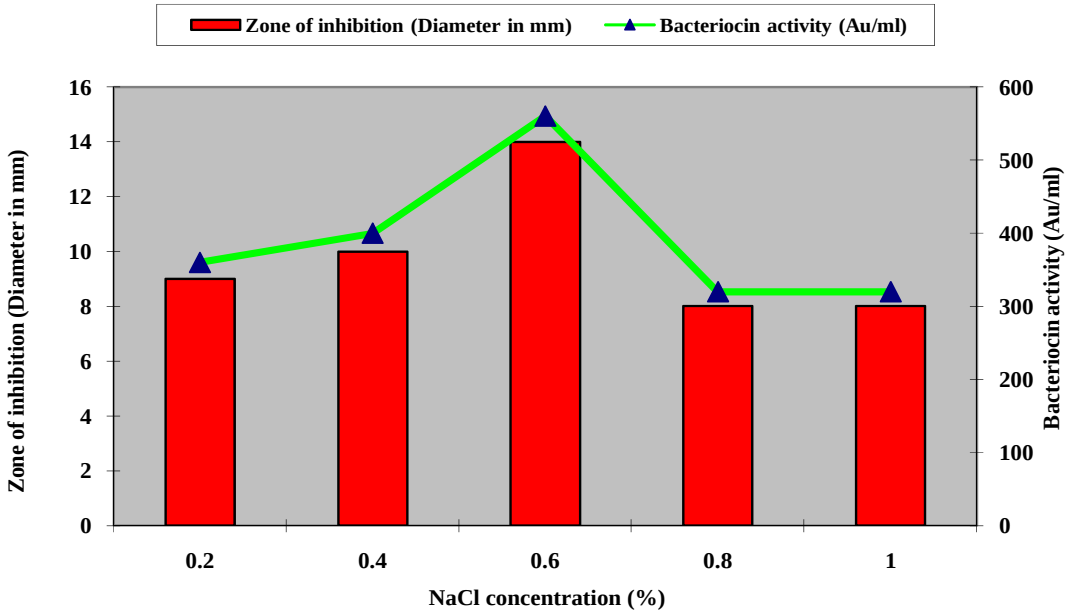


Fig.4 Production of bacteriocin at different yeast concentration (Indicator strain - *Escherichia coli*)

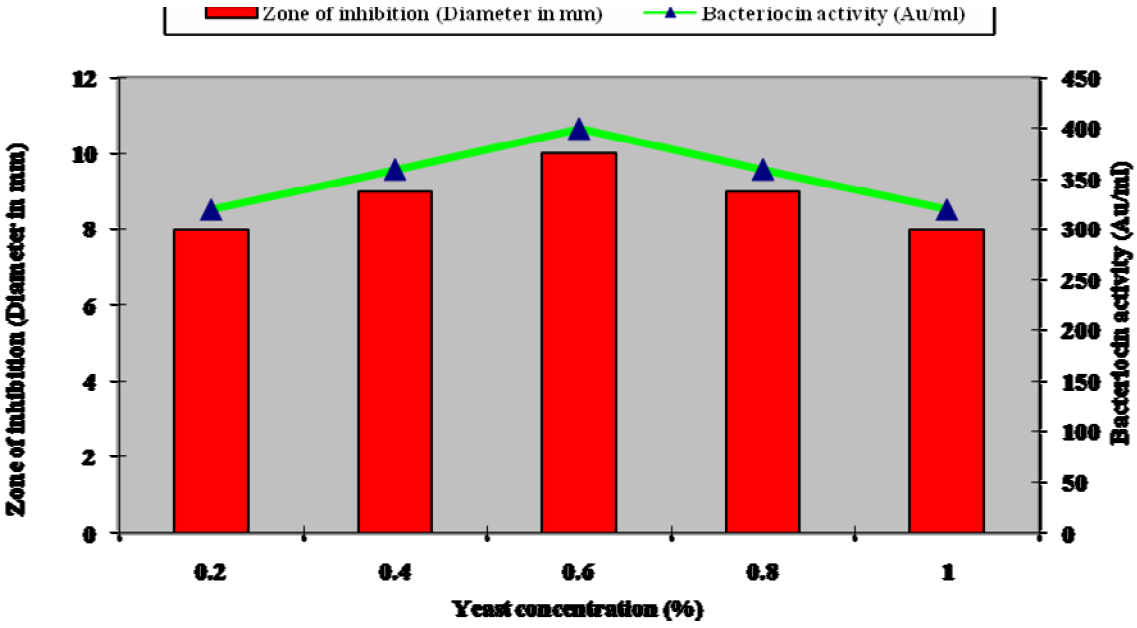


Fig.5 Production of bacteriocin at different yeast concentration (Indicator strain - *Staphylococcus aureus*)

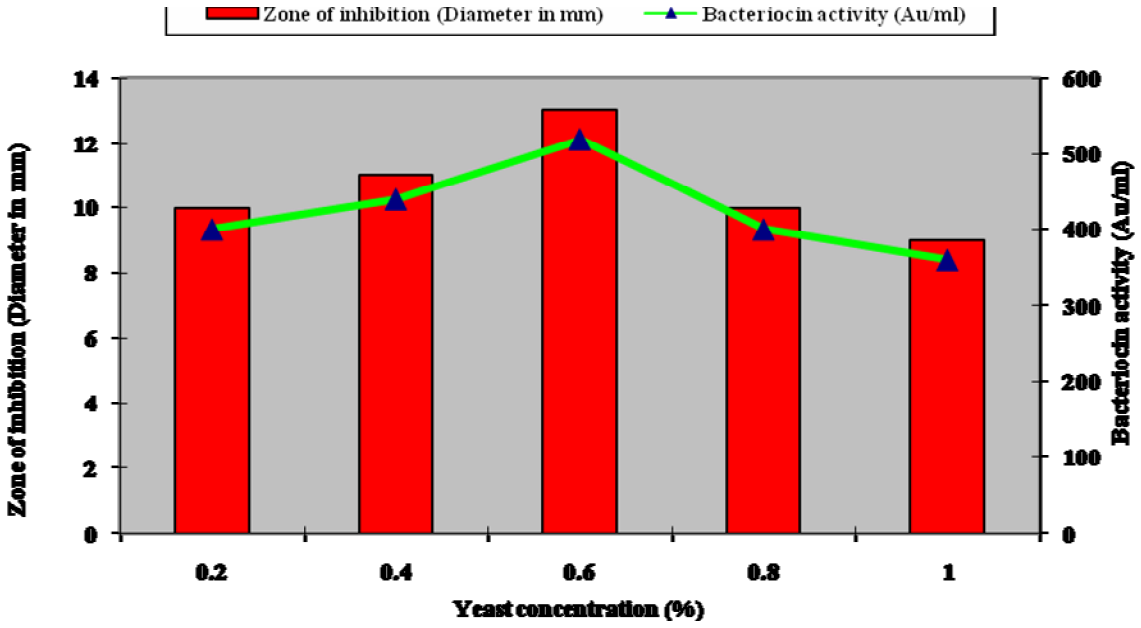


Fig.6 Production of bacteriocin at different tryptone concentration (Indicator strain - *Escherichia coli*)

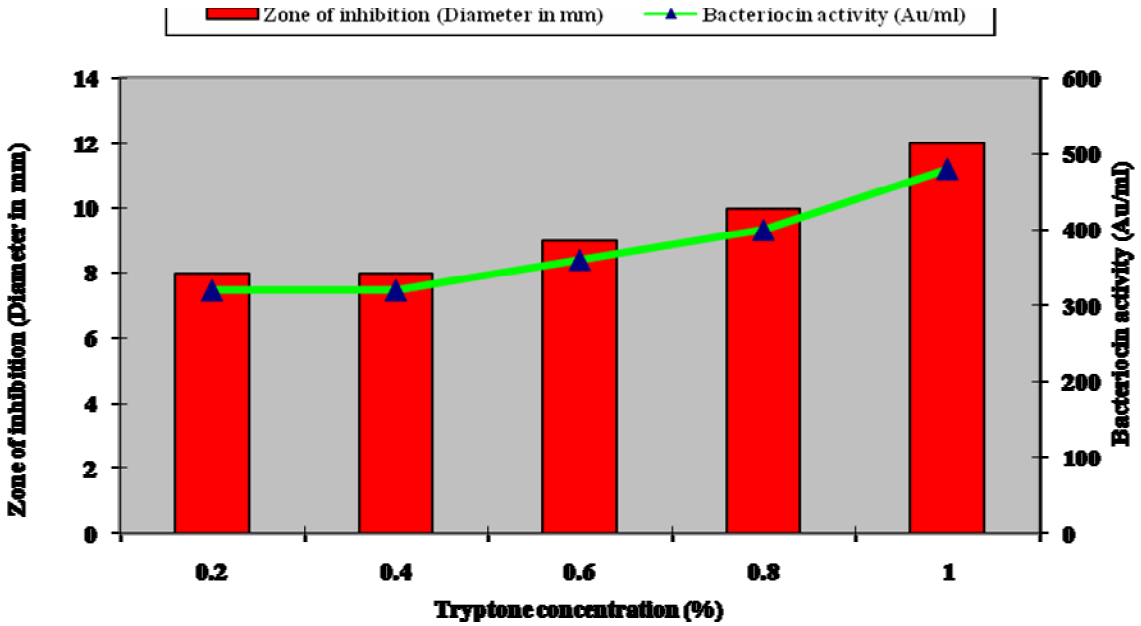
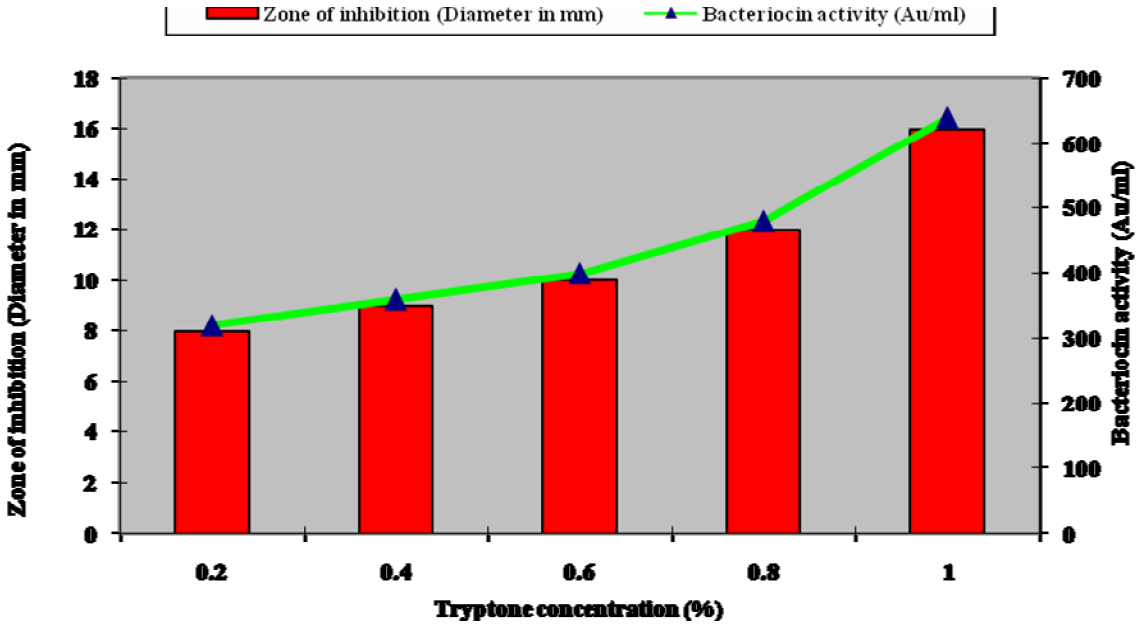


Fig.7 Production of bacteriocin at different tryptone concentration (Indicator strain - *Staphylococcus aureus*)



In case of tryptone concentration maximum bacteriocin production was observed at 1% (Fig. 6 & 7). Likewise Ansari *et al.* (2012) reported that the maximum bacteriocin production of *Bacillus subtilis* was achieved at 1% tryptone, 0.5% yeast extract and 0.5% NaCl concentration. The overall investigation can be concluded that the isolated endophyte *Bacillus subtilis* (BMP01) as a potential source for bacteriocin production.

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