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Prevalence of Bacteria in Tasar Growing Soils and their Isolation and Characterization

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A B S T R A C T

Sericulture is an agro-based industry providing employment to millions of rural as well as urban population. The Sericulture industry depends on the production of good quality of cocoon. The quality and quantity of Cocoon depends on the nutrients of the leaf. The nutrient of the leaf comes from the soil. The rhizobacteria present in their soil plays important role in solubilizing the insoluble form of nutrient making them available to plant. The current study was carried on the microbial population of Tasar growing region. The isolate was examined on the basis of their color and surface morphology. The biochemical test starting from gram staining test, carbohydrate fermentation test (Glucose, Lactose, Mannitol and Sucrose), Litmus milk test, Catalase test, IMViC test, Urease test, Gelatin hydrolysis test, Nitrate reduction test Starch hydrolysis test and Triple sugar test. 16rRNA analysis was carried out for 4 isolates. According to morphological, biochemical characterization it was found that the isolates belong to the genus *Bacillus spp., Pseudomonas spp., Planoccocus spp*., and *Azotobacter spp*. On the basis of 16S rRNA analysis of 4 isolates were found to be *Planococcus halotolerans* SCU63(T), *Bacillus tequilensis* KCTC13622(T), *Bacillus megaterium* NBRC15308(T) and *Bacillus zhangzhouensis* DW5-4(T). This finding suggest that identified strain may be utilized as biofertilizers after further screening their ability of nitrogen fixation and phosphate solubilization capability because soil fertility is the major factor in the sericulture industry for the production of good quality crops.

***Key words*:** Cocoon, Sericulture, Agro-based industry, *Azotobacter sp., Pseudomonas sp.*

Tasar Silk and Kosa silk (Sanskrit) both are same (https://en.wikipedia.org/wiki/Tussar_silk). Larvae produced from the moth of silkworm genus *Antheraea* including *A. paphia*, *A. assamensis*, *A. mylitta*, *A. roylei*, *A. pernyi*, *A. yamamai*, *A. mylitta* and *A. paphia* are reared mostly in central and eastern portions of India [1]. *Antherae mylitta D* is a polyphagous insect feeds primarily on Sal (*Shorea robusta* Gaertn), Arjuna (*Terminalia arjuna* W&A) and Asan (*Terminalia tomentosa* W&A) and so many of secondary food plants found in the natural forest of tropical Tasar belt [2] of which Jamun (*Syzygium cumini* L. Skeels), Ber (*Ziziphus jujube* Gaertn), Bahada (*Terminalia belerica* Gaertn Roxb.), Dha (*Anogeissus latifolia* Wall), and Sidha (*Lagerstroemia parviflora* Wall), are the most ample species. Silk production depends on the nutritionally enriched leave which directly impact the better growth and development, Silkworm larvae and thus the production of cocoon [3]. The production of cocoon depends on the quality of leave, nutrient is supplied to the silkworm larvae through leaf. So, it's important to have good quality of leave with high protein content [4]. The good quality of cocoon can be produced when only we will provide balanced nutrient to the plant, continuous application of chemical fertilizer has deleterious effect on the soil biological and chemical health [5]. Microorganism present in the soil can enhance the productivity, crop yield and health status. Interaction of soil microbial community with plants plays vital role in growth of plant [6]. Various activities like nitrogen mineralization and decomposition rates are positively associated with soil fertility [7-11]. Rhizospheric bacteria are alternative to chemical fertilizer that helps in promoting plant growth [12]. Rhizospheric soil is conquered by various bacterial communities [13], which protects plant from deleterious effects of heavy metals, flooding, salt and drought to which plant are exposed [14]. They also develop a mutual interaction with plant roots, improving the growth and development of plants through various mechanism [15-18] like biological nitrogen fixation, production of growth hormones, phosphate solubilization and bio-control agent [19-21]. The phosphate solubilizing bacteria solubilize bound phosphate by releasing organic acid (e.g., glycolic acid, lactic acid, formic acid, acetic acid and succinic

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acids etc.) by chelating the bounded cations of the phosphate thereby converting phosphorus into soluble forms [22]. Potassium solubilizing bacteria solubilizes the K which is present in the soil in the mineral form improving K transition [23]. Biological nitrogen fixation is done by archaea and bacteria, the organic nitrogen is available to plants in form of ammonium or nitrate [24]. The aim of the current study was to analyze the soil bacterial community present in the rhizospheric region of the Tasar growing soil and their biochemical characterization and identification through molecular characterization by 16S rRNA gene.

MATERIALS AND METHODS

Description of study area

The research was conducted at Microbiology and Plant Pathology laboratory of University Department of Botany, Ranchi University, Ranchi. Which is placed at latitude of 23.34ºN and longitude of 85.30ºE (https://www.latlong.net/).

Soil sample collection

The soil sample were obtained from the area of Central Tasar Research and Training Institute, Piska-Nagri, Ranchi, Jharkhand state. Sample were obtained from the depth of 5-17 cm from the rhizospheric area of different plant. All samples were collected in sterilize polythene bags and carried to laboratory aseptically and stored at 4°C for further process. The soil sample were air dried and grounded to pass through 2mm sieve before examination [25].

Isolation of bacteria and biochemical characterization

23 bacterial strains were isolated from soil sample of Tasar growing field. 1 gm of soil samples were serially diluted and spreaded on the plate in triplicate on NAM media containing Peptone 5.0g; Beef extract 3.0g; Agar 15.0g and Distilled water 1000ml; pH 6.8±0.2for inoculation 1 gm of soil sample were diluted with 9ml of sterilized distilled water and shaken for 15min. Then serial dilution was prepared as technique of [26-27]. Then 0.1ml soil dilution sample was taken as inoculums on the media and was spreaded on the media using a spreader. the plate were incubated at 30ºC for 24-48 hrs. Bacterial colonies were isolated based on morphology of the colony. The colonies were selected and streaked on the Agar slant. Repeated sub-cultures were made for pure culture and the colonies were designated as C_1, C_2, C_3, C_4, C_5 ……. C_{23}.

Biochemical characterization

Some of the biochemical characters were studied to identify the bacteria such as Microscopic study, Glucose, Lactose, Sucrose and Mannitol fermentation, Triple sugar test, Starch hydrolysis, Catalase activity, Methyl Red test, Citrate test, VP test, Litmus milk, Indole test, Urease, Gelatin hydrolysis and Nitrate Reduction etc. were determined [28]. Characterization was done as Bergey's manual (PVII).

Morphological character

For morphological characterization of the isolates, shape, size, elevation, surface form, margins and surface texture, pigmentation were observed [29].

Molecular characterization of the bacterial strain

4 Isolates were characterized using 16S rRNA gene. The identification report was generated using EzBiocloud Database and the identification was based on availability and homology shown by ~700 bp sequence [30].

RESULTS AND DISCUSSION

21 bacterial isolates were obtained from the soil sample collected from the Tasar growing region and 2 isolates were obtained from the biofertilizer bought from BAU, Ranchi. The identification was based on morphological, biochemical characteristics and 16S rRNA gene sequence.

Morphological characteristic

Colonies of the 23 isolates on the NAM media after 24 hours of growth were shown to have different pigmentation viz. white, milky white, orange, light yellow, pale yellow, off-white; convex, flat or raised elevation; smooth or rough surfaces; opaque or transparent in opacity and entire and undulating margin [31]. Gram staining results showed that all the colonies were rod shaped except C_8 which was found to be cocci. The results of morphological test are shown in (Fig 1, Table 1).

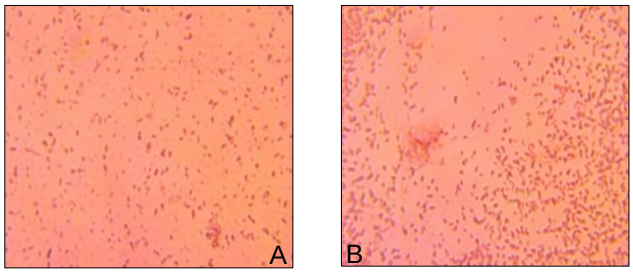

Fig 1 Showing the result of gram staining

Table 1 Showing the result of morphological test

Colony no.	Gram staining	Shape
C_1	+	Bacilli
C_2	-	Bacilli
C_3	+	Bacilli
C_4	+	Bacilli
C_5	-	Bacilli
C_6	+	Bacilli
C_7	-	Bacilli
C_8	+	Cocci
C_9	+	Bacilli
C_{10}	+	Bacilli
C_{11}	+	Bacilli
C_{12}	+	Bacilli
C_{13}	+	Bacilli
C_{14}	+	Bacilli
C_{15}	+	Bacilli
C_{16}	+	Bacilli
C_{17}	+	Bacilli
C_{18}	+	Bacilli
C_{19}	-	Bacilli
C_{20}	+	Bacilli
C_{21}	+	Bacilli
C_{22}	+	Bacilli
C_{23}	+	Bacilli

Biochemical characterization

The bacterial strains were characterized by sugar fermentation (Glucose, Lactose, Sucrose and Mannitol), Starch Hydrolysis, Citrate utilization, Nitrate Reduction, Catalase Test, Litmus Milk Test, Urease, Indole Test, Gelatin Hydrolysis, MR test, VP test, Triple Sugar test (Fig 2, Table 2). On the basis of biochemical test, it was found that C_1, C_3, C_4, C_6, C_9, C_{10}, C_{11}, C_{12}, C_{14}, C_{15}, C_{16}, C_{18} and C_{23} were found to be

Bacillus spp. and C_2, C_5, C_8, C_{19} and C_{20} were found to be *Pseudomonas sp*. C_{17} was *Azotobacter sp*. the identification was done according to Bergey's Manual of Systematic Bacteriology [32].

16S rRNA gene sequence analysis

From the 23 isolates 4 samples were send to National Centre for Microbial Resource, Pune. The samples were C_7, C_{13}, C_{21} and C_{22}. The sequence obtained were studied using FASTA format and identification report were generated using EzBiocloud Database (www.ezbiocloud.net). It was found that C_7 has 99.02% homology with *Planococcus halotolerans* SCU63(T) with Accession no MH266202 [33]. C_{13} showed 99.87% homology with *Bacillus tequilensis* KCTC13622(T) with Accession no AYTO01000043 [34]. C_{21} has 99.62% homology with *Bacillus megaterium* NBRC15308(T) with accession no JJMH01000057 [35]. C_{22} has100% homology with *Bacillus zhangzhouensis* DW5-4(T) with accession no JOTP01000061 [36].

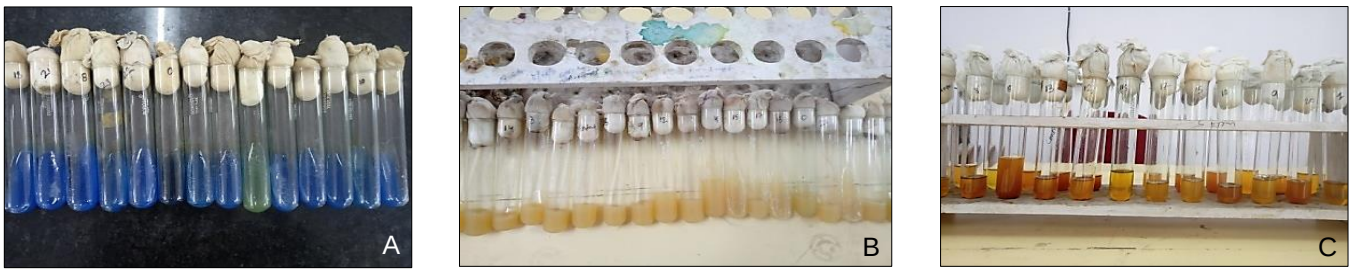

Fig 2 Showing (A) Citrate utilization test (B) Gelatin hydrolysis (C) Nitrate reduction

Table 2 Showing biochemical test of the isolates

S. No.	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}	C_{17}	C_{18}	C_{19}	C_{20}	C_{21}	C_{22}	C_{23}
Glucose	-	-	+	+	+	+	+	-	-	-	-	-	+	+	-	-	+	+	+	+	-	+	+
Lactose	+	-	+	+	-	-	-	-	-	+	+	-	+	-	+	-	+	+	-	+	-	+	+
Mannitol	-	-	+	-	-	-	-	-	+	+	+	-	+	+	+	-	-	-	-	-	+	-	+
Sucrose	-	+	+	+	+	-	+	+	-	-	-	-	-	-	-	+	+	+	+	-	+	+	-
Litmus milk	+	-	+	+	-	+	-	-	+	-	-	-	+	-	+	-	+	+	-	+	+	+	-
Catalase	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+
Citrate test	+	+	-	+	-	+	-	+	+	-	-	+	-	-	-	-	+	-	-	+	-	-	+
Indole test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Urease	-	-	-	+	-	+	-	+	+	-	-	+	+	-	+	-	+	+	-	-	-	+	-
Gelatin hydrolysis	+	+	+	+	-	-	-	-	-	+	+	-	+	+	+	-	+	-	+	-	+	-	-
MR test	+	+	-	+	+	+	+	-	+	+	+	-	-	+	-	-	-	-	-	-	-	+	-
VP test	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+
Nitrate reduction	+	-	+	+	-	+	+	-	+	+	-	+	+	+	-	+	+	+	-	+	+	-	+
Starch hydrolysis	+	+	+	+	+	+	-	+	+	-	+	+	+	+	-	-	+	-	+	+	+	+	+
Triple sugar test	+	-	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+

CONCLUSION

The 21 bacterial strains obtained from the Rhizosphere soil of Tasar growing Region were identified according to morphological, Biochemical and 16S rRNA gene analysis. Conferring to morphological and biochemical study the isolates were found to belong to the genus *Pseudomonas spp., Bacillus spp., Azotobacter spp.* and*Planococcus spp.*04 isolates were identified as *Bacillus Megaterium* NBRC15308(T), *Bacillus zhangzhouensis* DW5-4(T), *Bacillus tequilensis* KCTC13622(T) and *Planococcus halotolerans* SCU63(T).

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