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Evaluating the Co-Existence of Resistance to Antibiotics and Heavy Metal Resistance by Poyhydroxy Butyrate Producers from Various Samples

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Abstract
Bacteria were isolated from various samples as agricultural soil, red soil and sewage water. The isolates were screened for Polyhydroxy butyrate production by sudan black dye staining method. The isolates were further confirmed by staining. The resistance and susceptibility pattern of the isolates to different antibiotics and heavy metals was analyzed. The zones obtained revealed that the isolates were susceptible to a few antibiotics, but were found to be resistant to Penicillin G, Ampicillin, Vancomycin and Cefixime, whereas the isolates were highly resistant to zinc sulphate and copper sulphate and moderately resistant to silver nitrate, thus revealing the co-existance of resistance to antibiotics and heavy metals.

Keywords
Polyhydroxy butyrate, antibiotics, sudan black dye

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INTRODUCTION
Explosion of population has led to increase in demand for production of many biobased products including biopolymers to be used in agriculture, health, and medicine. Several years have been spent on extensive research to develop biodegradable polymers as a promising substitute for petrochemical-based polymers due to their eco-friendly nature [1]. Among most commonly known bio-products is the bioplastic. PHB production is increased by excess of carbon source and limiting the nutrients such as nitrogen, phosphorus, sulfur, magnesium, iron, oxygen, and potassium. It is an intracellular polymer accumulated under stress conditions but with excess carbon source. When carbon source nutrients are available in excess relative to other nutrients in the growth environment, certain strains of bacteria produce and accumulate different types of polyester recognized as bioplastics, which have unique characteristics like biodegradability and biocompatibility [2-6]. Among widely known bioplastics, polyhydroxybutyrate (PHB) is the most prevailing polyester owing to its great resemblance to synthetic petroleum–derived synthetic plastic polymers [7,8].

Interestingly, production of biopolymer during pilot studies or in industrial scale is greatly contaminated by environmental heavy metal especially in fermenters. De Lima et al. [9] mentioned that PHA biopolymers production in industrial scale is strongly affected by heavy contamination from environment in the fermenter, also production may be hindered by addition of antibiotics. Hence, it is highly crucial to exploit the potent biopolymer producing strains

regarding antibiotics and heavy metal resistance for controlling contamination before industrial production of the polymer [9-12].

MATERIALS AND METHODS

Sampling, Isolation and Screening for PHB Production

The bacteria used in this study were collected from agricultural soil, red soil and sewage water for screening of best PHB producing bacteria. Various samples which were collected were serially diluted and the 10-5 dilution was plated on nutrient media, with Peptone- 2gm, Yeast extract-2gm, NaCl-1gm, Agar-4gm, distilled water- 1lt, the media was then autoclaved at 121 ^{o}C for 20mins at 15 lbs pressure to avoid contamination. These plates containing soil sample were incubated overnight. Among the three samples used, sewage water gave more number of isolated single colonies. All obtained colonies were again grown in nutrient broth and then again incubated overnight and then preserved for rest of the experiment.

Screening for PHB producing bacteria

All the bacterial isolates were qualitatively tested for PHB production following the viable colony method of screening using Sudan Black Dye [13]. For this screening of PHB producers, nutrient agar media supplied with 1% of glucose was autoclaved at 121 ^{o}C for 20 min at 15lbs pressure. This media was poured into sterile Petri plates and allowed for solidification. The plates were divided into 5 equal parts and bacterial isolates were spotted. These plates were incubated for 24 hours. Ethanolic solution of 0.02% Sudan Black B was spread over the Petri plates containing colonies and was kept undisturbed for 30 min. They were washed with 96% ethanol to remove excess stain from colonies.

Antibiotics and Heavy Metals Profiling

Potent PHB-producing strains were screened for antibiotic sensitivity through sensitivity test using Mueller Hinton agar medium as well as Agar Sensitivity Test medium (BioWorld, USA) [14]. Ten different antibiotics used in this study namely, Penicillin, Ampicillin, Gentamycin, Kannamycin, Vancomycin, Tetracycline, Bacitracin, Novobiocin, Cefitaxidime and Cefixime. For heavy metal resistance, profiling different concentrations of silver, copper and zinc were incorporated in the growth medium of bacteria.

MTC of heavy metals

To determine the Maximum Tolerable Concentration (MTC) of the metal, several dilutions of the metal salt was prepared based on preliminary screening (10µg, 20 µg, 30 µg, 40 µg, 50 µg, 75 µg, 100 µg). To each set a bacterial culture was inoculated and plates were incubated and observed for growth by streaks on Nutrient agar plates. Strains that could not grow on 1.0 µg were termed as sensitive to the metal, while that which grew in 50 µg were further tested for higher concentration.

RESULTS AND DISCUSSION

Among the bacterial isolates from various samples screened for PHB production, three isolates showed promising results. The sensitivity and resistance pattern of the three isolates are shown in Table 1 and Fig.1. Isolates 2&3 were found to be resistant to six among the twelve chosen antibiotics. Isolates 2&3 are found to be multidrug resistant, since they are resistant to Penicillin, Ampicillin, Vancomycin, Bacitracin, Novobiocin and Cefixime, whereas the isolate 1 was found to be resistant only to Penicillin, Vancomycin and Cefixime. The demonstration of antibiotic resistance by PHB producers is of significance. All the three isolates were found to exhibit resistance to zinc and copper even at a high concentration of 100µg/ml, whereas they were found to resist silver upto a concentration of 50µg/ml.

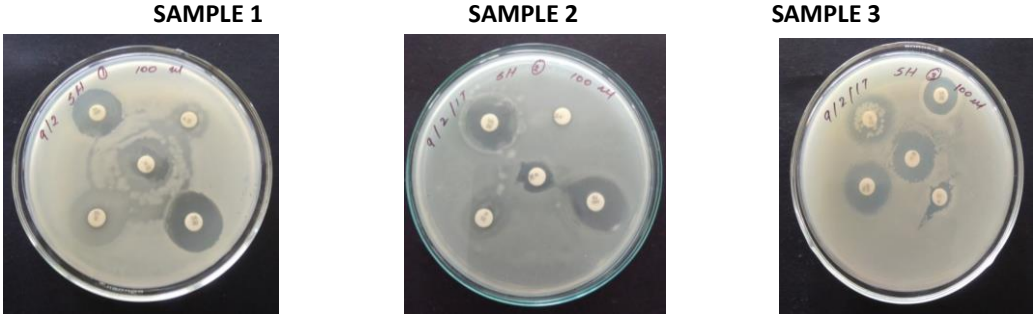

Fig.1: Isolates showing zones of inhibition against antibiotics

Table 1: Antibiogram pattern of the three isolates

S.No.	Antibiotics	Zones of Inhibition (dia in mm)		
		Isolate 1	Isolate 2	Isolate 3
1	Penicillin.G	-	-	-
2	Ampicillin	11	-	-
3	Kanamycin	17	13	11
4	Gentamycin	17	13	13
5	Vancomycin	-	-	-
6	Tetracycline	19	15	21
7	Bacitracin	9	-	-
8	Novobiocin	15	-	-
9	Ceftaxidime (CAC)	13	15	15
10	Cefixime (CFM)	-	-	-

Table 2: MTC of heavy metals

Metal concentration	Isolate 1			Isolate 2			Isolate3		
	Zinc	Copper	Silver	Zinc	Copper	Silver	Zinc	Copper	Silver
10µg/ml	+	+	+	+	+	+	+	+	+
20µg/ml	+	+	+	+	+	+	+	+	+
30µg/ml	+	+	+	+	+	+	+	+	+
40µg/ml	+	+	+	+	+	+	+	+	+
50µg/ml	+	+	+	+	+	-	+	+	-
75µg/ml	+	+	-	+	+	-	+	+	-
100µg/ml	+	+	-	+	+	-	+	+	-

+ indicates growth **- indicates absence of growth**

CONCLUSION

Thus, the three isolates obtained were found to harbour resistance to antibiotics and heavy metals and this requires more investigation.

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