"Isolation of bacteria associated with burrowing Wolf Spider, *Pardosapseudoannulata*"

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ABSTRACT:

The present study deals with the isolation and identification of the bacteria associated with the burrowing Wolf spider, *Pardosa pseudoannulata*. The spiders were collected and identified up to species level with the help of morphological characters. The spider specimens were inoculated into Nutrient broth media. The test tubes containing collected spiders in nutrient broth were incubated at 37°C for 24 hours. After incubation the samples were serially diluted and spread on nutrient agar plates and incubated at 37°C for overnight. The isolated colonies were selected and colony characteristics of bacterial isolates were recorded. The identification was carried out with the help of VITEK 2 automated microbiology system. The present investigation is the first report of isolation of bacterial species from burrowing wolf spider *Pardosa psuedoannulata*. All the four bacterial isolates recovered from spiders were Gram positive bacteria. The isolate SP-1, SP-2 and SP-4 were identified as *Leconostoc mesenteroides* dextrancium and isolate SP-3 was identified as *Staphylococcus sciuri*.It is observed that *Leconostoc mesenteroides* dextrancium has the greatest rate of bacterial colonization on spider in addition to *Staphylococcus sciuri*.

Keywords: Spiders, bacterial community, *Pardosa psuedoannulata*.

Introduction:

Spiders are carnivorous arthropods, found all over the world in almost every kind of habitat. Approximately 1,20,000 species are supposed to be present of which 46,731 species of spiders belonging to 4,058 genera and 113 families are so far reported worldwide (World Spider Catalog, 2017). Lycosidae or wolf spiders are a clearly delimited and well-defined spider family, possessing a unique eye pattern and a typical egg sac and spiderlings carrying behavior. Family Lycosidae consists of 123 genera and 2,404 species of spiders distributed throughout the world (World Spider Catalogue, 2017).In India, the family Lycosidae is represented by 133 species belonging to 19 genera (Keswani et al. 2012). *Pardosa* is the genus of burrowing wolf spiders established by C. L. Koch, (1847) consisting of 550 species distributed worldwide (world spider catalogue, 2017) and 44 species in India (Sebastian and Peter, 2009). *Pardosa psuedoannulata* (Phartale et al. 2014) and *Pardosa brevivulva* (Phartale et al. 2019) were reported from mango fields of Latur District. Many arthropods possess a number of symbiotic bacteria exerting a strong effect on host adaptation and, hence, host evolution (Engelstädter and Hurst, 2009: Serbus et al. 2008).Due to the recent advances in molecular methods, it becomes easy for a comprehensive quantification of microbial communities in a diverse set of species (Andreotti et al. 2011: Kautz et al. 2013). The majority of arthropod groups still remain largely unexplored. The same is applied for spiders, for which only little research on their microbial community has currently been performed. Although, there are few studies on

microbial assemblages associated with spiders, they are concentrated on the medical importance of spiders as vectors of potentially human pathogenic bacteria. As spiders are one of the important pest control agents in agroecosystems, if the bacterial communities present on spiders are identified, it will be useful to study their role in pest control. By considering this scenario, the present study is designed to study the bacteria associated with the burrowing Wolf spider, *Pardosa pseudoannulata*.

Materials and methods:

Collection of spiders:

Adult spiders of both sexes were collected from the mango fields of Bhada village Tq. Ausa, District- Latur. Collection was done early in the morning and spiders were identified up to genus level on the basis of keys described by Sebastian and Peter (2009).

Culture media and reagents used:

Nutrient broth (NB) and Nutrient agar (NA) media were used for isolation of bacteria (Yardley, 2004).

Enrichment and isolation of the bacteria:

The spider specimens were inoculated into Nutrient broth media. The test tubes containing collected spiders in nutrient broth were incubated at 37°C for 24 hours. After incubation the samples were serially diluted 10^{-1} to 10^{-6} and spread on NA agar plates and incubated at 37°C for overnight. The isolated colonies were selected and colony characteristics of bacterial isolates were recorded. The isolated and purified colonies were maintained on Nutrient agar slants.

Identification of bacterial isolates:

Identification of bacteria isolated from *Pardosa pseudoannulata* was carried out with the help of VITEK 2 automated microbiology system.

Suspension Preparation:

A sterile swab or applicator stick is used to transfer sufficient number of colonies of a pure culture and to suspend the selected microorganisms in 3.0 mL of sterile saline (aqueous 0.45% to 0.50% NaCl, pH 4.5 to 7.0) in a 12 x 75 mm clear plastic (polystyrene) test tube. The turbidity is adjusted accordingly (0.50-0.63) and measured using a turbidity meter called the DensiChekTM.

Inoculation:

Identification cards are inoculated with microorganism suspensions using an integrated vacuum apparatus. A test tube containing the microorganism suspension is placed into a special rack (cassette) and the identification card is placed in the neighboring slot while inserting the transfer tube into the corresponding suspension tube. The cassette can accommodate up to 10 tests or up to 15 tests. The filled cassette is placed either manually (VITEK 2 compact) or transported automatically (VITEK 2 and VITEK 2 XL) into a vacuum chamber station. After the vacuum is applied and air is re-introduced into the station, the organism suspension is forced through the transfer tube into micro-channels that fill all the test wells.

Card Sealing and Incubation:

Inoculated cards are passed by a mechanism, which cuts off the transfer tube and seals the card prior to loading into the carousel incubator. The carousel incubator can accommodate up to 30 or up to 60 cards.

All card types are incubated on-line at 35.5 + 1.0ºC. Each card is removed from the carousel incubator once every 15 minutes, transported to the optical system for reaction readings, and then returned to the incubator until the next read time. Data are collected at 15-minute intervals during the entire incubation period.

Optical System:

A transmittance optical system allows interpretation of test reactions using different wavelengths in the visible spectrum. During incubation, each test reaction is read every 15 minutes to measure either turbidity or colored products of substrate metabolism. In addition, a special algorithm is used to eliminate false readings due to small bubbles that may be present. Test Reactions Calculations are performed on raw data and compared to thresholds to determine reactions for each test. On the VITEK 2 Compact, test reaction results appear as "+"," –", "(–)" or "(+)".

Results and Discussion:

Collection & Identification of*Pardosa pseudoannulata* spiders:

In the present study, the adult spiders of both sexes were collected from the mango fields of Bhada village, Tq. Ausa, District Latur. Spiders were identified up to the species level by using some diagnostic features, on the basis of keys described by Sebastian and Peter, (2009). *Pardosa pseudoannulata* are most likely to be encountered in the field. The adults are usually found near the base of plants.

Fig-1: *Pardosa psuedoannulata*.

Description:

Total length of the spider is 18.58 mm; cephalothorax was longer than wide, convex, present fine hairs on cephalic region, measuring about 8.45 mm in length and 5.65 mm in width. The length of abdomen is 9.75 mm and 5.66 mm. Short fovea surrounded by light mid longitudinal band is present in the centre region of cephalothorax (Fig-1), lateral margins of cephalothorax are also with the deep brown longitudinal border. The length of the abdomen is 9.75 mm and width is 5.66 mm, slightly longer than wide. 4 to 5 transverse light bands present on the dorsal side of the abdomen. Sternum is oval in shape pointed behind, pale and covered by spine-like hairs. Legs are moderately strong yellowish brown long slender covered with spines and hairs. The leg formula is 4,1,2,3. 3rd pair is smallest of all leg pairs. 4th pair is longest of all and followed by 1st pair while 2nd pair (Table-1). Eyes are arranged in three rows (Fig-2), posterior median eye (PME) measuring about 0.87 mm in diameter and larger than posterior lateral eye (PLE) which was 0.71 mm in diameter. Anterior median eye (AME) is slightly larger than anterior lateral eye (ALE). AME is measuring about 0.56 mm in diameter while the diameter of ALE is 0.46 mm (Table-2).

Table-1: Measurements of legs of spider in mm

Leg	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	2.44	1.41	3.30	1.33	1.00	9.48
II	2.12	1.29	3.75	1.02	1.07	9.25
III	2.50	1.17	2.21	2.18	1.00	9.06
Iv	2.95	3.99	1.32	2.00	1.77	12.03

Table-2: Measurements of eyes of spider in mm

Mode	Radius	Diameter	Perimeter	Area
PLE	0.35	0.71	2.25	0.40
PME	0.43	0.87	2.73	0.59
ALE	0.23	0.46	1.45	0.16
AME	0.28	0.56	1.77	0.25

PME- posterior median eye**, PME**- posterior median eye, **ALE**- anterior lateral eye, **AME**-Anterior median eye

Fig-2: Eye arrangement of *Pardosa psuedoannulata*.

Isolation and identification of bacteria:

The primary objective of the study was to isolate and identify the microorganisms that can be found on the spider *Pardosa psuedoannulata*. Four bacterial isolates were recovered from *Pardosa psuedoannulata*. Gram staining was carried out as described by Baker (1967). Morphological characteristics of four bacterial isolates were recorded (Table-3). It was observed that the bacterial isolates recovered were Gram positive bacteria.

Table-3: Morphological Characteristics of Isolates

Isolate	Morphological characteristics
SP-1	Gram positive, spherical, coccoid cells in pairs and chains, short rods, non-motile
SP-2	Gram positive, spherical, short rod with rounded ends in long chains, non-motile

SP-3	Gram positive, non-motile, non-sporing, occurring singly and forming pairs and tetrads
SP-4	Gram positive, spherical, short rod, non-motile

Biochemical properties of isolates:

Identification of bacterial isolated was carried out with the help of VITEK 2 automated microbiology system. Isolate SP-1, SP-2 and SP-4 showed positive and negative results in various biochemical tests (Table-4). Based on the biochemical tests the isolate SP-1, SP-2 and SP-4 were identified as *Leconostoc mesenteroides* dextrancium and isolate SP-3 was identified as *Staphylococcus sciuri.*

Table-4: Biochemical characteristics of bacteria isolated from spider *Pardosa psuedoannulata*

Name of the Test	Isolates			
	SP-1	**SP-2**	**SP-3**	**SP-4**
+D- Amygdalin	-	-	+	-
Phosphatidylinositol Phospholipase C	-	-	-	-
D-Xylose	-	-	-	-
Arginine Dihydrolase 1	-	-	-	-
Beta-Galactosidase	-	-	-	-
Alpha-Glucosidase	-	-	+	-
Ala-Phe-Pro Arylamidase	-	-	-	-
Cyclodextrine	-	-	-	-
L-Aspartate Arylamidase	-	-	-	-
Beta Galatopyranosidase	-	-	-	-
Aplha Mannosidase	-	-	-	-
Phosphatase	-	-	+	-
Leucine Arylamidase	-	-	-	-
L-Proline Arylamidase	-	-	-	-
Beta Glucuronidase	-	-	-	-
Alpha-Galactosidase	-	-	-	-
L-PyrrolidonylArylamidase	-	-	-	-
Beta Glucuronidase	-	-	-	-
Alanine Arylamidase	-	-	-	-
Tyrosine Arylamidase	-	-	-	-
D-Sorbitol	-	-	+	-
Urease	-	-	-	-
Polymixin B Resistance	+	+	-	+
D-Galactose	-	-	+	-

D-Ribose	+	+	+	+
L-Lactate alkalinization	-	-	+	-
Lactose	-	-	-	-
N-Acetyl-D-Glucosamine	+	+	+	+
D-Maltose	+	+	+	+
Bacitracin Resistance	+	+	+	+
Novobiocin Resistance	-	-	+	-
Growth in 6.5 % Nacl	+	+	+	+
D- Mannitol	-	-	+	-
D-Mannose	+	+	+	+
Methyl-B-D-Glucopyranoside	-	-	+	-
Pullulan	-	-	-	-
D-Raffinose	-	-	-	-
O/129 Resistance (Comp. vibrio.)	+	+	+	+
Salicin	-	-	+	-
Saccharose/Sucrose	-	-	+	-
D-Trehalose	+	+	+	+
Arginine Dihydrolase 2	-	-	-	-
Optochin Resistance	+	+	+	+

Conclusion:

Four bacterial strains were isolated on *Pardosa psuedoannulata*. The isolate SP-1, SP-2 and SP-4 were identified as *Leconostoc mesenteroides* dextrancium and isolate SP-3 was identified as *Staphylococcus sciuri.* It was observed that the bacterial isolates recovered were Gram positive bacteria. *Leconostocmesenteroides*dextrancium has the greatest rate of bacterial colonization on spider in addition to *Staphylococcus sciuri*.

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