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ISOLATION AND IDENTIFICATION OF *CHROMOBACTERIUM VIOLACEUM* FROM DIFFERENT DISTRICTS OF MEGHALAYA, INDIA

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ABSTRACT

Objectives: The study was aimed at isolating and identifying violacein-producing bacteria from the different districts of the Indian state of Meghalaya. **Methods:** The violet colour pigment (violacein) producing bacteria was isolated and identified through different microbiological techniques. **Results:** A total of 41 bacterial *Chromobacterium violaceum* were isolated from different water sources across different districts in Meghalaya. **Conclusion:** The result of this investigation points to the fact that C. violaceum is likely to occur in significant concentrations in particular groundwaters and springwaters, which is of potential health relevance. Further bacteriological studies are crucial to clarify its environmental behaviour, distribution and the potential public health implications.

KEYWORDS: *Chromobacterium violaceum*, Violacein, antibiotic.

INTRODUCTION

Chromobacterium violaceum is a facultative anaerobic gram-negative bacillus possessing a single polar flagellum and, usually, one or multiple lateral flagella. It grows well on Nutrient media, including MacConkey agar, at 35–37°C for 24-48 hours. It is positive for oxidase and catalase reactions.[1,2] It is mainly a saprophytic bacterium in soil and water in the tropical and subtropical regions.[3]

Violacein possesses important biological activities and pharmacological properties. Violacein has strong antibacterial properties making it a promising candidate as a future antibiotic. When administered in combination with other antibiotics, the impact is more effective against the bacteria than the use of antibiotics.[4]

The pigment violacein shows a superior anti-microbial activity against a majority of deadly pathogenic bacteria such as *Staphylococcus, Streptococcus, Bacillus, Mycobacterium and Pseudomonas.* Besides, violacein has a strong bactericidal[5], antitumor[6], antiviral[7], antioxidant[8], and antiparasitic activities.[9-12] Violacein is also used as a biological dye, the pigment is also produced by other bacteria, such as the psychotropic bacterium RT102.[13] *Janthinobacterium lividum* (formerly known as *Chromobacterium lividum*)[14-17] and *Alteromonas luteoviolacea*.[18]

Meghalaya, the 21st State of India, emerged as a full-fledged State on the 21st January 1972. It is located between the latitudes 25°47'N and 26°10'N and longitudes 89°45'E and 92°i7'E. Meghalaya is bounded by Nowgonq, Kamrup and Goalpara districts of Assam on the north, Mikir and North Cachar Hill districts of Assam on the east, and Bangladesh on the south and west. Being a hilly region with a vast flora and fauna species biodiversity, novel microorganisms are still yet to be unearthed.

There are numerous sources of water for drinking and domestic purposes. These include rainwater, surface water (springs, streams, rivers, lakes etc,) and underground water (shallow wells and deep wells and springs). Surface water gets easily polluted, through direct contamination by man and animals, or indirectly when rain washes faeces and other pollutants from the banks into the water body. Seepage of surface water can easily pollute shallow well.[19] To safeguard public health and to ensure that water is safe for public use, any form of water intended for drinking treated or untreated, piped or un-piped must meet certain microbiological standards.[20,21] Therefore the benefits of this study outlay the limitations, hence, this study aimed to isolate *Chromobacterium violaceum* from all districts of Meghalaya and study its prevalence.

MATERIALS AND METHODS

Sample collection

81 water samples were collected from 11 districts of Meghalaya from February 2019 up to May 2019.

Isolation of bacteria

The bacteria were originally isolated by plating the dilutions of water samples in the nutrient agar and incubated at 37°C for 24-48 hrs. Individual colonies of bacteria with the violet colour colonies were picked up, and the pure culture was sought by streaking in the nutrient agar. The bacterial isolates were stored in the nutrient agar at 4°C and subcultured once every 1 week.

Identification of bacterium

The bacteria were cultured on MacConkey's agar, Nutrient agar, blood agar and other selective media followed by the identification of the isolates based on their cultural characteristics, gram staining, reactions in standard biochemical tests and confirmatory was done by Vitex MS.

Antimicrobial agents

All the isolates were tested for antimicrobial susceptibility by the Kirby-Bauer disk diffusion technique on Muller Hinton Agar by Filter Paper disks impregnated with antibiotics (HiMedia^{TM}). A pre-diffusion time of 30 min was allowed at room temperature and the plates were incubated at 37°C for 24 h. The diameter of the zone of inhibition was measured and the results were interpreted as sensitive, or resistant, according to Clinical Laboratory Standard Institute 2014 guidelines.

RESULTS

In the present study, a total of 81 water samples were collected from various districts of Meghalaya of which 41 (50.62%) were positive for *Chromobacterium spp*. The isolates were numbered as S1-S81. The colonies were round convex, smooth, and violet. The intensity of the purple colour is increasing on prolonged incubation from light to dark purple colour.

Table 1: Number of positive *C. violaceum* across all districts in Meghalaya.

District	Capital	Presence of *C. violaceum*
East Khasi Hills	Shillong	0
West Khasi Hills	Nongstoin	5
South West Khasi Hills	Mawkyrwat	4
West Jaintia Hills	Jowai	0
East Jaintia Hills	Khliehriat	11
Ri-Bhoi	Nongpoh	0
West Garo Hills	Tura	0
East Garo Hills	Williamnagar	7
South Garo Hills	Baghmara	7
South West Garo Hills	Ampati	0
North Garo Hills	Resubelpara	7

Table 2: Percentage of isolated *Chromobacterium from various districts of Meghalaya*.

Number	Positive	Negative
	41	40
%	50.62	49.38

The biochemical characterization of bacterial isolates has been done to confirm the genus. Based on the morphological and partial biochemical characteristics, isolates found to be a motile, non-spore forming, Gram-negative, catalase and oxidase-positive, rod-shaped bacterium with the violet colonies in Nutrient agar, MacConkey agar, blood agar plates.

Vitex MS

Confirmation test was performed by Vitex MS on all 41 positive samples suspected to be Chromobacterium spp. Vitex MS confirmed all 41 samples to be *Chromobacterium violaceum.*

Antibiotic susceptibility test

The overall C. *violaceum* recorded the highest susceptibility to Ciprofloxacin 35 (85.37%), followed by Sparfloxicin and Levofloxacin each with 30 (73.17%). The resistance was seen for Ampicillin and Cefixime each with 40 (97.56%).

Table 3: Susceptibility pattern of the *C. violaceum* isolated.

Antibiotic	Susceptible	Intermediate	Resistant	Antibiotic conc. tested
Sparfloxicin (SPX)	30	0	11	5mcg/mL
Cefixime (CFM)	0	1	40	5mcg/mL
Amoxicillin with Clavulanic acid (AMC)	0	2	39	30mcg/mL
Ampicillin (AMP)	0	1	40	10mcg/mL
Cefuroxime (CXM)	0	3	38	30mcg/mL
Ciprofloxacin (CIP)	35	1	5	5mcg/mL
Amikacin (AK)	10	1	30	30mcg/mL
Gentamicin (GEN)	11	0	30	10mcg/mL
Levofloxacin (LE)	30	1	10	5mcg/mL
Ceftriaxone (CTR)	8	0	33	30mcg/mL

DISCUSSION

In the remote regions of North-east states of India, *Chromobacterium violaceum* has been understudied due to its former non-pathogenic nature and limited resources to drive research into it, however, new research in other parts of the world signifies that this particular bacteria has immense beneficial potentials in areas of medicine, food and textile industries due to its pigment "violacein". Synthetic Pigments are extensively used in food, cloth, painting, cosmetics and pharmaceuticals. These cause environmental as well as health hazards. Hence, violacein acts as an alternative natural pigment with the biodegradable and ecofriendly properties. Violacein, a violet pigment has attracted much attention in recent years due to its antibiotic, antitumoral, and anti-Trypanosoma activities. Out of the 81 water samples, 41 (50.62%) were positive of *C. violacein.* The present study had revealed the high prevalence rate of *C. violaceum* in water bodies such as spring water, groundwater, surface water in South West Khasi Hills and East Jaintia hills as compared to others districts which could be suggestive of the disturbed environment due to coal mining and urbanization.

Rare sepsis caused by *C. violaceum* as reported by Ray P et al. (2004) is indicative of the emerging pathogenic nature of *C. violaceum.* The isolates were then checked for antibiotic susceptibility which showed that the highest susceptibility was to Ciprofloxacin 35 (85.37%), followed by Sparfloxicin and Levofloxacin each with 30 (73.17%). The resistance was seen for Ampicillin and Cefixime each with 40 (97.56%). Due to some reported cases of *C. violaceum* infections around the world, more research needs to be focused on pathogenicity and virulence factors.

CONCLUSION

Chromobacterium violaceum has a balanced duality. The pigment produced by these bacteria has enormous potential in the field of pharmaceutical sciences, industries as well as environmental benefits such as bioremediation.Violacein has shown to have anti-cancer as well as anti-microbial properties which could be extensively extracted and purified for medicinal purposes. However, reports have shown that this same bacterium causes lethal infections such as septicemia and other superficial infections of the skin. In the present work, an attempt was made to isolate violacein producing bacteria from water samples from the entire 11 districts of the Indian state of Meghalaya. The result of this investigation points to the fact that C. violaceum is likely to occur in significant concentrations in particular groundwaters and springwaters, which is of potential health relevance. Further bacteriological studies are crucial to clarify its environmental behaviour, distribution and the potential public health implications.

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