ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF RECENTLY ISOLATED OXALATE UTILIZING BACTERIA

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ABSTRACT

Susceptibilities of eight strains of aerobic, oxalate-utilizing bacteria and two oxalate-utilizing reference strains Pseudomonas oxalaticus DSM 1105 and Methylobacterium extorquens DSM 1337 to 18 antimicrobial agents were determined by the National Committee for Clinical Laboratory Standards (NCCLS) disk diffusion method. All strains were sensitive towards Carbencillin and resistant to Rifampicin. Penicillinase production was detected in 80% of all strains tested.

The results show that in addition to the normal determination methods it is possible to separate some of the strains of these oxalate utilisers with the help of antimicrobial susceptibility patterns.

INTRODUCTION

Oxalate is commonly found in soils and is primarily derived from root exudates, break-down products from plants, animal, microbial tissues and metabolites from bacteria and fungi. Several species of Penicillium and Aspergillus convert sugar into calcium oxalate with 90% yields under optimum conditions. It should not be surprising that microbes able to attack oxalate are widely distributed in natural ecosystems [Allison et al., 1995]. On the death and decay of plants containing oxalate, the oxalate can accumulate in the soil where its chelating properties will prove toxic and interfere with plant growth. The prevention of this accumulation is attributed to the activities of oxalate metabolizing soil microorganisms [Chandra et al., 1977].

Oxalate oxidase known to catalyse the aerobic oxidation of oxalic acid into CO₂ and H₂O and it has been found in bacteria, fungi, mosses
and some higher plants. So far, a membran bound oxalate oxidase has been purified from *Pseudomonas* sp. The degradation of endogenous oxalate in rat by immobilized oxalate oxidase has opened a new vistas in enzyme therapy of hyperoxaluria [Pundir et al., 1993].

Several species of aerobic bacteria are known to be able to grown with oxalate [Tamer, 1982] and the oxidation of oxalic acid was studied intensively with *Pseudomonas oxaliticus* [Quayle et al., 1961; Dijkhuizen et al., 1977]. Although *Pseudomonas oxaliticus* and *P. extorquens* are the best studied oxalate metabolism, their taxonomic status are not clear. They are mentioned as “species incertae sedis” in the eight edition of Bergey's Manual of Determinative Bacteriology [Jenni et al., 1988]. However, data on the antimicrobial susceptibility patterns of recently described species are very scanty. The resistance or susceptibility to inhibitors are also generally stable characters and can serve as diagnostic aids. Furthermore, the patterns of susceptibility to antibiotics can be useful in distinguishing similar species from each other as well as the strains of the same species [Trüper and Schleifer, 1992].

The intention of the present study was to provide the comprehensive antimicrobial susceptibility data for aerobic oxalate utilizing species for the first time.

**MATERIALS AND METHODS**

**Strains**

The strains, collection numbers and references are listed in Table 1. The strains were isolated by enrichment on 4 g/L potassium oxalate in mineral medium under air, from soil litter close to *Arum maculatum*, *Oxalis acetocella* and *Rumex* sp. live plants, as described by Tamer and Aragno [1980].

**Susceptibility testing**

The susceptibility towards antibiotics was tested with impragened disks (Oxoid) by the Kirby-Bauer method. Inhibition zones were assessed
Table 1. Bacterial strains studied.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Collection No.</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas oxalaticus OX1</em></td>
<td>DSM 1105</td>
<td>Khambata et al., 1953</td>
</tr>
<tr>
<td><em>Methyllobacterium extorquens</em></td>
<td>DSM 1337</td>
<td>Bassalik et al., 1960</td>
</tr>
<tr>
<td><em>Alcaligenes paradoxis SA 29</em></td>
<td>DSM 645</td>
<td>Davis et al., 1969</td>
</tr>
<tr>
<td><em>Pseudomonas sp. TA 08</em></td>
<td>NEU 51</td>
<td>Tamer, 1982</td>
</tr>
<tr>
<td><em>Pseudomonas sp. TA 21</em></td>
<td>NEU 101</td>
<td>Tamer, 1982</td>
</tr>
<tr>
<td><em>Pseudomonas sp. TA 25</em></td>
<td>NEU 105</td>
<td>Tamer, 1982</td>
</tr>
<tr>
<td><em>Pseudomonas sp. TA 17</em></td>
<td>NEU 98</td>
<td>Tamer, 1982</td>
</tr>
<tr>
<td><em>Pseudomonas sp. TA 23</em></td>
<td>NEU 103</td>
<td>Tamer, 1982</td>
</tr>
<tr>
<td><em>Pseudomonas sp. TA 24</em></td>
<td>NEU 104</td>
<td>Tamer, 1982</td>
</tr>
<tr>
<td><em>Methyllobacterium sp. TA 05</em></td>
<td>NEU 48</td>
<td>Tamer, 1982</td>
</tr>
</tbody>
</table>

DSM: Deutsche Sammlung von Mikroorganismen, Göttingen, Germany.
NEU: Laboratoire de Microbiologie de l'Université, Neuchâtel, Suisse

on Mueller-Hinton agar plates and evaluated after 24 and 48 hours of incubation at 27°C. Size of inhibition zones were interpreted by referring to the tables which represent the NCCLS subcommitee's recommendations [Anonymus, 1990]. Tests were performed in triplicate. Strains which fall into “intermediate” category were given “resistant”. All susceptibility tests could be read without difficulty after 24h of incubation. Only for *Methyllobacterium* sp. strains were growth more easily observed after 48h.

Data Processing

Numerical analysis of the data was performed by using the simple matching coefficient [Sokal et al., 1958]. The matrix of distance was computed into phenogram by using single linkage clustering method [Sneath, 1957].

RESULTS AND DISCUSSION

No comprehensive data regarding the antimicrobial susceptibility patterns of the ten species examined in this study could be found in the literature. The limited data given for *Methyllobacterium extorquens* [Green, 1992] isolates that were sensitive to especially the tetracyclines whereas most are resistant to cephalothin, nalidixic acid, penicillin, bacitracin,
carbenicillin, colistin sulfate and polymyxin B. *Methylobacterium extorquens* was the first oxalate degrading bacteria to be described in the literature and was initially named as *Bacillus extorquens* by Bassalik in 1913. However, the taxonomic position of this organism and other pink-pigmented, facultative methylootrophs (PPFM) was uncertain. Thus, they were subsequently assigned to several different genera (e.g., *Vibrio, Pseudomonas, Protomonas*) until it was proposed that PPFMs be grouped in the genus *Methylobacterium* and that *Protomonas extorquens* be reclassified as *M. extorquens* [Bousfield et al., 1985].

The results of the susceptibility testing were shown in Table 2. All strains were found to be sensitive towards carbenicillin and resistant to

**Table 2. Susceptibility of aerobic oxalate-degrading bacteria to antibiotics.**

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Conc. (ml⁻¹)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
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</thead>
<tbody>
<tr>
<td><strong>Beta-Lactames</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Penicillin G</td>
<td>10 IU</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>10 mg</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Carbenicillin</td>
<td>100 mg</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Cephalaxin</td>
<td>30 mg</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<tr>
<td><strong>Aminoglycosides</strong></td>
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<tr>
<td>Streptomycin</td>
<td>10 mg</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Neomycin</td>
<td>30 mg</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>30 mg</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<tr>
<td>Gentamycin</td>
<td>10 mg</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
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</tr>
<tr>
<td>Tobramycin</td>
<td>10 mg</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
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<tr>
<td><strong>Other antibiotics</strong></td>
<td></td>
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<tr>
<td>Bacitracin</td>
<td>10 IU</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>30 mg</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>30 mg</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
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<td>S</td>
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<tr>
<td>Erythromycin</td>
<td>15 mg</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<tr>
<td>Chloramphenicol</td>
<td>30 mg</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
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<td>R</td>
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<tr>
<td>Rifampicin</td>
<td>30 mg</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<td>R</td>
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<tr>
<td>Polymyxin B</td>
<td>300 IU</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>30 mg</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Trimethoprim-sulphamethoxazole</td>
<td>1.25 mg+</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
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<tr>
<td></td>
<td>23.75 mg</td>
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</tbody>
</table>

S, Sensitive; R, Resistance. **Strains:** 1= *Pseudomonas oxalaticus* DSM 1105; 2= *Pseudomonas TA 08 NEU 51* ; 3= *Pseudomonas TA 21 NEU 101* ; 4= *Pseudomonas TA 25 NEU 105* ; 5= *Methylobacterium TA 05 NEU 48* ; 6= *Methylobacterium extorquens* DSM 1137; 7= *Pseudomonas TA 17 NEU 98* ; 8= *Pseudomonas TA 23* ; 9= *Pseudomonas TA 24* ; 10= *Alcaligenes paradoxus SA 29 DSM 645*
rifampicin. Penicillinase production was detected in 80% of all strains. Tobramycin, erythromycin, polymyxine B and trimethoprim-sulphamethoxazole were the most effective antibiotics. Two *Methylobacterium* strains were sensitive to all antibiotics except rifampicin. Yellow pigmented strain TA 17 was found to be resistant towards the three aminoglycoside antibiotic (streptomycin, neomycin, kanamycin) and penicillin G, ampicillin, cephalaxine, bacitracine, tetracycline, erythromycin, chloramphenicol, rifampicine and vancomycin such multiresistant characteristics clearly discriminate the starin TA 17 from the other oxalate utilizers [Fig. 1].

**Figure 1.** Phenogram based on the susceptibility tests listed in Table 2. The scale shows the similarities in %.

![Phenogram](image-url)
Results from this study were examined in Figure 1 from the phenograms. The strains are clustered in phenogram in a similar manner; thus genomic [Tamer et al., 1993] and numerical phenetic [Tamer et al., 1980] approaches show a good correlation. Characterization of oxalate utilizing aerobic, non-lithotrophic bacteria has indicated three different types. One of these, pink-pigmented strains (DSM 1337 and TA 05), exemplified by "Pseudomonas extorquens" is related to Methylobacterium. Yellow pigmented strains (TA 17, TA 23 and TA 24) probably constitute a new taxon at species level. Others, non-pigmented ones are strains of "P. oxalaticus" [Tamer et al., 1993].

The resistance of the oxalate utilizing bacteria towards rifampicin might be used to isolate such strains with an improved selective medium. Antimicrobial susceptibility patterns may be useful in distinguishing between similar strains of oxalate bacteria but further study is needed on more strains which are isolated from different environmental sources.

REFERENCES


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