

## Utilisation of Alicyclic Compounds by Soil Bacteria

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**Abstract:** Alicyclic compounds are recalcitrant hydrocarbons, they are a major component of crude oil and their fraction in the oil may be as high as 37%. They are used as industrial chemicals and are obtained via extraction from petroleum or by synthesis. A number of alicyclic compounds are, in addition to the petrogenic source, continually synthesized biologically as constituents of plants and microorganisms. Despite the wide occurrence of these compounds in nature, very little works had been carried out on their utilization by microorganisms. Species of *Pseudomonas*, *Acinetobacter*, *Arthrobacter* and *Nocardia* able to utilize cyclohexanone as sole carbon source were isolated from soil by enrichment technique. The isolates also grew on cyclohexanol, succinic and acetic acids as sole carbon sources. DNA profiles of the organisms did not reveal the presence of any plasmid. Growth in acriflavin-supplemented broth did not result in loss of ability to utilize the compounds. The genetic control of alicyclic metabolism in these organisms appeared to be chromosomal in nature. [Nature and Science. 2006;4(3):65-68].

**Keywords:** Hydrocarbons; Alicyclic; Degradation; Bacteria; Microorganisms

### Introduction

Alicyclic hydrocarbons are the most resistant molecules to microbial attack among saturated hydrocarbons (Sikkema *et al.*, 1995; Ko and Lebeault, 1999), and are major components of 'drilling oils' (Chaîneau *et al.*, 1995). These are becoming increasingly important as industrial solvents to replace benzene (Sikkema *et al.*, 1995). Studies have shown that the alkanes are the most susceptible to attack followed by the isoalkanes with cycloalkanes as the most recalcitrant (Perry, 1984; Leahy and Colwell, 1990). The wide spread occurrence of alicyclic hydrocarbons in the biosphere over the preceding millions of years suggests that the capability for utilization of these compounds as sole source of carbon and energy would be broadly distributed over the microbial world but this is not so. Where one can readily demonstrate  $10^6$  bacteria capable of growth of n-hexadecane thrive in a gram of soil, reports on the successful enrichment for axenic cultures of cycloalkane-utilizing organisms are limited. Their very low water solubility and potential membrane toxicity to microbes are some of the reasons for their persistence and paucity of reports concerning their biodegradation (Anderson *et al.*, 1980; Sikkema *et al.*, 1995). Ilori (1999) reported the ability of lagoon water isolates identified as species of *Pseudomonas*, *Acinetobacter*, *Vibrio*, *Micrococcus* and *Flavobacterium* to utilise cyclohexanol as sole source of carbon and energy. To our knowledge, only *Brevibacterium* sp. strain HCU (Brzostowicz *et al.*, 2000) has been reported to grow on cyclohexanone while reports on the potentials of other organisms to utilise the compound as sole source of carbon and energy is scanty. This work

reports the ability of some soil microorganisms to utilize cyclohexanone as sole source of carbon and energy.

### Materials and Methods

#### Isolation of and identification of organisms

Cotton wool pieces were soaked in cyclohexanone, wrapped with aluminium foil and sterilised by autoclaving. One of the cotton wool was buried in an uncontaminated soil around Faculty of Science while another one was buried in spent oil-polluted soil at the AP filling station in University of Lagos. The cotton wool pieces were removed after 3 weeks and incubated in an enrichment medium described by Murray *et al.* (1974) for one week on rotary shaker (50 rpm) at 30 °C. Aliquots of the culture was thereafter plated on cyclohexanone agar and incubated at room temperature for one week. Pure cultures were obtained from this by picking distinct colonies and streaking each on separate cyclohexanone agar plates before transferring onto cyclohexanone slants. Identification of isolates was as described by Holt *et al.* (1994).

#### Growth on alicyclic compounds and organic acids

The isolates were tested for their ability to oxidize cyclohexanone, cyclohexanol, cyclohexane, succinic and acetic acids. These substrates were supplied in minimal salts medium as the sole carbon source at a concentration of 0.1%.

#### Screening for plasmids and curing

Screening for plasmids was as described by Birnboim and Doly (1979) while curing was as described by Miller (1972).

## Results

A mixed culture of bacteria, which grew on exogenously supplied cyclohexanone as sole source of carbon and energy were obtained. They were subsequently characterized and found to belong to four genera namely *Pseudomonas*, *Arthrobacter*, *Acinetobacter* and *Nocardia*. All the isolates grew on cyclohexanone, cyclohexanol, succinate and acetate (Table 1), while only the *Nocardia* species grew on cyclohexane.

The time course growth profile of the organisms on cyclohexanone is as shown in Figure 1. *Nocardia* sp had the best growth on the compound while the least growth was recorded with *Acinetobacter* sp. Figure 2 shows the content of the DNA extracts of the isolates after agarose gel electrophoresis. Growth in acriflavine supplemented nutrient broth for 48 h revealed that all the isolates retained the ability to grow on cyclohexanone.

Table 1. Growth of Isolates on Alicyclic Compounds and Organic Acids

Isolates	Cyclohexanone	Cyclohexanol	Cyclohexane	Succinate	Acetate
<i>Acinetobacter Calcoaceticus</i>	++	++	-	+	+
<i>Arthrobacter</i> sp.	+++	+++	-	+	+
<i>Pseudomonas fluorescens</i>	+++	+++	-	+	+
<i>Nocardia</i> sp.	+++	+++	+	++	+

+++ , Heavy growth; ++, moderate growth; +, poor growth; -, no growth.

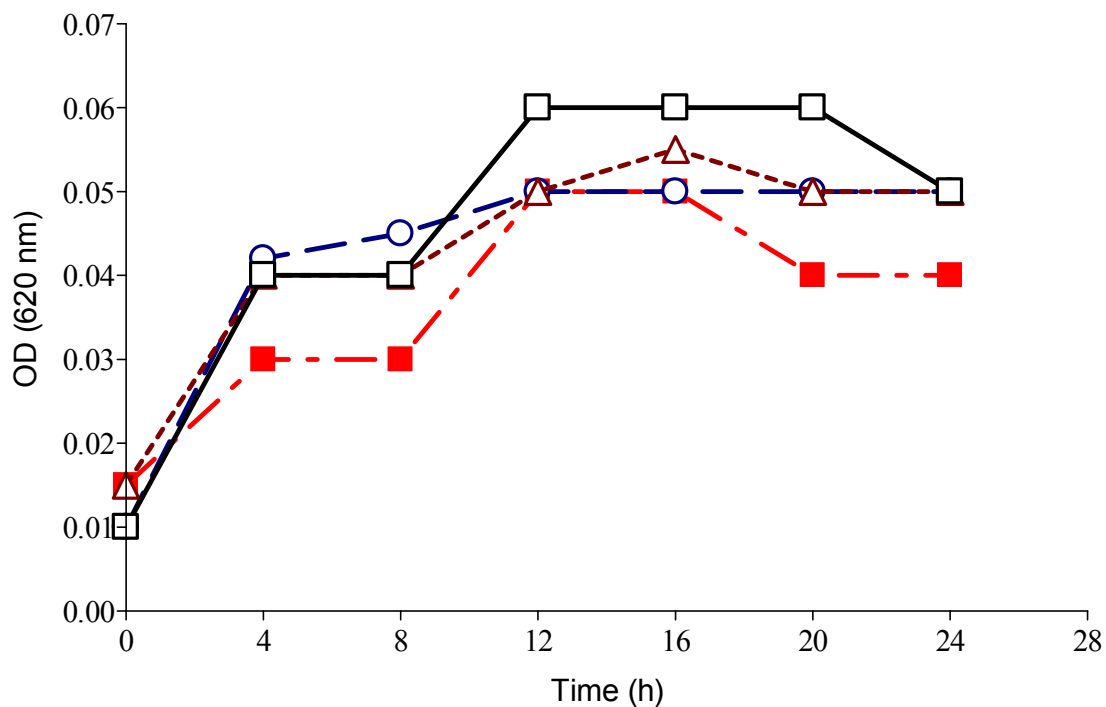


Figure 1. Growth Profile of the Isolates on Cyclohexanone. ○, *Pseudomonas fluorescens*; ■, *Acinetobacter calcoaceticus*; □, *Nocardia* sp.; Δ, *Arthrobacter* sp.

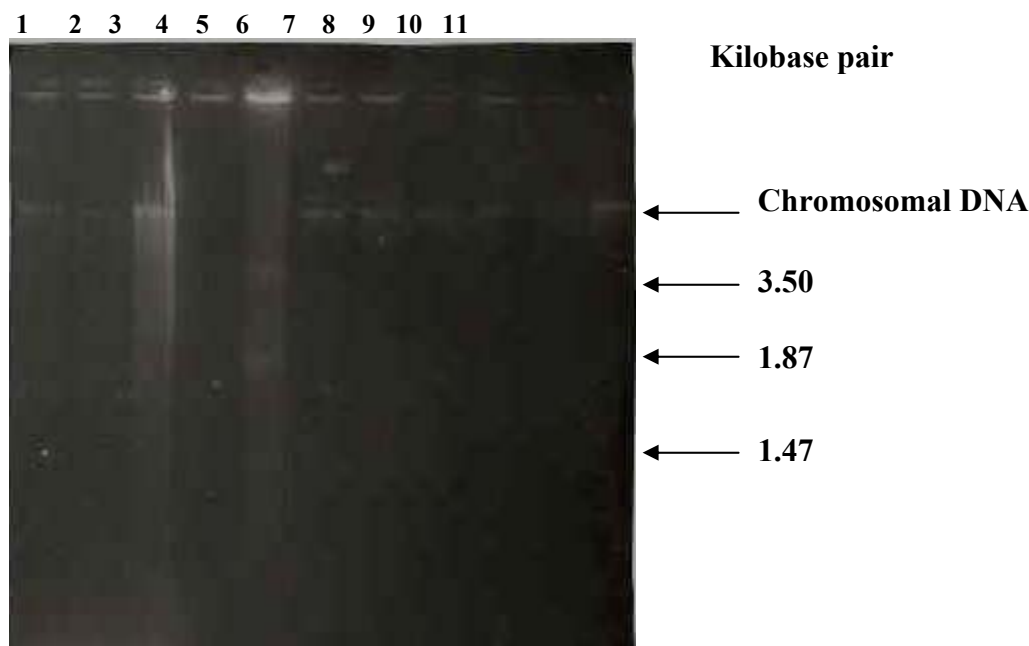


Figure 2. Agarose Gel Electrophoresis of DNA Extracts. Lanes: 1, 2, 4, 6, 7, 8, 9, *Pseudomonas* spp.; 3, *Nocardia* spp.; 5, *Escherichia coli* V517; 11, *Acinetobacter calcoaceticus*.

### Discussion

Alicyclic compounds are widely distributed in nature, they include monocyclic and other more complex terpenes, sterols and a wide range of more exotic molecules from pyrethrins. It is possible that, in natural mixed microbial populations, the degradation of such compounds may yield cyclohexanone or closely related compounds like cyclohexanol which are themselves not of significant natural occurrence as metabolic intermediates. The organisms grew on both cyclohexanone and cyclohexanol at nearly equivalent rates. This is similar to the findings of Donoghue and Trudgill (1975) who reported that this ability is compatible with a role for cyclohexanone in cyclohexanol oxidation. The failure of the organisms to grow on succinate and acetate at equivalent rates reflect the inability of these more polar compounds to penetrate the bacteria rapidly. Cyclohexane is a component of crude oil but reports of microorganisms able utilize this compound as sole source of carbon have been very few (Beam and Perry, 1973; Anderson *et al.*, 1980). Cyclohexane is very volatile and does not persist long enough for potential oxidizers to develop. Furthermore, the compound may be toxic to microorganisms in both liquid and vapour forms thereby making it resistant to microbial attack (Donoghue and Trudgill 1975; Sikkema *et al.*, 1995). The time course growth experiment showed that no appreciable period of time is required for the metabolism of cyclohexanone by the

organisms. The involvement of plasmid-encoded genes in the metabolism of both n-alkane and aromatic hydrocarbons has been documented (Chakrabarty, 1980; Foght and Westlake, 1996). No such report has however appeared on the catabolism of cycloalkanes. Therefore, cyclohexanone is probably catalysed by chromosomally encoded enzymes as shown by the results of plasmid screening and curing experiments.

### Conclusion

Many intermediates in the aliphatic and aromatic break-down by bacteria can be conveniently catabolized by chromosomally encoded enzymes.

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