ISOLATION AND CHARACTERISATION OF CIPROFLOXACIN-DEGRADING BACTERIA FROM PHARMACEUTICAL EFFLUENT

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OUTLINES

- Introduction
- Statement of Research Problem
- Significance of the Study
- Aim of the Study
- Objectives of the Study
- Methodology
- Results and Discussion
- Conclusion
- Selected References

INTRODUCTION

- Antibiotics in general have been described by several authors to have potential risks in the environment.

- Studies on antibiotics have shown that up to 95% of antibiotic compounds can be released unaltered into the sewage system.

- High concentrations of antibiotics discharged in the environment can lead to alterations in microbial community structure and ultimately impact food chains. They have been described as ‘emerging contaminant’.

Statement of Research Problem

- The indiscriminate disposal of improperly or untreated pharmaceutical and antibiotic production wastewater which contain sub-lethal concentration of antibiotics into the environment triggers the selection of resistant bacteria in the environment, which have been regarded as "emerging contaminants" (Samal *et al*., 2022).

Significance of the Study

- It is therefore important that bacteria able to metabolize antibiotic residue in wastewater be characterized and the products of their metabolism understood so that they can be utilized for removal of residual antibiotics in pharmaceutical effluent and production wastewater

Aim of the study

This study is aimed at isolating bacteria that have the capability to utilize and degrade ciprofloxacin as their sole carbon sole from pharmaceutical effluent .

Objectives of the study

The objectives of this study were to:

i. To isolate, characterize and identify bacteria with the ability to utilize on ciprofloxacin as carbon source from pharmaceutical effluent.

ii. To determine the growth pattern of bacterial isolates on single carbon source (SCS)-ciprofloxacin medium.

iii. To employ the isolated bacteria for biodegradation study of ciprofloxacin

METHODOLOGY

Collection of Sample

Water sample was collected from the wastewater channel of a pharmaceuticals company in Ilorin in sterile sampling bottles, following the modified method of Adekanmbi *et al*.

Culture Media and Formulation

Single Carbon Source (SCS) media was formulated according to the modified method of Dantas *et al*. as informed by Walsh *et al*. The SCS medium contain 5 g $(NH_4)_2SO_4$, 3g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 4.5 mg $ZnSO_4 \cdot 7H_2O$, 4.5 mg $CaCl_2 \cdot 2H_2O$, 3 mg $FeSO_4 \cdot 7H_2O$, 1 mg $MnCl_2 \cdot 4H_2O$, 1 mg H_3BO_3, 0.4 mg $Na_2MoO_4 \cdot 2H_2O$, 0.3 mg $CuSO_4 \cdot 5H_2O$, 0.3 mg $CoCl_2 \cdot 6H_20$ and 0.1 mg KI per litre at pH 5.5.

Bacteria Isolation

SCS medium was sterilized and allowed to cool to 40^oC. Ciprofloxacin purchased from Sigma-Aldrich, Germany (≥98.0% purity) was added to the medium at 1 g/L and 18 ml was dispensed into 100 ml conical flask. The water sample (2 ml) was dispensed into the SCS-ciprofloxacin medium and the culture was incubated on an orbital shaker at 120 rpm for a period of 5-7 days. A loopful of the culture broth was streaked on the formulated SCS-ciprofloxacin medium which was supplemented with agar agar and incubated at 37^oC for 24 to 48 hours.

METHODOLOGY CONT'D

Determination of Growth Pattern

A 24-48 hours old bacteria inoculum was suspended in 0.9% normal saline and adjusted to 0.5 McFarland standard. A 1:10 dilution was achieved by adding 2 ml of the suspension in 18 ml of SCS-antibiotic media. The growth pattern of the bacteria isolates was determined with spectrophotometer at 600 nm for a period of 4 days.

Degradation Study

The isolate with the most consistent growth pattern was selected for analysis of ciprofloxacin degradation from the growth media using High Performance Liquid Chromatography (HPLC). 1 ml of the culture prepared using 0.5 McFarland standard was inoculated into 9 ml SCS-ciprofloxacin medium. Sample of the culture was monitored for degradation for 4 day sat 280 nm using HPLC. The flow rate was set at 1 ml/min and injection volume at 20 µL. The mobile phase consisted of a mixture of 2% acetic acid and acetonitrile (84:16, v/v).

The total bacteria count was carried out during the 4 days period and turbidity of the media was also taken with spectrophotometer at 600 nm

RESULTS AND DISCUSSION

Table 1: Identity of the Isolates

S/N	Code	Isolate
1	RR11	*Paenibacillus sp.*
2	RR12	*Paenibacillus sp.*
3	RR13	*Paenibacillus sp.*
4	RR14	*Paenibacillus sp.*

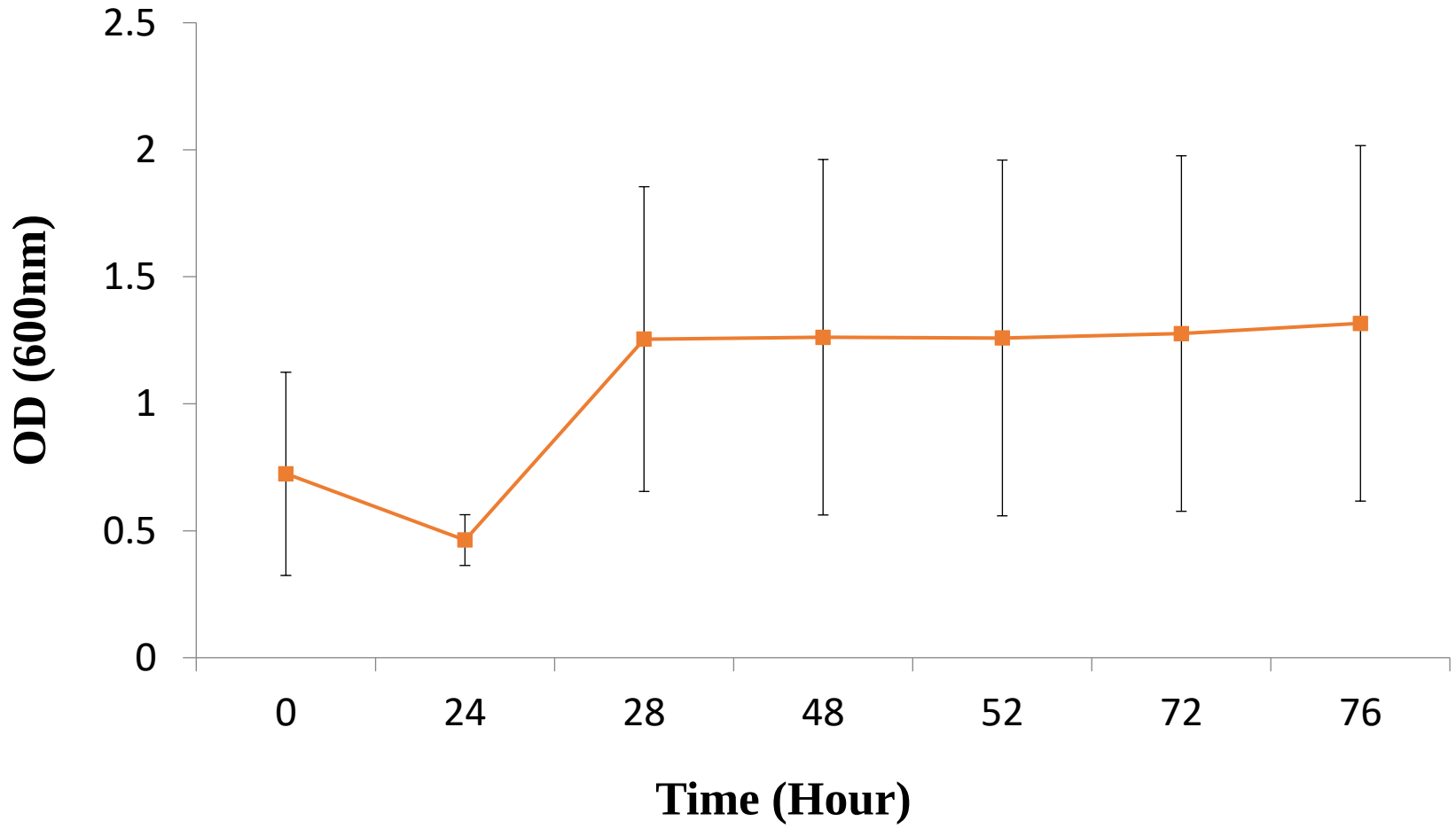
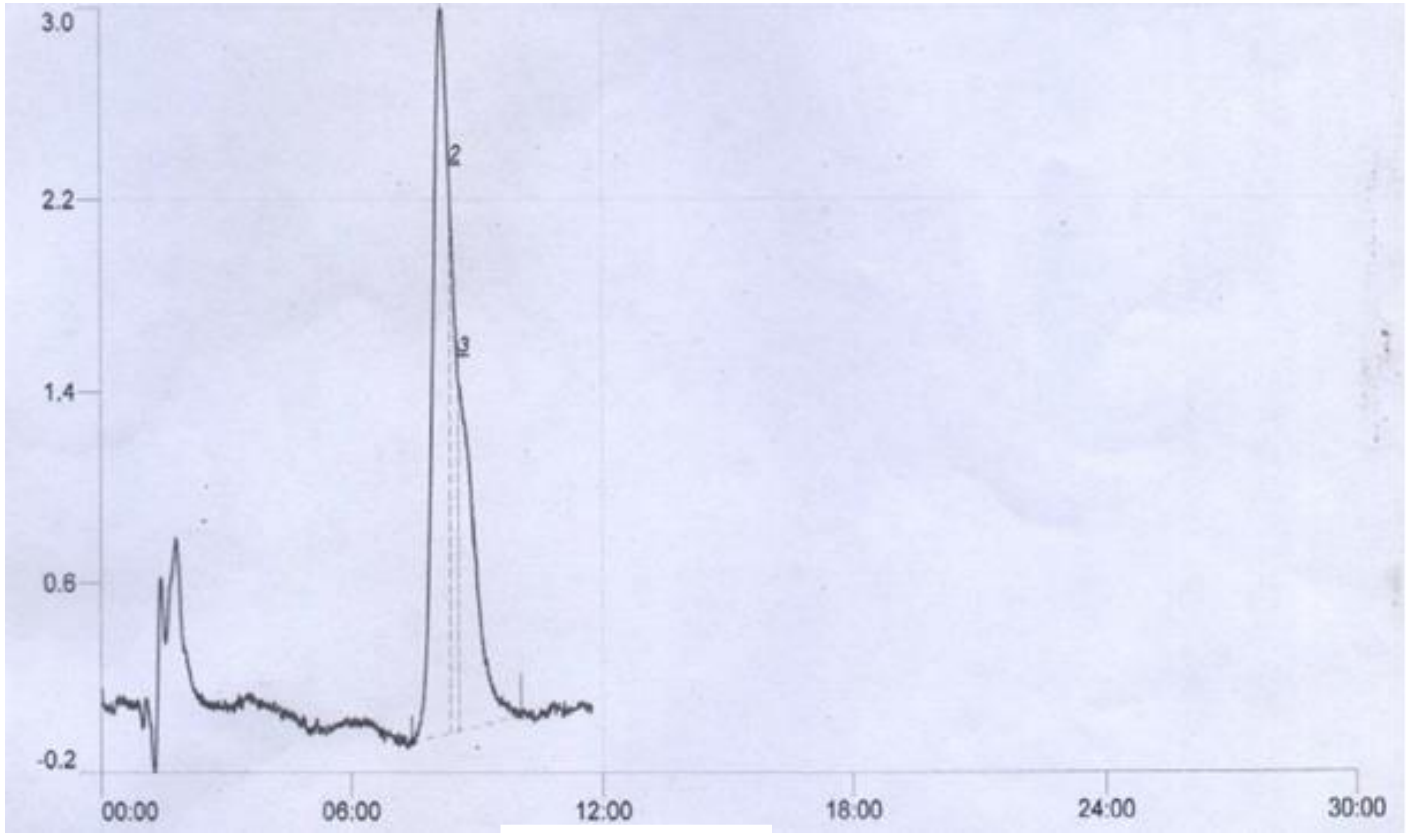


Fig. 1 Growth Pattern of RR14 (*Paenibacillus* sp) on SCS-ciprofloxacin media (1 g/L)

RESULTS AND DISCUSSION CONT'D

Abundance

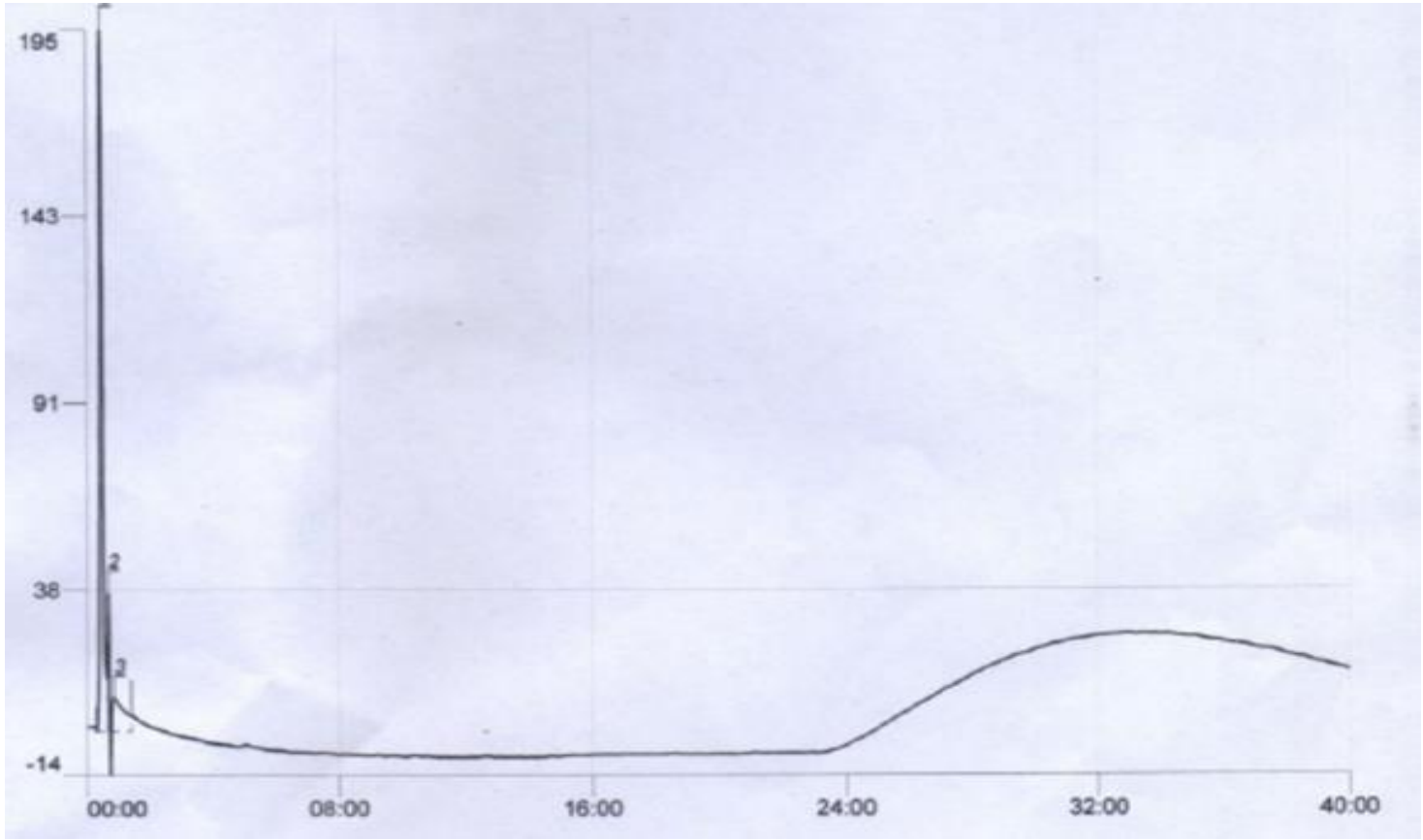


Time (min)

Fig. 2a shows the chromatogram of ciprofloxacin, of the standard (control/blank) prepared at a concentration of 4µM

RESULTS AND DISCUSSION CONT'D

Abundance



Time (min)

Fig. 2b The chromatogram of ciprofloxacin degradation by *Paenibacillus* sp RR14 on SCS-ciprofloxacin medium

CONCLUSION AND RECOMMENDATION

In conclusion, *Paenibacillus* sp isolated in the present study is capable of utilizing ciprofloxacin as its carbon source and could be useful in the treatment of effluent contaminated with antibiotic.

It is recommended that effluent should be properly treated and screened to eliminate antibiotic residues before discharge into the environment.

It is also recommended that the metabolic pathway and product of metabolism of ciprofloxacin be elucidated.

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