Isolation and Characterization of Naga king chilli rhizobacteria

Comment [1]: You need to go through the whole manuscript and correct the grammatical errors.

Abstract

The Northeast region of India is recognized for its chilli diversity with number of variants noted from this region. Amongst the many landraces of chilli that are cultivated in this region, the Naga King Chilli (*Capsicum chinense* Jacq.) is one of the best known worldwide. Rhizosphere is the soil around the root system of a plant considered to be rich in nutrients due to the accumulation of various plants exudates, providing a rich source of nutrients for soil microbes and is reflected on the number of microbes being higher in this region when compared with the bulk soil. The bacteria colonizing this region of the soil are called rhizobacteria and are believed to play a vital role in plant growth and development. An investigation was carried out in order to know the indigenous rhizobacterial population of Naga King Chilli rhizosphere collected from the farmers' field Nagaland, where the crop is popularly grown. 27 rhizobacteria were isolated on nutrient agar and king's B agar medium and characterized based on their colony characters and some biochemical test including gram reaction, KOH test, gelatine hydrolysis and starch hydrolysis test. The results suggested that the rhizosphere of Naga king chilli is dominated by gram negative bacteria with 21 isolates being gram negative. All the isolated shows positive catalyst and gelatine hydrolysis activity with 18 isolates being able to hydrolyse starch.

Comment [2]: Should be 27 soil samples containing bacteria were used to isolate the rhizobacteria

Comment [3]: The introductory section of the abstract is huge so it is good to reduce it. In relation to the size of the whole manuscript the abstract is bigger.

Keywords: Characterization, Naga King chilli, Rhizobacteria

Introduction

Comment [4]: In the introduction section, You need to provide some details regarding the role of rhizobacteria on chilli growth and production for better justification of the study. Besides this, it is good to mention and describe some commonly existing bacteria along with the description of their behavioral characteristics in relation to plant growth advancement.

The Northeast region of India is recognized as hot-spot for chilli diversity (Mathur *et al*., 2000) and amongst the many landraces of chilli that are cultivated in this region, the Naga King Chilli (*Capsicum chinense* Jacq.) is the best known worldwide. It is a self-pollinated plant; however, considerable cross pollination (upto 10%) may occur when insect population is high. It behaves as a semi perennial herb if grown under favourable condition (Borgohain and Devi, 2007). Chilli fruits constitute large amounts of beneficial compounds including antioxidants,

carbohydrates, minerals, phytochemicals, proteins, amino acids, and vitamins (Olatunji and Afolayan, 2018). The narrow zone of soil directly surrounding the root system is referred to as rhizosphere (Walker *etal*., 2003). This zone is rich in nutrients when compared with the bulk soil due to the accumulation of a variety of plant exudates, such as amino acids and sugars, providing a rich source of energy and nutrients for bacteria (Gray and Smith, 2005). The rhizosphere is populated by a diverse range of microorganisms and the bacteria colonizing this habitat are called rhizobacteria (Schroth and Hancock, 1982) which increases the absorption capacity of nutrients and protection against phytopathogens (Pérez-García *et al*., 2023). Knowledge of the native bacterial population, their characterization, and identification is required for understanding the distribution and diversity of indigenous bacteria in the rhizosphere of specific crops (Chahboune *et al*., 2011).

Comment [5]: Specify the correlation of different rhizobacteria with chilli plants.

Materials and method

Comment [6]: You need to indicate the number of isolates which can interchangeably called as your "treatments"

A field survey was undertaken for the collection of rhizobacteria from the farmer's field of Peren district, Nagaland. Where, rhizospheric bacteria were isolated from the rhizosphere of healthy plant at flowering stage.

Comment [7]: Describe fully what you did during surveying the field. Avoid vague information and you need to mention details of what ever was done during surveying.

Isolation and maintenance of bacteria

Soil dilution plate technique described by Waksman, 1927 was followed for the isolation of rhizobacteria and the suspension was serially diluted to 10^{-8}. 0.1 ml of the last 3 serial dilution were spread with a glass spreader on nutrient agar and King's B medium plates and incubated for 48h at 26±1°C. Colonies with different morphological appearances were selected from the countable plates and re-streaked on a new plate containing the same media to obtain pure colonies (Patel *et al.*, 2014).

Comment [8]: Mass of soil sample used should be indicated. Specify the nature of the diluting substance eg: Distilled water or deionized water was used for dilution.

Comment [9]: Give some details about Kings B medium

Comment [10]: If the same media is used then what is the need of mentioning NA medium?

Characterization of rhizobacteria

Morphology of bacterial colonies were characterized based on various traits such as size, shape, elevation, surface, optical properties, margin, and pigmentation on bacterial cultures previously grown for 48 h on NA medium and incubated at 28°C.

Comment [11]: Describe the abbreviation of NA and

A series of biochemical tests were conducted using the criteria of Bergey's Manual of Systemic Bacteriology (1994).

Catalase test

To detect the production of catalase a loop full of 24-48 hours old test bacterium was smeared on the slide and then covered it with a few drops of 3% hydrogen peroxide. The smear was observed for bubble production. (Koche and Gade, 2013)

KOH test

A loopful of bacteria was mixed in a drop 3% aqueous KOH solution for not more than 10 seconds. The inoculating loop was raised a few centimetres from the microslide and the formation of a mucoid thread was recorded. Gram positive bacterium do not produce strands even on repeated strokes of the inoculating loop while gram negative bacterium do.

Comment [12]: ??? determine the quantity

Comment [13]: Give exact value

Starch hydrolysis

Starch hydrolysis was evaluated using nutrient agar amended with 0.2% starch. After incubating the test bacterium on the medium for 7 days, the agar plates were then flooded with Lugol's iodine and allowed to act for few minutes. The cultures showing a clear zone was considered to be positive reaction.

Gelatine liquefaction test

Stab method described by Koche and Gade. (2013) was followed for gelatine liquefaction test (Peptone-10g; Beef extract-5g; Gelatin-20g and Distilled water - 1000ml). Inoculation was done by stabbing a straight inoculating needle charged with 48 hours old growth of the test bacterium. The tubes were incubated at 20^{o}C and observation were recorded for liquefaction of gel column.

Result and discussions

Morphological characters of the rhizobacterial isolates are presented in Table 1. The isolates were observed to be predominantly smooth, round, either orange or milky white in colour, smooth surface, convex with entire margin and translucent Result on biochemical characterization are shown in Table 2. It was Observed that out of the 27 isolated rhizobacteria eight were found to be Gram positive and 19 Gram negative, five isolates were recorded to utilize lactose whereas, only six isolates were found to

Comment [14]: Lactose was not mentioned in the methodology, so you need to replace it possibly by starch.

be negative for KOH test. All the isolates were recorded to liquify gelatine. However, eight isolates could not hydrolyse starch.

The findings of the present investigation were found to be in contrary to the findings of Banerjee *et al*. (2011) who reported that the bacterial population in chilli rhizosphere was dominated by gram positive bacteria with white, irregular, opaque colonies as the Naga king chilli rhizosphere was found to be dominated by Gram negative, orange coloured, opaque colonies. This difference in observations may be due to the fact that different ecological and environmental conditions favour the growth, development and establishment of different bacterial population and hence reflected in the population of the rhizobacteria. The results on catalase productions were found to be in conformity with the findings of Patel and Desai (2015) who observed that all rhizobacterial isolates were positive for catalyst production and hence, are aerobic in nature.

Table 1: Morphological characterization of rhizobacteria

Isolates	**Size**	**Shape**	**Colour**	**Surface**	**Elevation**	**Margin**	**Opacity**
T_1	Small	Round	White	Smooth	Flat	Erose	Translucent
T_2	Small	Round	White	Smooth	Convex	Entire	Translucent
T_3	Small	Round	Creamy white	Smooth	Convex	Entire	Opaque
T_4	Small	Irregular	Light yellow	Smooth	Raised	Undulated	Translucent
T_5	Small	Round	Orange	Smooth	Convex	Entire	Opaque
T_6	Small	Round	Light orange	Smooth	Convex	Entire	Opaque
T_7	Small	Irregular	Orange	Corrugated	Raised	Undulated	Opaque
T_8	Small	Round	Reddish	Smooth	Convex	Entire	Opaque
T_9	Small	Round	Yellow	Smooth	Pulvinate	Entire	Opaque
T_{10}	Smal	Round	Yellow	Smooth	Convex	Entire	Translu

	l						cent
T_{11}	Small	Round	Pale yellow	Smooth	Convex	Entire	Opaque
T_{12}	Small	Round	Greenish yellow	Smooth	Convex	Entire	Opaque
T_{13}	Medium	Round	White	Smooth	Convex	Entire	Opaque
T_{14}	Small	round	Yellow	Smooth	Raised	Entire	Opaque
T_{15}	Small	Round	Milky white	Smooth	Convex	Entire	Translucent
T_{16}	Medium	Round	Orange	Smooth	Convex	Entire	Opaque
T_{17}	Medium	Round	Dull white	Smooth	Convex	Entire	Translucent
T_{18}	Small	Round	Orange	Corrugated	Raised	Undulated	Opaque
T_{19}	Small	Round	Light orange	Smooth	Flat	Entire	Translucent
T_{20}	Small	Round	Pale white	Smooth	Convex	Entire	Translucent
T_{21}	Small	Round	Orange	Smooth	Convex	Entire	Opaque
T_{22}	Small	Round	Yellow	Smooth	Flat	Entire	Opaque
T_{23}	Small	Round	Orange	Smooth	Convex	Entire	Opaque
T_{24}	Small	round	Pale white	Smooth	Flat	Entire	Translucent
T_{25}	Small	Round	Creamy white	Smooth	Convex	Entire	Opaque
T_{26}	Medium	Round	Dull white	Smooth	Convex	Entire	Opaque
T_{27}	Small	Round	Pale yellow	Smooth	Convex	Entire	Opaque

Table 2. Biochemical characterization of the rhizobacteria

Isolates	Gram staining	Starch Hydrolysis	KOH test	Gelatine liquification	Catalyst test
T_1	-	-	+	+	+
T_2	-	-	+	+	+

T_3	-	+	+	+	+
T_4	-	+	+	+	+
T_5	+	-	-	+	+
T_6	-	+	+	+	+
T_7	-	+	+	+	+
T_8	-	+	+	+	+
T_9	-	+	+	+	+
T_{10}	-	+	+	+	+
T_{11}	-	-	+	+	+
T_{12}	-	-	+	+	+
T_{13}	-	+	+	+	+
T_{14}	-	+	+	+	+
T_{15}	+	+	-	+	+
T_{16}	-	-	+	+	+
T_{17}	+	+	-	+	+
T_{18}	-	+	+	+	+
T_{19}	-	-	+	+	+
T_{20}	+	+	-	+	+
T_{21}	-	-	+	+	+
T_{22}	+	+	-	+	+
T_{23}	-	+	+	+	+
T_{24}	-	+	+	+	+
T_{25}	+	+	-	+	+
T_{26}	-	+	+	+	+
T_{27}	-	+	+	+	+

Conclusion

Comment [15]: You need to write the a brief conclusion of your results.

References

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Comment [16]: You need to have consistency while writing the list of references by following the recommended citation styles like APA, Chicago .....etc as per what is mentioned in the author guidelines of the journal.

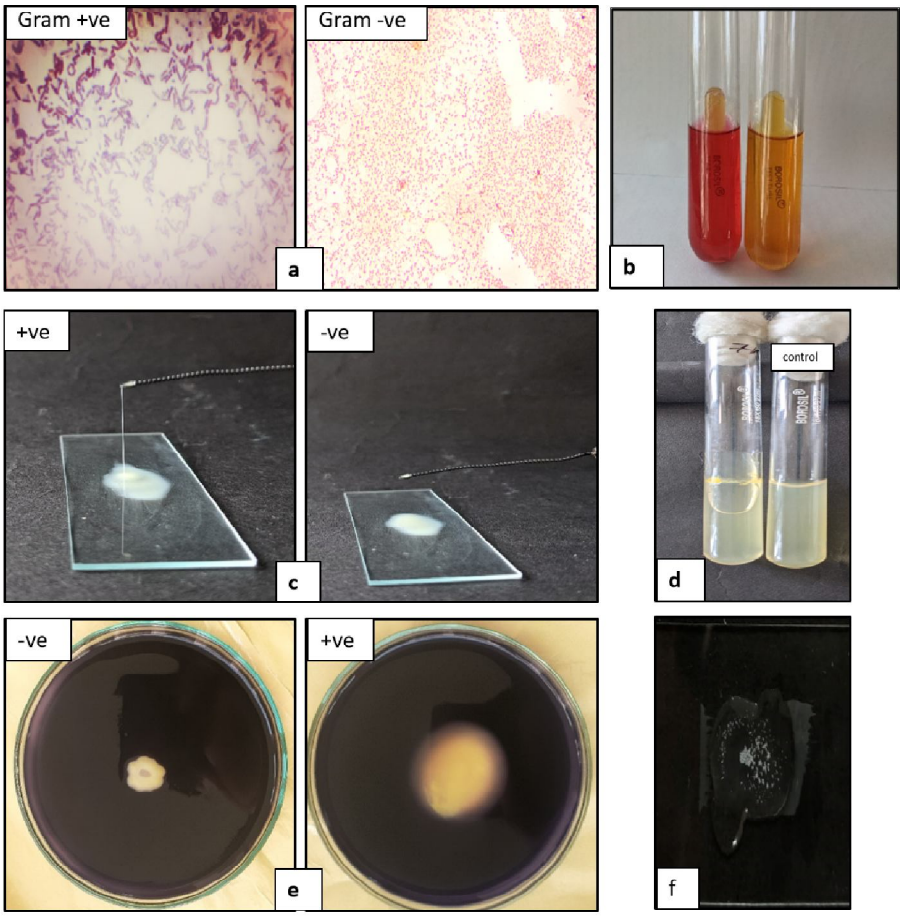

Plate 1. Biochemical test

a. Gram reaction
b. Lactose utilization test
c. KOH test
d. Gelatine liquification test
e. Starch Hydrolysis test
f. Catalase test