Commun. Fac. Sci. Univ. Ank. Series C
V. 16. pp. 1-7 (1998)

ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF RECENTLY ISOLATED OXALATE UTILIZING BACTERIA

Nurettin ŞAHİN* and A. Üsame TAMER**

* *Department of Biology, Faculty of Arts and Science, Muğla University, Kötekli/MUĞLA*
** *Department of Biology, Faculty of Arts and Science, Celal Bayar University, MANİSA*

(Received Sep. 22, 1997; Revised June 12, 1998; Accepted July 22, 1998)

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 Standarts (NCCLS) disk diffusion method. All strains were sensitive towards Carbenicillin 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 exduates, 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 suprising that microbes able to attact 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 oxdation of oxalic acid into CO_2 and H_2O_2 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 similer 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.

Strains	Collection No.	Reference
Pseudomonas oxalaticus OX1	DSM 1105	Khambata et al., 1953
Methylobacterium extorquens	DSM 1337	Bassalik et al., 1960
Alcaligenes paradoxus SA 29	DSM 645	Davis et al., 1969
Pseudomonas sp. TA 08	NEU 51	Tamer, 1982
Pseudomonas sp. TA 21	NEU 101	Tamer, 1982
Pseudomonas sp. TA 25	NEU 105	Tamer, 1982
Pseudomonas sp. TA 17	NEU 98	Tamer, 1982
Pseudomonas sp. TA 23	*NEU 103*	Tamer, 1982
Pseudomonas sp. TA 24	NEU 104	Tamer, 1982
Methylobacterium sp. TA 05	NEU 48	Tamer, 1982

DSM: Deutshe Sammlung von Mikroorganismen, Göttingen, Germany.
NEU: Laboratoire de Microbiologie de l'Université, Neuchatel, 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 *Methylobacterium* 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 susceptibilitiy patterns of the ten species examined in this study could be found in the literature. The limited data given for *Methylobacterium 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 methylotrophs (PPFMs) 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

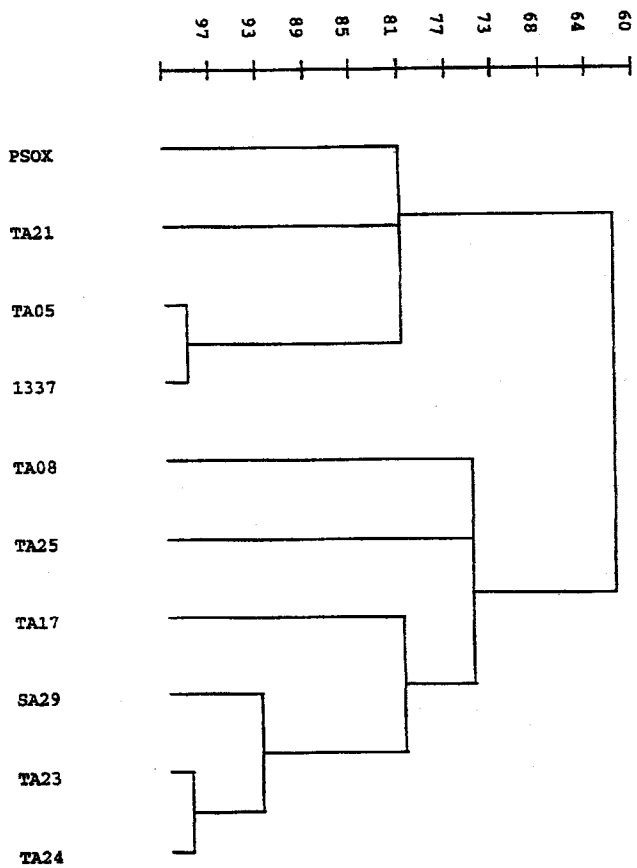
Table 2. Susceptibility of aerobic oxalate-degrading bacteria to antibiotics.

Antibiotics	Conc. (ml^{-1})	Strains 1	2	3	4	5	6	7	8	9	10
Beta-Lactames											
Penicillin G	10 IU	R	R	R	R	S	S	R	R	R	R
Ampicillin	10 mg	S	R	S	R	S	S	R	R	R	R
Carbenicillin	100 mg	S	S	S	S	S	S	S	S	S	S
Cephalexin	30 mg	S	S	S	S	S	S	R	R	R	R
Aminoglycosides											
Streptomycin	10 mg	S	R	S	R	S	S	R	R	R	R
Neomycin	30 mg	S	R	S	R	S	S	R	R	R	R
Kanamycin	30 mg	S	R	S	R	S	S	R	R	R	R
Gentamycin	10 mg	S	R	S	R	S	S	S	R	R	R
Tobramycin	10 mg	S	R	R	R	S	S	S	S	S	S
Other antibiotics											
Bacitracin	10 IU	R	R	S	R	S	S	R	R	R	R
Tetracycline	30 mg	S	S	S	R	S	S	R	R	R	R
Doxycycline	30 mg	S	S	S	S	S	S	S	R	R	R
Erythromycin	15 mg	R	S	S	R	S	S	R	R	R	R
Chloramphenicol	30 mg	S	S	S	R	S	S	R	R	R	S
Rifampicin	30 mg	R	R	R	R	R	R	R	R	R	R
Polymyxin B	300 IU	S	S	S	R	S	S	S	S	S	S
Vancomycin	30 mg	R	R	R	R	S	S	R	R	R	R
Trimethoprim-sulphamethoxazole	1.25 mg+ 23.75 mg	S	S	S	S	S	S	S	R	R	R

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. Tobramycine, erythromycine, polymyxine B and trimethoprim- sulphamethoxazole were the most effective antibiotics. Two *Methylobacterim* strains were sensitive to all antibiotics expect rifampicin. Yellow pigmented strain TA 17 was found to be resistant towards the three aminoglycoside antibiotic (streptomycine, neomycine, kanamycine) and penicilline G, ampicilline, cephalexine, bacitracine, tetracycline, erythomycine, chloramphenicol, rifampicine and vancomycine 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 %.



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, nonlithotrophic 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

[1] ANONYMUS, 1990. National Committee for Clinical Laboratory Standards (NCCLS). Performance Standards for Antimicrobial Disc Susceptibility Tests. 4 th. Ed. M2-A4, Vol., 10, No: 7. *National Committee for Clinical Laboratory Standards,* Villanova, Pa.

[2] ALLISON, M.J., DANIEL, S.L., CORNICK, N.A., 1995. Oxalate degrading bacteria. In: Calcium oxalate in biological systems, (Ed) Saeed Khan, CRC Press, Boca Raton, FL.

[3] BASSALIK, K., 1913. Uber die verarbeitung der oxalsaure durch *Bacillus extorquens* n. sp. Jahrb. Botan., 53:255-302.

[4] BASSALIK, C., JANOTA-BASSALIK, L., BRISOU, J., 1960. Etude sur *Flavobacterium extorquens* (ex. *Pseudomonas extorquens*). Ann. Inst. Pasteur, 98:165-168.

[5] BOUSFIELD, I.J., GREEN, P.N., 1985. Reclassification of bacteria of the genus Protomonas Urakami and Komagata 1984 in the genus *Methylobacterium* (Patt, Cole and Hanson) emend. Green and Bousfield 1983. Int. J. Syst. Bacteriol., 35:209.

[6] CHANDRA, T.S., SHETHNA, Y.I., 1977. Microbial metabolism of oxalate and one-carbon compounds. J. of Indian Inst. of Sci., 59:26-52.

[7] DAVIS, D.H., DOUDOROFF, M., STANIER, R.J., MANDEL, M., 1969. Proposal to reject the genus *Hydrogenomonas*: Taxonomie implications. Int. J. System. Bact., 19:375-390.

[8] DIJKHUIZEN, L., WIERSMA, M., HARDER, W., 1977. Energy production and growth of *Pseudomonas oxalaticus* Ox1 on oxalate, formate. Archiv. Microbiol., 115:229-236.

[9] GREEN, P.N., 1992. The genus *Metnylobacterium*. In: Balows, A., Trüper, H.G., Dworkin, M., Harder, W., Schleifer, K.H. (Eds). The Prokaryotes, 2nd ed: a handbook on the biology of bacteria; ecophysiology, isolation, identification, applications. Springer-Verlag, New York.

[10] JENNI, B., REALINI, L., ARAGNO, M., TAMER, A.Ü., 1988. Taxonomy of non H_2-lithotrophic, oxalate-oxidizing bacteria related to *Alcaligenes eutrophus*. System. Appl. Microbiol., 10:126-133.

[11] KHAMBATA, S.R., BHAT, J.V., 1953. Studies on a new oxalate-decomposing becterium, *Pseudomonas oxalaticus*. J. Bact., 66:505-507.

[12] PUNDIR, C.S., VERMA, U., 1993. Isolation, purification, immobilization of oxalate oxidase and its clinical applications. Hindustan Antibiot. Bull., 35:173-182.

[13] QUAYLE, J.R., KEECH, D.B., TAYLOR, G.A., 1961. Carbon assimilation by *Pseudomonas oxalaticus* Ox1. Metbolism of oxalate in cell-free extracts of the organism grown on oxalate. Biochemical J., 78:225-236.

[14] SOKAL, R.R., MICHENER, C.D., 1958. A statistical method for evaluating systematic relationships. Univ. Kansas Sci. Bull., 38:1409-1438.

[15] SNEATH, P.H.A., 1957. The application of computers to taxonomy. J. Gen. Microbiol., 17:200-226.

[16] TAMER, A.Ü., ARAGNO, M., 1980. Isolement, caractérisation et essai d'identification de bactéries capables d'utiliser l'oxalate comme seule source de carbon et d'énergie. Bull. Soc. Neuch. Sci. Nat., 103:91-104.

[17] TAMER, A.Ü., 1982. Oksalatı karbon ve enerji kaynağı olarak kullanılan bakteriler üzerinde taksonomik ve fizyolojik bir araştırma (Ph.D thesis). Ege Üniversitesi, Fen Fakültesi, Mikrobiyoloji Bölümü, Bornova-İZMİR.

[18] TAMER, A.Ü., ARAGNO, M., JENNI, B., 1993. Genomic relation among nine strains of oxalate utilizing bacteria. Doga Tr. J. of Biology. 17:115119.

[19] TRÜPER, H.G., SCHLEIFER, K.H., 1992. Prokaryote characterization and identification. In: Balows, A., Trüper, H.G., Dworkin, M., Herder, W., Schleifer, K.H. (Eds). The Prokaryotes, 2nd ed: A handbook on the biology of bacteria; ecophysiology, isolation, identification, applications. Springer-Verlag, New York.

C O M M U N I C A T I O N S

DE LA FACULTE DES SCIENCES
DE L'UNIVERSITE D'ANKARA

FACULTY OF SCIENCES
UNIVERSITY OF ANKARA

Séries C: Biology, Geological Engineering and Geophysical Engineering

INSTRUCTIONS TO CONTRIBUTORS

This series of the Communications accepts original research articles in the fields of biological and geological sciences and publishes in English. Review articles can also be accepted, provided they are written by emminent scientists.

Manuscripts must be submitted in triplicate, typed double-spaced throughout with 4 cm left and 2 cm right margin and single-sided on white A4 standard paper. Inclusive of figures and tables, manuscripts must at most be 25 type-written pages long.

After the manuscript has been accepted for publication the author will not be permitted to make any new additions to the manuscript. Before publication the galley proof is always sent to the author for corrections. Thus, it is solely the author's responsibility for any typographical mistakes which occur in their article as it appears in the Journal.

1. **Title page:**

   Title must be typed on a separate page along with the name(s) and affiliations of the author(s).

2. **Abstract:**

   A short abstract, should not exceed 150 words, must addended to each submitted article. Below the abstract provide 3-10 KEY WORDS.

3. **Sections:**

   The consecutive sections should be introduction, material and methods, results, discussion and conclusion. Acknowledgement(s) should be given at the end of the text.

4. **Tables and Figures:**

   All illustrations except tables must be labeled as "Figure". All tables and figures must have a number and a caption or legend. All tables and figures must be numbered consecutively throughout the paper. Captions and legends must be double-space typed on a separate sheet and labeled according to which table or figure they belong.

   Figures must be originals and drawn neatly in Indian ink on white paper or printed on smooth tracing/drawing paper. Photographs must be clear, black and white, and printed

on glossy paper. The manuscript title and the name(s) of the author(s) must be written in pencil on each figure or photograph.

5. References:

References must be typed on a separate sheet in alphabetical order of author names. References must be denoted in the text by the surname(s) and the year of publication.

They should be given as follows;

Articles in Periodicals: a) Surname(s) and initial(s) of author(s), b) Year of publication (in parenthesis), c) Title of the article, d) Abbreviated form of the periodical as used in international publications, e) Volume number, f) Page numbers (numbers of first and last pages).

Books: a) Surname(s) and initial(s) of author(s), b) Year of publication, c) Title of the book, d) Editor's name (if any), e) Volume number, f) Place of the publication and name of the publisher, g) Page number.

Theses: a) Surname an the initials of the author, b) Year in which the research was carried out, c) Title of the thesis, d) Type of the thesis (M.Sc. or Ph.D.), e) Name and address of institute where the research was carried out.

6. Notes:

The author(s) will receive 25 reprints without charge. Irrespective of their acceptance, manuscripts will not be returned to authors.

7. Computer Disk:

If you are able to initially prepare your manuscript in a MS Word Programme (Macintosh or PC) file including the figures translated into the picture environment of Encapsulated PostScript format (EPS), we advise that you do so. Then, only if and after your manuscript is accepted for publication, we will ask you to submit a revised disk copy of your manuscript which will enable us to more efficiently and accurately prepare proofs. (This is not a requirement but is highly encouraged.)

8. Charges:

Each paper is due to be charged for the amount of which is determined by the administration each year.

9. Address:

Texts should be sent to the following address:
Prof.Dr. Öner ÇAKAR - Editor, Communications
Ankara Üniversitesi, Fen Fakültesi
06100, Beşevler-ANKARA

COMMUNICATIONS

DE LA FACULTE DES SCIENCES
DE L'UNIVERSITE D'ANKARA

FACULTY OF SCIENCES
UNIVERSITY OF ANKARA

Volume: 16 **Number: 1-2** **Year: 1998**

CONTENTS