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Isolation, Screening and Optimization of Penicillin Degrading Bacterial Strains from Poultry Manure, Municipal and Industrial Waste

Rachana V. Nair[1-2], R. Manikandan[3], Maghimaa Mathanmohun[4] and S. Selvakumar*[5]

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A B S T R A C T

Penicillin is regularly used in food animal production for the prevention of diseases in husbandry, which is a major source of penicillin contamination in the environment. The current study aimed to isolate, screen, and optimize penicillin degrading bacteria from poultry manure, municipal waste, and industrial wastes. Four bacterial strains ARB1, RPMB1, KPRB1, and TMRB2 were screened. Among the four isolated bacterial strains, ARB1 and RPMB1 bacterial strains have the highest sensitivity to penicillin. In addition, ARB1and RPMB1 bacterial strains had a great performance in penicillin degradation when the penicillin concentrations were 50 and 100mg/L. These results indicated that the isolated ARB1 and RPMB1 bacterial strains are effective in degrading penicillin and can be used for bioremediation of antibiotic contaminated soil. In the future research is required to confirm the performance of isolated ARB1 and RPMB1 bacterial strains in field conditions.

***Key words*:** Antibiotics, Bioremediation, Penicillin, Sensitivity, Bacteria

Antibiotics are extensively used as growth promoters and treat diseases in livestock and aquaculture [1]. Recently, antibiotic residues are increased rapidly in the environment due to the applications of antibiotics in food animal production and wastewater treatment plants. This concern is enhancing the toxicity and multidrug resistance microbial communities in the environment [2]. Among the antibiotics, penicillin is used to treat various bacterial infections and penicillin is very active than others. The large concentrations of penicillin have been present in animal residues, wastewater, and soil, which might pose severe threats to human health and promote the penicillin-resistant bacteria strains in the environment [3]. Therefore, it is an urgent need to identify a good way to degrade the penicillin residues in the environment. Numbers of physical and chemical methods such as activated carbon adsorption, different membrane filtration, and chemical hydrolysis have been explored to degrade the penicillin residues in the environment. These methods are cost-effective and produce secondary toxic products [3-4]. Biological degradation is an alternative method to remove and degrade the antibiotics in the environment [445]. For example, Opris *et al*. [1] reported that the isolated two strains are potentially degrading penicillin-contaminated soils. Similarly, Zhao *et al*. [6] investigated that the effectively penicillin degrading bacterial strains isolated from pig manure. Hence, the present study was aimed to isolate and characterize penicillin degrading bacterial strains from poultry manure, municipal waste, and industrial wastes.

* **S. Selvakumar**
✉ selvaphdpaper@gmail.com

[1,5] Department of Microbiology, The Kavery Arts and Science College for Women, Salem, Tamil Nadu, India

[2] Department of Microbiology, Periyar University, Salem, Tamil Nadu, India

[3] Department of Biotechnology, Padmavani Arts and Science College for Women, Salem, Tamil Nadu, India

[4] Department of Microbiology, Muthayammal College of Arts and Science, Rasipuram, Namakkal, Tamil Nadu

MATERIALS AND METHODS

Sample collection, isolation, and enrichment of penicillin degrading bacterial strains

Isolation and screening of penicillin degrading bacterial strains by Liu *et al*. [7] and Feng *et al*. [8] modified methods. Poultry waste (Manure), Municipal waste, and industrial waste samples were collected from various places of Salem district, Tamil Nadu, India. Collected samples were placed in sealed sterile bags and kept at a temperature above -20°C before the experiment. The collected sample was serially diluted and inoculated in 100 ml of Nutrient broth with different concentrations of penicillin (5 to 500 µl) and incubated at 37°C for 24 h. After 24 h of incubation cultured by spread plate method and incubated at 37°C for 24 h. After 24 h incubation again the growth was made by quadrant streak method using

Nutrient agar media plate and incubated at 37°C for 24 h. After 24 hours of incubation again made streak for pure culture.

Primary screening of penicillin degrading bacterial strains

In the present study, the agar well diffusion technique was performed for primary screening of penicillin degrading bacterial strains. The isolated bacterial strains were spread onto the nutrient agar medium and made four wells on the agar surface and added different concentrations (5, 10, 15, and 20mg/L) of penicillin on each well finally incubated at 30°C for 48hr. After the incubation, the size of the inhibition zones has reflected the sensitivity of test bacteria to penicillin (smaller size circle zone is more reactive bacteria in penicillin degradation).

Optimization of penicillin degrading bacterial strains

Based on the primary screening study, ARB1 and RPMB1 have the maximum penicillin degradation ability. Therefore, ARB1 and RPMB1 were used for the optimization of penicillin with different pH (3, 5, 7, 9, and 11), and penicillin concentrations (0, 50, 100, 200, and 300 mg/L) was optimized in 100ml of nutrient medium and incubated at 30°C and 150rpm/min for 10 days. After incubation the penicillin degrading was evaluated by using a UV- spectrophotometer at 360nm [9-10].

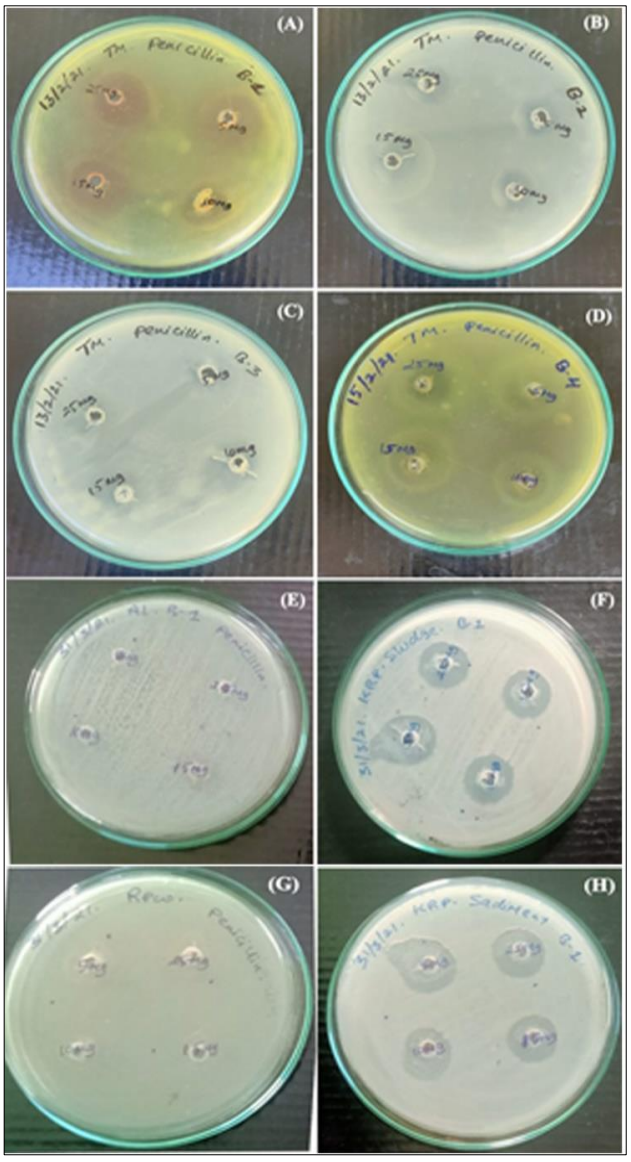

Fig 1 Sensitive test of isolated bacterial strains, A-TMRB1, B-TMRB2, C-TMRB3, D-TMRB4, E-ARB1, F-KPRB2, G-RPMB1 and H-RPMB2

RESULTS AND DISCUSSION

Isolation and primary screening of penicillin degrading bacteria

A total of four penicillin-degrading bacterial strains (ARB1, RPMB1, KPRB1, and TMRB2) were isolated from the Poultry waste (Manure), Municipal waste, and industrial waste samples. All the isolates were first subjected to primary screening which was carried out through antibiotic sensitivity tests against penicillin (Fig 1-2, Table 1). The inhibitory zone diameter of KPRB1 and TMRB2 was significantly larger than that of the other two strains. Therefore, KPRB1 and TMRB2 were considered to be not effective in degrading penicillin. Whereas ARB1 and RPMB1 do not produce an inhibitory zone, these results indicated that ARB1 and RPMB1 are good abilities to degrade penicillin. Similarly, Yang *et al*. [11] isolated and screened the penicillin V potassium degrading bacteria strains from polluted soil of a pig farm.

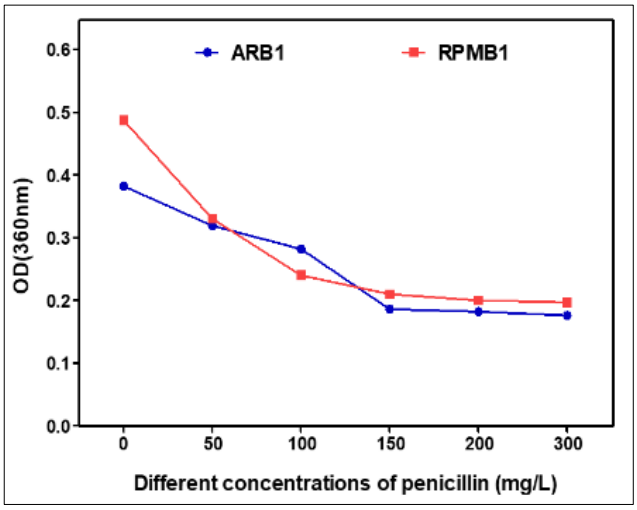

Fig 2 The evaluation of isolated ARB1 and RPMB1 bacterial strains on the degradation of penicillin

Optimization of penicillin degrading bacterial strains

Data presented in (Fig 2) revealed that the optimization of penicillin degrading ARB1 and RPMB1 strains on the degradation of penicillin under the different concentrations of penicillin contained media at the end of fermentation. It was found that isolated ARB1 and RPMB1 bacterial strains grow well and active growth at 50 and 100 mg/L of penicillin. The penicillin degradation efficiency was dependent on total microbial activity and growth of ARB1 and RPMB1 bacterial strains. The present study results are in agreement with Larcher and Yargeau [12] report that the mixed bacterial strains on sulfamethoxazole degradation.

The pH value of the bacterial growth medium plays an important role in bacterial growth and the production of secondary metabolites. In the present study, the ARB1 bacterial strain is survived and sustains high degradation ability at 5 and 9 pH (Fig 3). Similarly, RPMB1 bacterial strain also exhibited a high degradation efficiency at 5 and 9 pH (Fig 4). Similar results were obtained by Liu *et al*. [13].

CONCLUSION

In the present study, two penicillin degrading ARB1 and RPMB1 bacterial strains were isolated from the poultry waste and industrial waste samples. ARB1 and RPMB1 do not produce inhibitory zone on different concentrations of

penicillin. The isolated bacterial strains may have the potential for bioremediation of penicillin-contaminated environments, such as soil and wastewater, but further research is needed to confirm the performance of these strains in field conditions.

Table 1 Penicillin sensitivity test of isolated bacterial strains

Conc. of penicillin (mg/L)	Zone of inhibition(mm)							
	TMRB1	TMRB2	TMRB3	TMRB4	ARB1	KPRB1	RPMB1	KPRB2
5	4	0	0	0	0	10	0	10
10	9	2	0	0	0	8	0	7.5
15	11	0	0	0	0	10	0	10
25	10	4	6	0	0	10	0	7.5

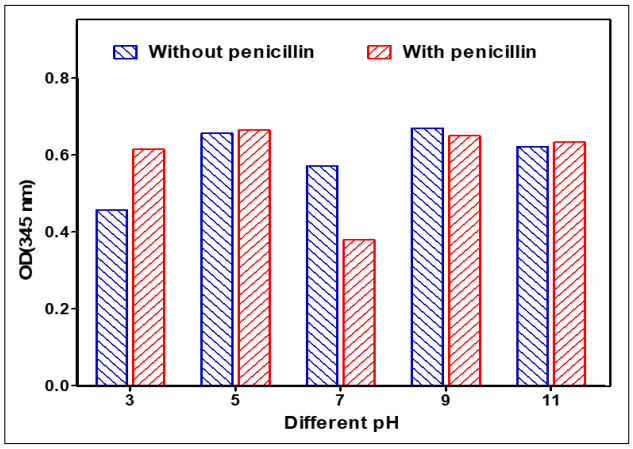

Fig 3 The effect of different pH conditions on penicillin degradation by ARB1 isolated bacterial strains at the end fermentation

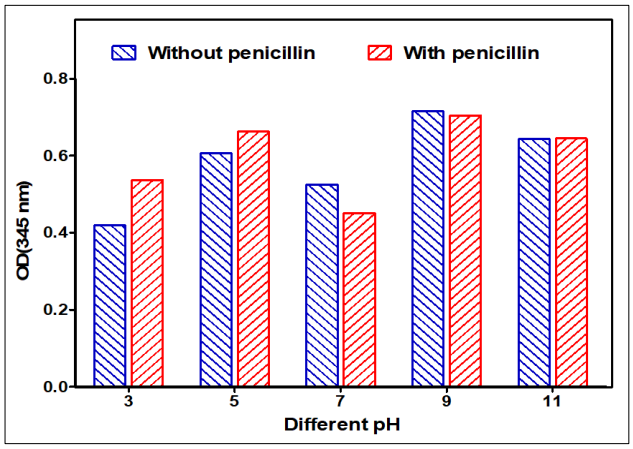

Fig 4 The effect of different pH conditions on penicillin degradation by RPMB1 isolated bacterial strains at the end fermentation

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