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CHARACTERIZATION OF *PSEUDOMONAS CEPACIA* USING BIOCHEMICAL, ANTIBIOTIC SUSCEPTIBILITY AND SEROLOGICAL TESTS

by

Beatrice Miceika

A Thesis Submitted to the Faculty of the Graduate School of Loyola University of Chicago in Partial Fulfillment of the Requirements for the Degree of Master of Science

May

1978

LOYOLA UNIVERSITY MEDICAL CENTER

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VITA

The author, Beatrice Grazina Miceika, is the daughter of Eugene P. Vilkas and Irena (Staras) Vilkas. She was born December 3, 1949 in Chicago, Illinois.

Her elementary education was obtained in parochial schools in the Chicago area and secondary education at Mother of Sorrows High School, Blue Island, Illinois, where she graduated in 1967.

She attended the University of California at Riverside for two years and Loyola University of Chicago for two years. She graduated magna cum laude with the degree of Bachelor of Science with a major in biology from Loyola University in June of 1971. She was elected a member of Phi Beta Kappa in 1969. In September of 1971 she entered Little Company of Mary Hospital's School of Medical Technology (Evergreen Park, Illinois) for a year's internship in medical technology. In 1972 she became a registered Medical Technologist. She enrolled in the Graduate College, Department of Microbiology, at Loyola University of Chicago in September of 1974.

In March, 1974, she joined the staff of the Clinical Microbiology Laboratory at Foster G. McGaw Hospital, Loyola University of Chicago. In November of 1977, she was appointed assistant section supervisor of the Microbiology-Serology Laboratory at Foster G. McGaw.

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LIST OF ABBREVIATIONS

Abbreviations Related To Antiobiotic Susceptibility Testing

CFU = Colony forming units
I = Indeterminate
MBC = Minimum bactericidal concentration
MIC = Minimum inhibitory concentration
Am = Ampicillin
An = Amikacin
Cb = Carbenicillin
Cl = Cephalothins
C = Chloramphenicol
Cm = Clindamycin
Cs = Colistin
E = Erythromycin
FD = Nitrofurantoin
Gm = Gentamicin
K = Kanamycin
N.A. = Nalidixic acid
SO = Oxacillin (Methicillin)
P = Penicillin G
Te = Tetracycline
Ti = Ticarcillin
NN = Tobramycin
TM-SXT = Trimethoprim-sulfamethoxazole
Va = Vancomycin

Abbreviations Related To Biochemical Testing

A = Acid
ADH = Arginine dihydrolase
AMY = Amygdalin
ARAB = Arabinose
BHIA = Brain heart infusion agar
CAT = Catalase
CIT = Citrate
DNAse = Deoxyribonuclease
FLUOR = Fluorescence
F/N = Fluorescence-denitrification agar
GEL = Gelatin hydrolysis
GLCN = Gluconate Oxidation
GLU = Glucose
HEMO = Hemolysis
IND = Indole production
INO = Inositol
K = Alkaline
LDC = Lysine decarboxylase
LECI = Lecithinase
LIP = Lipase
MAC = Growth on MacConkey agar
MAN = Mannitol
MEL = Melibiose
MOT = Motility
NITR = Nitrate reduction
ODC = Ornithine decarboxylase

Additional Abbreviations Related To Biochemical Testing

OF = Oxidative-Fermentative

OF Con = OF Control

OF Dex = OF Dextrose

OF-F = OF Fermentation

OF Lac = OF Lactose

OF Malt = OF Maltose

OF Mann = OF Mannitol

OF-O = OF Oxidation

OF Rham = OF Rhamnose

OF Xyl = OF Xylose

ONPG = β-galactosidase production

OXI = Oxidase

PHENYL = Phenylalanine deaminase

RHA = Rhamnose

SOR = Sorbitol

SS = Growth on SS agar

SUC = Sucrose

TDA = Tryptophan deaminase

TSB = Trypticase soy broth

TSI = Triple sugar iron agar

URE = Urease

VP = Voges-Proskauer reaction

(+) = Positive reaction

(-) = Negative reaction

CHAPTER I

INTRODUCTION

Pseudomonas cepacia is a gram negative nonfermentative bacillus also referred to in the past as Pseudomonas multivorans, King group EO-1, and Pseudomonas kingii. A number of investigators have done biochemical, antibiotic susceptibility and DNA homology studies on P. cepacia. Since the late 1960's, literature citing P. cepacia as an organism causing nosocomial infection outbreaks in hospitals throughout the world has been increasing.

Biochemical methods currently used for identification of this organism are time-consuming and errors can readily occur during laboratory manipulations. Although serological methods have proven useful for the rapid identification of a variety of other human pathogens, very little work has been done with respect to the antigenic characteristics of this organism. The purpose of this study was to perform a detailed biochemical and serological analysis of Pseudomonas cepacia and to determine whether differences in serological reactivity can be correlated with differences in antibiotic susceptibility and biochemical reaction patterns among diverse strains of P. cepacia.

A. Pathogenicity

Group A streptococci and staphylococci were the major pathogens encountered in hospital-associated infections before the antibiotic era began in the 1940's (72). Following the introduction of sulfonamides, penicillins, tetracyclines and erythromycin, there was a considerable decrease in death-rates from major infections such as bacteremias. Antibiotic-resistant staphylococci began to emerge as a major hospital problem so that the 1950's came to be called the "staphylococcal decade" (72). In the early 1960's penicillinase-resistant semisynthetic penicillins came into use and, about the same time, epidemic staphylococcal disease within hospitals appeared to subside. Since then, another phenomenon has been emerging. There has been a striking increase in nosocomial infections caused by gram negative bacilli (72). Studies in the mid-1960's showed *Staphylococcus aureus* as the cause of 34% of nosocomial infections (72); by 1975, the frequency of nosocomial *S. aureus* infections had dropped to 10.4% (Center for Disease Control: National Nosocomial Infections Study Report, Annual Summary 1975, Issued October 1977). Gram negative rods, particularly *Escherichia coli* and members of the *Klebsiella-Enterobacter* group and *Pseudomonas* species are now the pathogens in more than 70% of nosocomial infections. A 1970 survey of community hospitals showed that 7% of 90,000 patients developed nosocomial infections and pseudomonads accounted for 10% of these hospital-acquired infections (26).

The steady increase in the isolation of gram negative rods, especially the Pseudomonas sp., as opportunistic pathogens appears to correlate with a general increase in the numbers of debilitated or immunologically compromised patients found in hospitals today. These pseudomonads, which are generally low grade in virulence, have a predilection to cause potentially serious disease in patients with burns, cancer (especially acute leukemia), cystic fibrosis, narcotic addiction and immune deficiency syndromes. A number of unusual Pseudomonas sp. have been shown to be a significant cause of infection in patients receiving prolonged courses of antibiotics, immunosuppressive drugs, steroids, cytotoxic agents or inhalation therapy (26). These organisms can also be a threat to patients undergoing respiratory or urinary instrumentation and to recipients of various transplants and prostheses (23). Newborns as well as the elderly are likely candidates to develop infections with these opportunistic pathogens.

Among the unusual saprophytic Pseudomonas sp. which are being isolated in hospitals as potential nosocomial pathogens, one of the most commonly isolated is Pseudomonas cepacia. P. cepacia was originally described as a plant pathogen causing onion bulb rot (8). In 1953 (68), and then again in 1961 (65), this organism was isolated from cases of septicemias and fatal bacterial endocarditis after cardiac

surgery, intravenous therapy, and genitourinary tract manipulations. One problem in evaluating the role of P. cepacia as a pathogen is the fact that the virulence of strains of the organism may be quite variable. Some strains of P. cepacia appear to act as primary invaders, since the organism has been isolated as the etiological agent in a subacute necrotizing granulomatous lung lesion resembling melioidosis in a previously healthy young male (17). P. cepacia strains have also been isolated as the pathogen in foot lesions from healthy soldiers training or working in the swamplands of Florida and the Mekong Delta in Vietnam (69). In general, however, the organism seems to have low invasiveness and to require a portal of entry such as intravenous cannula or bladder catheter to cause deep infections (23).

Between 1960 and 1970, P. cepacia was recovered from septicemias (32,46,55,76), pneumonitis (17,75), wound infections (3,32), contaminated detergent solutions in urinary catheter kits (35,48), renal calculi (62), urinary tract infections (32,35,48), and from soil and water (2). In some patients, infections caused by this organism resolved without any treatment. However, in debilitated patients, or in patients with compromised defense mechanisms, the infections could only be eradicated with the use of proper antibiotics (75).

Recently, there has been a marked increase in nosocomial infections caused by *P. cepacia*. Ultrasonic inhalation therapy nebulizers, which have a water reservoir, have occasionally been found to be colonized with *P. cepacia* (28). The optimum growth rates and maximum population yields for *P. cepacia* in distilled water occur at 37°C but high population levels of 10^6-10^7 organisms/ml are maintained at temperatures of 18-42°C (11). It is not surprising, then, that contaminated distilled water was found to be the source of infection in several studies (9,61,64). Contamination of inhalation therapy equipment has led to a lung abscess in a diabetic patient (59), and to pneumonia following cardiac surgery (75). *P. cepacia* has been recovered from a bacteremia occurring in a hydrocephalic child after insertion of a Holter valve (4), from a meningitis following a meningomyelocele repair (18), and from a case of septic arthritis following injection of contaminated methylprednisolone into the ankle (42).

There has also been an increased incidence of isolation of *P. cepacia* as the causative agent of endocarditis in heroin addicts (16,50,51). The broad resistance of *P. cepacia* to antibiotics, particularly to bactericidal agents, hinders the achievement of sufficient levels of antibiotic in the blood to eradicate organisms implanted within vegetations, either natural heart valves or on prosthetic valves (60). Skin lesions suggestive of ecthyma

gangrenosum, which is usually associated with infection with P. aeruginosa, have been reported in two of these heroin addicts with P. cepacia endocarditis (44,51). It was suggested that P. cepacia may also be able to produce bacterial toxins which would cause the cutaneous vasculitis (44). However, toxin production by P. cepacia has not been extensively studied, to date.

P. cepacia strains are able to destroy preservative additives, such as methyl propyl-*p*-hydroxy benzoates, added to cosmetics (15), thus making colonized cosmetics a potential danger to debilitated people. Photoreactivation of P. cepacia strains after ultraviolet irradiation used to sterilize industrial and potable water supplies has also been reported as a potential source of contamination if such ultraviolet-treated water is used in the hospital environment (12)

B. Taxonomy

P. cepacia is an aerobic unicellular gram negative bacillus which has been assigned to the pseudomallei group in the family Pseudomonadaceae (33). Major nutritional characteristics which are found useful in distinguishing P. cepacia from other members of the Pseudomonadaceae are: utilization of a wide variety of monosaccharide and disaccharide sugars, variable oxidase activity, accumulation of poly-β-hydroxybutyrate, production of lysine decarboxylase and the absence of arginine dihydrolase.

In the past, there has been a great deal of controversy regarding the taxonomic position of P. cepacia and its relationship to other organisms, including P. multivorans, EO-1 King group and P. kingii. On the basis of nutritional studies of the phytopathogenic pseudomonads, Ballard et al. (2) concluded that P. cepacia is the same as P. multivorans. The pseudomonad EO-1 of King (40), designated P. kingii by Jonsson (39), was shown by Gilardi (30) and Pickett (57) to be indistinguishable from P. cepacia. Weaver et al. (63) also studied the fatty acid compositions of P. cepacia (P. multivorans) and P. kingii and found them to be identical.

When the rRNA/DNA homology groupings of the Pseudomonas sp. are examined (54), P. cepacia falls into a single group which includes the following organisms: P. cepacia, P. marginata, P. caryophylii, P. pseudomallei, P. mallei, P. pickettii, P. solonacearum. All the members of this pseudomallei-cepacia rRNA/DNA homology group, with the exception of P. pickettii are pathogens for animals or plants. The P. cepacia "species" vary a great deal in their phenotypic properties and in DNA homology. This indicates that a further subdivision of the species may be warranted.

C. Isolation and Identification

1. Biochemical characteristics

P. cepacia shares a few phenotypic characters with the other members of the pseudomallei-cepacia DNA homology group, including: accumulation of poly-β-hydroxybutyrate as carbon reserve material, utilization of arginine and betaine as sole carbon sources and ability to grow at 40°C in complex media (20). Some of the P. cepacia strains are the most nutritionally versatile in the genus Pseudomonas. P. cepacia along with the fluorescent pseudomonads, use the β-keto-adipate pathway for the degradation of various aromatic compounds such as mandelate, benzoate, *p*-hydroxybenzoate and tryptophan. However, the enzymes of P. cepacia and the fluorescent group in the pathway are immunologically unrelated (54).

P. cepacia strains are motile by means of polar multitrichous flagellation (one to three flagella). Many strains produce a conspicuous sulfur-yellow, water-soluble, nonfluorescent pigment in the colonies and surrounding medium. Some strains can also produce green, brown, red, violet or purple phenazine pigments, depending on the carbon source used for growth (20,37).

P. cepacia strains do not denitrify but they can produce nitrites from nitrate. Most strains are lipolytic and some can hydrolyze gelatin. Most P. cepacia strains can

use from 95 to more than 105 different organic compounds as sole carbon sources for growth. The optimal temperature for growth is about 30-35°C and they will not grow at 4°C (20).

2. Antibiotic susceptibility patterns

Gilardi (31) proposed that antimicrobial susceptibility can be used as a diagnostic aid in identification of nonfermenting gram negative bacteria. Gilardi and other workers (23,49,52,57) have consistently reported *P. cepacia* to be a highly antibiotic resistant organism. *P. cepacia* strains have been found to be resistant to colistin, gentamicin, tobramycin, and carbenicillin, which are commonly used drugs-of-choice against *Pseudomonas aeruginosa* infections. Most strains of *P. cepacia* have been found to be sensitive to chloramphenicol and trimethoprim-sulfamethoxazole with a lesser percentage also sensitive to nalidixic acid and/or kanamycin.

3. Purpose of this study

a. Biochemical analysis

The media and test conditions used in the identification and differentiation of nonfermentative gram negative bacteria are diverse. Most clinical laboratories presently use one of three general schemata for identification of the nonfermentative bacilli. These are based on modifications described by Weaver et al. (74), Hugh and Gilardi (37), and Pickett and Pedersen (57,58). The first two use basically similar media and methods as originally described by Eliza-

beth King (40) and the third of these (Pickett) uses a buffered substrate method. All of these methods are time-consuming, vulnerable to much error during laboratory manipulations and difficult to standardize. In an effort to find a more rapid method to identify *P.cepacia* strains, the API 20 Enteric Strip, made by Analytab Products, Inc., was analyzed for its ability to correctly identify diverse strains of *P. cepacia* and to differentiate this organism from biochemically similar nonfermentative organisms. Biochemical reactions of 38 strains of *P. cepacia* tested with the API 20E micromethod were compared with results obtained by testing the same strains with conventional media as used by King, Weaver and Gilardi.

b. Antibiotic susceptibility testing

Most of the studies on antibiotic susceptibility patterns of gram negative nonfermentative rods (33,34,52), have used the Kirby-Bauer (5) antimicrobial testing method. Therefore, only resistance or sensitivity to the battery of antimicrobials tested are reported. Only a few workers (60) have reported minimum inhibitory concentrations (MIC's) for *P. cepacia* strains. Since *P. cepacia* strains show variable antibiograms (49) and are generally found to be resistant to most antibiotics by the Kirby-Bauer technique, it was thought of importance to determine the MIC levels that these strains of *P. cepacia* exhibit. There-

fore, antibiograms obtained for the 38 strains using the Kirby-Bauer disk diffusion method were compared with those derived from MIC values obtained using a micromethod developed by Micro-Media Systems, Inc. (1435 Koll Circle, #106, San Jose, Ca. 95112).

c. Serological studies

Serological methods have proven useful for the identification of many species of the Enterobacteriaceae (25) as well as other bacteria (73). They provide an invaluable tool along with antibiograms, bacteriophage typing and pyocin typing in epidemiological investigations. Recently, for example, commercial antisera for *P. aeruginosa* strains (26) have become available and this has proven to be an aid in epidemiological and clinical studies.

Very little work has been done with respect to the antigenic characteristics of *P. cepacia*. Therefore, a serological study of this organism seemed to be a logical and necessary step. In this study, formalin-killed antigens were used to immunize rabbits and prepare antisera against 29 *P. cepacia* strains. The antisera were then used in agglutination studies to determine presence of cross-reacting antigens among the various strains.

CHAPTER II

MATERIALS AND METHODS

A. Organisms

Thirty-six strains of P. cepacia used for study in this work were all clinical isolates obtained from various workers in this field. The following strains were received from Dr.R.E.Weaver, the Center for Disease Control in Atlanta, Georgia: B4134, B4648, B5910, B5912, D6566, D6460. Dr. G.L.Gilardi from New York sent the following strains: 2332, 2416, 2160, 2106, 2078, 2075, 2076, 2077, 2331, 2369, 2368, 2371. Strains B-1 and B-2 were received from Dr. E.J.Bottone in New York. Dr. D.Taplin from Florida sent us three strains: CJ62, FB96 and FB143. Nine strains were received from Dr. M.J.Pickett at UCLA in California: K1036, K1021, K981, K998, K1075, K808, K358, K359, K360. Two strains were also received from the API Reference Laboratory in New York: 75-3351 and 75-2013.

The following strains were obtained from the American Type Culture Collection (ATCC) to be used as reference strains: P. aeruginosa #10145, P. cepacia #10856, P. cepacia #25416, P. maltophilia #13637, P. fluorescens #13525, Flavobacterium meningosepticum #13253, Acinetobacter calcoaceticus #23055, Xanthomonas campestris #6402,

P. stutzeri #17588, and Achromobacter xyloseoxidans #27061.

Two strains, LU2800 and LU3468, were obtained from clinical specimens from the Clinical Microbiology Laboratory of Foster G. McGaw Hospital of Loyola University of Chicago.

B. Isolation and Maintenance

All of the strains used in this study were isolated on BBL Blood agar plates (Trypticase Soy Agar with 5% defibrinated Sheep Blood) and were incubated at 35°C in incubators flushed with 100% CO_2 (Ohio Medical Products) regulated to maintain a constant 5% CO_2 atmosphere. Although increased carbon dioxide tension was not an absolute requirement for the growth of these organisms, it did enable us to obtain larger yields for subsequent biochemical studies. The organisms were subcultured to fresh Blood agar plates everyday for short-term maintenance of their viability.

For long-term maintenance, all of the organisms used in this study were quick-frozen in defibrinated sheep blood utilizing a modified CDC Quick-freeze technique (41). Disposable glass tubes (16X100 mm) with plastic caps were autoclaved and then appropriately labeled. One ml of defibrinated sheep blood was sterilely added to each tube. A Dacron swab (Scientific Products) was used to remove as much growth as possible from a 24-hour culture of the or-

ganism on a Blood agar plate and a heavy suspension of the organism was made in each tube. Prior to freezing, each tube was checked for contamination by subculturing onto a Blood agar plate which was incubated overnight at 35°C in 5% CO_2. Each tube was then quick-frozen in a freezing bath consisting of a metal container filled with acetone (100 ml) to which a small piece of dry ice had been added. The frozen tubes were then placed in a freezer and maintained at -55°C.

C. Biochemical Tests According to Conventional Methods as Used by King, Weaver, Gilardi, Pickett and Others.

Difco OF basal medium (prepared according to manufacturer's instructions) with 10% added dextrose, sucrose, maltose, lactose, xylose, mannitol or rhamnose was used to test for carbohydrate utilization (27,36). OF basal medium without carbohydrate was used as the control with each set of tests. The transfer broth used to inoculate the carbohydrates was 2.5 ml of Trypticase soy broth (Difco TSB, prepared according to manufacturer's instructions). A Dacron swab was used to make a heavy (milky) suspension of a 24-hour old culture of the organism taken from Blood agar plates. The oxidation tubes were inoculated with approximately 0.3 ml of the bacterial suspension by stabbing with a pasteur pipet and releasing the suspension slowly while withdrawing the pipet from the medium.

The reactions of the OF carbohydrates were read after 48 to 72 hours of incubation at 35°C. A change in the color of the bromothymol blue indicator in the semi-solid OF media from green to yellow at the surface of the medium denoted acidity (A) due to oxidation of the carbohydrate. A change in color of the media from green to blue (K) denoted alkalinization due to deamination of the peptones in the basal medium.

The oxidase test was performed by rubbing a colony from a Blood agar plate onto a filter paper which had been premoistened with a drop of Kovac's reagent-1% tetramethyl-ρ-phenylenediamine dihydrochloride (Eastman) and then observing for development of a purple color within 10 seconds (70).

Catalase production was tested for by placing a drop of 30% H_2O_2 on a glass slide, adding a loopful of the organism, and checking for the production of bubbles (53).

Growth on MacConkey agar was detected by inoculating MacConkey agar slants (Difco, prepared by manufacturer's instructions) and MacConkey agar plates (BBL) with a six-hour TSB culture of the organism and incubating at 35°C (33). The slants and the plates were checked for growth at 24 and 48 hours.

ONPG disks (Difco) were used according to manufacturer's instructions to test for the presence of β-D-galactosidase.

Six-hour TSB cultures were used to inoculate Triple Sugar Iron (TSI), urea, citrate and tryptone media (Difco, prepared according to manufacturer's instruction). TSI agar slants were used to determine the ability of an organism to attack lactose, sucrose and glucose with or without the production of gas, along with the determination of possible hydrogen sulfide (H_2S) production from sodium thiosulfate (25). Citrate agar slants were inoculated to determine a certain bacteria's ability to utilize citrate as its sole source of carbon (66). Urease agar is used to determine an organism's ability to produce the enzyme urease, which splits urea, forming two molecules of ammonia (14). TSI, urea and citrate were checked at 24 and 48 hours. Xylene and Erlich's reagent (53) were added to the tryptone broth after 48 hours to test for indole production (70).

Motility was determined in Difco's Motility Test Medium, prepared according to manufacturer's instructions. Growth was taken from a 6-hour TSB culture with an inoculating needle, the media was stabbed half way down and incubated at 25°C. Motility (indicated by diffuse growth emanating from the stab line in the semi-solid medium) was checked for at 24 and 48 hours (25).

Esculin hydrolysis was detected by inoculating bile esculin slants (Difco, prepared according to manufacturer's instructions) with a heavy TSB suspension of the organism. Organisms able to hydrolyze esculin turned the bile escu-

lin slant black in 24 to 48 hours (33).

Gelatin liquefaction was detected with gelatin strips (Key Scientific) following manufacturer's instructions. A heavy suspension of the organism was made in 1 ml of distilled water in a 13 X 100 mm tube. A gelatin strip was added and the tube was incubated at 35°C for 24 to 48 hours. Liquefaction was shown by separation of the gelatin from the strip resulting in the appearance of the blue supporting base.

Nitrate broth (Pfizer) was used to detect the ability to reduce nitrate to nitrite. To a 48-hour nitrate broth culture of the organism one drop of 6M HCl was added followed by 12 drops of nitrate reagent (Key Scientific). The development of a pink color was a positive test. A small amount of zinc dust was added to all negative tubes to check for denitrification of nitrate to nitrogen gas. If still no color developed nitrate was denitrified to nitrogen gas or other products (37).

F/N (Fluorescence-denitrification agar) slants (Key Scientific) were used to detect the ability to reduce nitrate and/or nitrite to N_2 gas (56). The media was used according to manufacturer's instructions. The slants were inoculated with 0.3 ml of transfer broth and the butts were stabbed with an inoculating needle. Gas bubbles in the butt of the F/N slant showed the ability of the organism to break nitrate and/or nitrite down to nitrogen gas. F/N

slants were also used to detect the presence of autofluorescence. After 48 hours of incubation at 35°C all tubes were placed under a UVSL·25 Mineralight in a dark room and checked against a positive (P. fluorescens) and a negative (P. maltophilia) control.

Gluconate oxidation was detected by adding a loopful of growth of the organism into a 13 X 100 mm tube containing 1.5 ml of sterile water and a gluconate substrate tablet (Key Scientific) and incubating at 35°C for 48 hours. Following manufacturer's instructions for performance of this assay a Clinitest (Ames) tablet was added to detect the presence of reducing sugars. A negative test was development of a blue color and a positive test ranged from green to dark orange.

Hemolytic activity of the organisms was detected on sheep Blood agar plates incubated at 35°C under 5% CO_2 (33).

Growth on Salmonella-Shigella (SS) agar was detected by streaking the organism on Pfizer SS agar and observing for growth after a 24-48 hour incubation at 35°C (27).

The production of phenylalanine deaminase was detected by inoculating Difco phenylalanine slants (prepared according to manufacturer's directions) with a few drops of a 6-hour TSB culture and incubating at 35°C. After 24 hours the slant was flooded with 10% (w/v) ferric chloride

solution and observed for the development of a green color (24).

Accumulation of poly-β-hydroxybutyrate (PBHB) was determined by staining organisms taken from 24-hour old cultures grown on Blood agar plates with Sudan Black (7,27). The stain was prepared by dissolving 0.3 g of Sudan Black B (Harleco) in 100 ml of 70% ethanol and allowing the solution to stand overnight before use. A thin smear of the organism was made and heat fixed. The slide was flooded with Sudan Black, left for ten minutes, drained and blotted dry. The smear was washed and cleared with xylol and counterstained with 0.5% aqueous solution of safranine (Difco) for 10 to 15 seconds. The highly refractile poly-β-hydroxybutyrate granules in the cells appeared blue-black, whereas the rest of the cell stained pink, when examined under oil immersion with a 100 X objective.

Deoxyribonuclease (DNAse) activity was detected by making heavy streaks of the organisms on DNAse Test agar (Difco) incorporated with 0.01% (w/v) of toluidine blue. After 24-48 hours of incubation at 35°C a change in the indicator to pink around the growth was observed (33).

Lecithinase production was tested by observing for a white precipitate around 48-hour growth on a TSA plate

supplemented with 10% (w/v) of Egg Yolk Enrichment (BBL) (33).

Lipase activity was tested for by observing for an irridescent sheen around 48-hour growth on the egg yolk enriched TSA plates (33).

Casein hydrolysis was determined by observing for a clear zone around 48-hour growth of the organism on TSA plus 10% (v/v) of Skim Milk Powder (BBL) (33).

Growth at 42°C was determined by inoculating pre-heated Brain Heart Infusion Agar (BHIA, Difco) slants and Trypticase Soy Broth with one colony of the organism and incubating in a 42 ± 0.05°C incubator for 24 hours (33).

Yellow pigment production by the organisms was observed on a variety of media including TSI slants, Blood agar plates and Mueller-Hinton plates which were used for determining antibiotic susceptibilities.

Lysine decarboxylase and ornithine decarboxylase production were detected by adding 1-2 drops of a heavy suspensionin the organism in saline to a 13 X 100 mm tube (one for each test) containing 1 ml of distilled water and a lysine decarboxylase test tablet (Key Scientific) or an ornithine decarboxylase test tablet (Key Scientific), respectively, (as directed by manufacturer's instructions). After 24 hours of incubation at 35°C 2 drops of 40% KOH followed by 1 ml of ninhydrine (Key Scientific) in chloro-

form were added to each tube. Development of a violet color in the lysine decarboxylase test and a rose-red color in the ornithine decarboxylase test in the lower chloroform layer in 10 to 15 minutes was considered a positive test.

D. Biochemical Tests Used in the API 20E Method

The API 20 Enteric Strips used in this study were obtained from Analytab Products, Inc., Plainview, New York. The API 20E System is a standardized, miniaturized version of conventional procedures for the identification of *Enterobacteriaceae* and other gram negative organisms. The system is a strip of plasticized paper consisting of 20 microtubes containing dehydrated media. The media are reconstituted by adding a bacterial suspension and then the strips are incubated so that the organisms could react with the contents of the tubes. Twenty tests are available on the strip: arginine dihydrolase, citrate, H_2S, urease, tryptophan deaminase, indole, Voges-Proskauer, glucose, mannitol, inositol, sorbitol, rhamnose, melibiose, ONPG, lysine decarboxylase, arabinose, amygdalin, ornithine decarboxylase, gelatinase and sucrose. Reactions of seven additional biochemical tests are used to help in the identification of nonfermentative rods with this system. The reduction of nitrate to nitrite or complete denitrification to nitrogen gas is tested for on the test strip in

the glucose microtubule which has potassium nitrate added to it. Conventional nitrate reagents are used with the tests. Motility of the organisms is tested according to conventional means using 0.3% semisolid agar or the hanging drop method. An organism's ability to grow on MacConkey agar produce cytochrome oxidase and oxidize or ferment OF dextrose are the four other reactions considered in the identification. These four tests are performed according to the conventional methods.

In this study the API 20E strips were inoculated and read as described in the manufacturer's instructions except that the inoculum used was prepared as follows: two 24-hour old colonies on Blood agar plates were touched with the tip of a sterile wooden applicator stick, the stick was inserted into a tube with 5 ml of sterile saline, pH 5.5-7.0 and then the stick was rotated in the saline to release and disperse the organisms. The microtubes were then filled with the inoculum (approximately 10^6 bacteria/ml) and incubated for 48 hours at 35°C in an incubator under atmospheric conditions. The same lot of API 20E strips was used throughout this study.

The computerized API Analytical Profile Index, which lists the biochemical patterns associated with each _Enterobacteriaceae_ species and other gram negative organisms,

is used to compare derived biochemical test results with and arrive at a genus and species identification of the test organism. This computerized index is continuously updated by API to include more and more possible biochemical patterns for gram negative bacilli as they discover new strains.

E. Antibiotic Susceptibility Testing

1. Kirby-Bauer disk diffusion method

Antibiotic susceptibility testing on all thirty-eight strains of *P. cepacia* was performed using the standardized Kirby-Bauer, single disk diffusion method (36). A few colonies (three to eight) of the organisms to be tested were transferred with a wire loop from a Blood agar plate to a test tube containing 2.5 ml of Trypticase soy broth. A 2-5 hour TSB culture of the organism was then diluted with saline to the density equivalent to that of a 0.5 McFarland standard, approximately 1.5 X 10^8 bacteria/ml (53). A sterile Dacron swab was then immersed into the broth culture, rolled against the side of the tube to remove excess liquid, and then used to swab a 150 mm Mueller-Hinton (BBL) plate (5-6 mm in depth) in three planes. Care was taken to cover the entire plate evenly. The following antibiotics were dropped onto the seeded Mueller-Hinton plates for each organism: one dispenser included (a) ampicillin (10 mcg, Pfizer), (b) cephalothin

(30 mcg, Pfizer), (c) chloramphenicol (30 mcg, Pfizer), (d) tetracycline (30 mcg, Pfizer), (e) gentamicin (10 mcg, Pfizer), (f) kanamycin (30 mcg, Pfizer), (g) carbenicillin (100 mcg, Pfizer), (h) colistin (10 mcg, Pfizer), (i) tobramycin (10 mcg, Pfizer); another dispenser included (a) nitrofurantoin (300 mcg, Pfizer), (b) trimethoprim (1.25 mcg) and sulfamethoxazole (23.75 mcg, Pfizer), (c) nalidixic acid (30 mcg, Pfizer), (d) vancomycin (30 mcg, Pfizer), (e) oxacillin (1 mcg, Pfizer), (f) erythromycin (15 mcg, Pfizer), (g) penicillin G (10 units, Pfizer), (h) clindamycin (2 mcg, Pfizer), (i) amikacin (10 mcg, Pfizer), and (j) ticarcillin (75 mcg, BBL). The plates were incubated overnight at 35°C without CO_2. Sensitivity or resistance to each antibiotic was determined by measuring the size of the zone of inhibition around each disk. These zone sizes were compared to the standardized required zone sizes for sensitivity to each antibiotic as determined by Kirby and Bauer (5). Standard control organisms of known susceptibility (Stock cultures of Staphylococcus aureus ATCC #25923, Escherichia coli ATCC #25922 and Pseudomonas aeruginosa ATCC #27853 were tested with each batch of sensitivities as a check on the activity of the disks and on the reproducibility of the tests.

2. Micro-Media microtiter antibiotic dilution susceptibility testing method

Minimum inhibitory concentrations of nine antibiotics against the 38 strains of *P. cepacia* were determined using microdilution MIC test panels prepared by Micro-Media Systems, Inc. The gram negative test panel used in these studies consists of a microtiter "V" well plate containing antimicrobic drug solutions serially diluted in Mueller-Hinton broth to concentrations bridging the range of clinical interest. Panels of seven dilutions of each antimicrobic are provided in sequential wells of each plate, along with sterility and growth controls. The panels are frozen after preparation and are kept frozen at -20°C or lower until ready to be used. One panel is used for each test organism. The antibiotics included in the gram negative panel are the following:

Antibiotic	mcg/ml Range
Ampicillin	16-0.25
Cephalothin	64-1.00
Gentamicin	16-0.25
Tetracycline	16-0.25
Carbenicillin	512-8.00
Chloramphenicol	32-0.50
Kanamycin	64-1.00
Tobramycin	16-0.25
Trimethoprim-Sulfa	32/608-0.50/9.50
Nitrofurantoin	64
Colistin	4

The MIC panels were inoculated and read as described in the manufacturer's instructions. The "turbidity standard technique" (47), whereby the bacterial suspension is brought

to the equivalent turbidity of a 0.5 McFarland standard before dilution to the desired concentration was used to prepare the inoculum. Three to five isolated colonies from a Blood agar plate were inoculated into 2.5 ml of TSB and incubated at 35°C for 2-4 hours in order that the bacterial suspension could reach log-phase. This suspension was then diluted to an equivalent turbidity of the 0.5 McFarland standard. Five-tenth ml of the standardized suspension was pipetted into a tube containing 25 ml of sterile distilled water supplemented with 0.02% Tween 80. This 1:50 dilution takes the bacterial suspension to approximately 2 X 10^6CFU/ml. Five microliters of this inoculum was then transferred to each well in the microtiter plate using a special seeding plate thus making a further 1:20 dilution resulting in a final inoculum of 10^5 CFU/ml in each well. The plates were incubated for 15-18 hours at 35°C in an incubator under atmospheric conditions. The lowest concentration (mcg/ml) of each antibiotic which demonstrated no visible growth was recorded as the minimum inhibitory concentration for the antibiotic against each test organism. The single wells for nitrofurantoin (64 mcg/ml) and colistin (4.0 mcg/ml) were recorded for inhibition or resistance by each organism to these two antibiotics.

The sterility and growth controls were checked before any test wells were even examined. Potency of the antimicrobic dilutions and reproducibility of the technique

used were checked with each batch of MIC's run by testing control organisms with know MIC values. E. coli ATCC #25922 and P. aeruginosa ATCC #27853 were run with each batch of gram negative panels. Culture purity was checked by subculturing the final diluted inoculum (2 X 10^6 CFU/ml) onto a Blood agar plate.

F. Preparation of Antigens for Immunization

Each P. cepacia strain was inoculated onto Brain heart infusion agar plates (BHIA) and incubated at 35 C for 48 hours. The bacteria were washed off the BHIA plates with 10 ml of 0.5% Formalin and incubated at 35°C for 72 hours. The suspensions were utilized if plating a loopful of the bacteria-formalin mixture onto another BHIA plate and incubating at 35°C for 48 hours resulted in no growth.

Bacteria were washed five times with saline containing 0.025% Formalin and 0.01% sodium azide. The bacterial suspensions were adjusted in sterile saline to a density equivalent to that of a no.7 McFarland standard (approximately 2.1 X 10^9 bacteria/ml) (53) and used for inoculations.

G. Immunization Procedure

New Zealand white rabbits weighing 2-2.5 kg were inoculated according to the following schedule: day 0, the rabbits were bled from the marginal ear vein for normal serum; day 1, 0.25 ml of the cell suspension incorporated in 0.25 ml of Freund's complete adjuvant (Difco) was injected intradermally

and subcutaneously into several sites of the footpads and back; day 8, 0.1 ml of the cell suspension incorporated in 0.1 ml of Freund's complete adjuvant was injected intramuscularly into each thigh; and day 15, 1.0 ml of the cell suspension was injected intravenously into the marginal ear vein. The rabbits were bled on day 22 and every 2 to 3 days thereafter for one week. The whole blood collected from the rabbits was allowed to stand at 25°C for approximately one hour, then overnight at 4°C. The clots were centrifuged at 2500 rpm several times to remove all blood cells from the sera. The sera collected each day from a rabbit were pooled and then quick-frozen in 1.0 ml aliquots and stored in a freezer at -55°C.

A total of 30 rabbits were immunized. The antisera were designated according to the antigen used for the immunization of a rabbit. (eg. Anti-25416 was prepared by immunization of a rabbit with P. cepacia strain 25416.)

Each antiserum was tested against its respective antigen for the presence of antibody by slide agglutination tests using a saline suspension of each organism from a BHIA plate. 75-100% (3-4+) agglutination was obtained for each antisera. The following gradations were used in scoring agglutination reactions: 1 + (<25%) - questionable clumping, 2 + (50%) - definite clumping, supernate cloudy, 3 + (75%) - definite clumping, supernate clearing, and 4 + (100%) - completely

precipitated, supernate clear (27). A saline control was run with each organism to detect self-agglutinating strains. None of the P. cepacia strains were found to be self-agglutinating.

H. Preparation of Antigens for Tube Agglutination Studies

Each P. cepacia strain was inoculated onto six Brain heart infusion agar plates and incubated at 35°C for 48 hours. The bacteria were then washed off the BHIA plates with sterile saline. This bacterial suspension was then washed twice with saline. After the last centrifugation, the supernatant was decanted and the packed cells were resuspended in 10 ml of sterile saline and boiled for one hour. The heat-killed cells were washed again with sterile saline and finally resuspended in fresh saline to a density of a no. 3 McFarland standard, (approximately 9 X 10^8 bacteria/ml) (53). This type of suspension of P. cepacia was then used in the tube agglutination studies.

I. Tube Agglutination Studies

All twenty-nine antisera prepared against the P. cepacia strains and one antiserum prepared against P. fluorescens #13525 were tested against each of the thirty-eight strains of P. cepacia and the P. fluorescens strain by doing tube agglutinations. Antiserum was prepared against the P. fluorescens reference strain to see if there would be any cross-reactivity between P. fluorescens and any of

the P. cepacia strains since this organism is often confused biochemically with P. cepacia.

Serial dilutions of each antiserum tested were made in the following way: 0.9 ml of saline was pipetted into the first of ten 13 X 100 mm test tubes; the following 9 tubes were filled with 0.5 ml of saline; 0.1 ml of the antiserum was placed into the first tube and mixed thoroughly; 0.5 ml of the 1:10 dilution in the first tube was transferred to the second tube and so forth; thus a two-fold serial dilution was carried out to a final dilution of 1:5120 in the 10th tube. An additional tube with 0.5 ml of saline was set up for a saline control. Normal serum from each rabbit used for the immunizations was also diluted out 1:10 and was used as a serum control.

Each organism that was used as an antigen was heat-killed and a proper suspension prepared as described above. Five-tenth ml of the organism was then added to each serially diluted antiserum saline control and normal serum control tubes. The final dilutions in the tubes ranged from 1:20 in the first to 1:10,240 in the tenth tube. All the tubes were then incubated for 18 hours at 35°C and examined for macroagglutination (10). The endpoint titer was the highest dilution giving a 2 + or greater agglutination reaction.

Each of the described assays, whether biochemical, antibiotic susceptibility or serological, was performed at least twice for each of the 38 strains of *P. cepacia* studied as well as for all of the control organisms. If a discrepancy in results occurred, the assay was repeated at least one more time and the result occurring most often was recorded.

CHAPTER III

RESULTS

A. Biochemical Characteristics of the P. cepacia Strains

The thirty-eight strains of *P. cepacia* tested showed a high degree of similarity in their biochemical and cultural characteristics based on 37 different tests using the conventional methods of King and others (37,58,74). Tables 1 and 2 summarize the results obtained when the two ATCC *P. cepacia* reference strains and the thirty-six clinical isolates of *P. cepacia* were tested. All 38 strains of *P. cepacia* gave the following results: alkaline slant and butt in TSI with no H_2S or gas production; oxidation of dextrose, maltose, lactose, xylose, but not rhamnose in OF media; positive motility; growth on MacConkey slants but no growth on SS agar; no denitrification of nitrate or nitrite to nitrogen gas; production of lipase but not DNAse, phenylalanine deaminase, indole or fluorescence; and utilization of citrate as a sole carbon source. Ninety-seven percent (37/38) of the strains produced lysine decarboxylase and showed no hemolysis on sheep Blood agar plates (Table 2). One strain, K981, showed β-hemolysis on sheep Blood agar. Using the Sudan Black B stain, 95% (36/38) of the strains were found to accumulate PBHB inside their cells. Ninety-two percent (35/38) of the strains oxidized

Table 1.
Biochemical Properties of 38 Strains of _P. cepacia_ As Determined With Conventional Methods of Identification [a,b,c]

STRAINS TESTED	HEMO	LDC	PBHB	LECI	OF MANN	ONPG	ESCULIN	OXI	CAT	MAC PLATE	CASEIN	OF SUC	GEL	URE	GLCN	PIGMENT	42°C	NO_3+NO2	ODC
2075	-	+	+	-	A	+	+	+	+	+	+	A	+	+	-	+	-	-	+
2076																			
2077																			
B4134	-	+	+	-	A	+	+	+	+	-									
2078																			
2416	-	+	+	-	A	+	-												
B4648	-	+	+	-	A	+	+	+	+	-	+	A	+	-					
K1021	-	+	+	-	K	-	+	+	+	+	+	K	+	+	+	-			
D6460	-	+	-																
ATCC10856	-	+	+	-	A	+	+	+	+	+	+	A	+	+	-	+	+		
B-2	-	+	+	-	A	+	+	+	+	-									
K808	-	+	+	-	A	+	+	+	-										
B5910	-	+	+	-	A	+	+	+	+	+	+	A	+	+	-	+	-	+	
2332	-	+	+	-	A	+	+	+	-	+	+	A	+	+	-	-			
B6566	-	+	+	-	A	+	+	+	+	-									
B5912																			
2368	-	+	+	-	A	+	+	+	+	+	+	A	+	+	-	+	+		
2369																			
2331	-	+	+	.	A	+	+	+	-										
75-3351	-	+	+	-	A	+	+	+	+	-									
K358	-	+	+	-	A	+	+	+	+	+	+	A	-	+	-	-			
K359																			
K360	-	+	+	-	A	+	+	+	+	+	+	A	-	-					
75-2013	-	+	+	-	A	-	+	+	+	-	+	A	+	+	-	+	-	-	-
LU3468	-	+	+	-	A	+	+	+	+	+	-								
K1075	-	+	+	-	K	+	-	+	+	-	+	A	-						
ATCC25416	-	+	+	-	A	+	+	+	+	+	+	A	+	+	+				
2160	-	+	+	-	A	+	+	+	+	+	+	A	+	-					
CJ62	-	+	+	-	A	+	+	-	+	-									
FB96																			
FB143																			
K981	+	+	+	-	A	+	+	+	-										
2106	-	+	+	-	A	+	+	+	+	+	-	A	+	+	-	+	+		
K998	-	+	+	-	A	+	+	+	+	-	+	A	-						
K1036	-	+	+	-	A	+	+	+	+	-	+	A	+	+	+				
LU2800	-	+	+	-	A	+	+	+	+	+	+	A	+	+	-	-	+	+	
2371	-	-	+	+	A	+	+	+	+	+	-	K							
B-1	-	+	-	+	A	+	-	+	+	+	-	K	-						

[a] All 38 strains of _P. cepacia_ gave the following reaction for TSI: KK— (slant/butt/gas/H_2S)

[b] All 38 strains showed a positive reaction for the following 8 tests: CIT, MOT, LIP, MAC SLANT, OF DEX, OF MALT, OF LAC, OF XYL.

[c] All 38 strains were negative for the following 8 tests: OF CON, OF RHAM, IND, PHENYL, SS, DNAse, FLUOR, $NO_3 \rightarrow N_2$ and $NO_2 \rightarrow N_2$.

Table 2.

Summary of Biochemical Properties of 38 Strains of

P. cepacia

As Determined With Conventional Methods of Identification

Substrate or character	# of Strains (+)	% of Strains (+)
TSI	38[a]	100
Citrate	38	100
Motility	38	100
Lipase	38	100
MacConkey agar slant	38	100
OF Dextrose	38	100
OF Maltose	38	100
OF Lactose	38	100
OF Xylose	38	100
OF Control	0	0
OF Rhamnose	0	0
Indole	0	0
Phenylalanine deaminase	0	0
SS agar	0	0
DNAse	0	0
Auto-fluorescence (F/N)	0	0
$NO_3 \rightarrow N_2$ gas	0	0
$NO_2 \rightarrow N_2$ gas	0	0
Lysine decarboxylase	37	97
PBHB	36	95
OF Mannitol	35	92
ONPG	35	92
Esculin hydrolysis	35	92
Oxidase	35	92
Catalase	34	89
Casein hydrolysis	34	89
OF Sucrose	34	89
Gelatinase	32	84
Urease	31	82
Yellow pigment	27	71
Ornithine decarboxylase	23	61
MacConkey agar plate	21	55
Growth at 42°C	16	42
$NO_3 \rightarrow NO_2$	14	37
Gluconate oxidation	9	24
Lecithinase	2	5
Hemolysis (β)	1	3

[a]TSI reaction: alkaline slant, alkaline butt, no gas, no H_2S.

mannitol, hydrolyzed esculin and produced detectable β-galactosidase (+ONPG) and cytochrome oxidase. Eighty-nine percent (34/38) of the strains oxidized sucrose and produced catalase and casein hydrolase. Thirty-two strains produced gelatinase and 31 produced urease. Seventy-one percent (27/38) of the strains produced yellow pigment either on sheep Blood agar, TSI slant or on Mueller-Hinton agar. Twenty-three of the strains (61%) produced ornithine decarboxylase. Although all 38 strains of P. cepacia grew on MacConkey agar slants, only 21 or 55% of the strains grew on MacConkey agar plates. Forty-two percent (16/38) showed growth on BHIA slants and in TSB at 42°C. 37% of the strains reduced nitrate to nitrite. Only 9 strains oxidized gluconate and only two strains produced lecithinase.

When tested with the API 20E method, the P. cepacia strains showed a slightly greater degree of biochemical variability than was observed with the conventional methods. Tables 3 and 4 summarize these results. All the strains oxidized glucose and could utilize citrate as a sole carbon source but were negative for the following twelve tests: arginine dihydrolase, H_2S, urease, tryptophan deaminase, indole, Voges-Proskauer, mannitol, inositol, sorbitol, rhamnose, melibiose and nitrogen gas production. Ninety-five percent (36/38) of the strains produced β-galactosidase and

Table 3.

Biochemical Properties of 38 Strains of P. cepacia As Determined With the API 20E Method [a,b]

STRAINS TESTED	ONPG	GEL	LDC	ARAB	AMY	$NO_3 \rightarrow NO_2$	SUC	ODC
2077	+	+	+	+	+	–	+	+
2369								
2368								
2331								
2076								
2075								
2078								
2416								
2332								
K808								
B6566	+	+	+	+	+	+		
LU2800								
K358								
K360	+	+	+	+	+	+	–	
K359								
B5910								
B5912								
D6460	–	+	–	+	+	–		
K1021	–	+	+	–	–			
75-2013	+	+	+	+	+	–	+	–
2106								
ATCC10856								
LU3468	+	–						
B4648	+	–	–	–				
B4134								
2160	+	–	+	+	–			
ATCC25416	+	+	–					
K981	+	+	+	–				
K1075								
B-2	+	+	–					
K1036								
K998	+	+	+	–	–	–	–	
CJ62								
FB96								
FB143								
B-1	+	–						
75-3351	+	+	+	+	+	+		
2371	+	–	–					

[a]All 38 strains were positive for the following reactions: GLU,CIT.

[b]All 38 strains were negative for the following reactions: ADH,H_2S,URE,TDA,IND,VP,MAN, INO,SOR,RHA,MEL, and $NO_3 \rightarrow N_2$ gas.

Table 4.

Summary of Biochemical Properties of 38 Strains of *P. cepacia* As Determined With The API 20E System

Biochemicals Tested [a]	# of Strains (+)	% of Strains (+)
Arginine dihydrolase	0	0
H_2S production	0	0
Urease	0	0
Tryptophan deaminase	0	0
Indole	0	0
Voges-Proskauer	0	0
Mannitol	0	0
Inositol	0	0
Sorbitol	0	0
Rhamnose	0	0
Melibiose	0	0
$NO_3 \rightarrow N_2$ gas	0	0
Glucose	38	100
Citrate	38	100
ONPG	36	95
Gelatinase	32	84
Lysine decarboxylase	31	82
Arabinose	26	68
Amygdalin	26	68
Sucrose	25	66
Ornithine decarboxylase	19	50
$NO_3 \rightarrow NO_2$	9	24

[a] The following additional conventional tests, which are not included in the API strip, are recommended by the manufacturers for use in the identification of the nonfermenters: OF-Oxidation, OF-Fermentation, Oxidase, Motility and Growth on MacConkey agar.

84% (32/38) liquefied gelatin with the API method (Table 4). Eighty-two percent (31/38) of the strains of P. cepacia produced lysine decarboxylase on the API strip and 66% (25/38) of the strains oxidized the sucrose in the API microtubes. Ornithine decarboxylase was detected in 50% of the 38 strains and 24% (9/38) of the strains were shown to reduce nitrate to nitrite. The same number of strains (26/38) utilized amygdalin and arabinose in the API 20E system. These two tests were not included in the conventional system.

B. Biochemical Characteristics of Control Organisms

Tables 5 and 6 summarize the results obtained with the eight control gram negative nonfermentative reference strains tested with the conventional King method and the API 20E method, respectively. The P. maltophilia, F. meningosepticum, P. aeruginosa, P. fluorescens, P. stutzeri and Achromobacter xyloseoxidans ATCC strains were correctly identified as such with the conventional method as well as with the API 20E system. The Acinetobacter calcoaceticus strain #23055 from ATCC was a poor grower. Although it could be identified using conventional media, it could not be properly identified using the API 20E system. The Xanthomonas campestris ATCC strain #6402 was identified with the conventional methods, while the API 20E system identified this organism as a CDC Group IIK-1.

Table 5.

Biochemical Properties of Control Organisms As

Determined With Conventional Methods of Identification

Substrate or Character	STRAINS TESTED							
	P. maltophilia ATCC13637	F. meningo-septicum ATCC13253	P. aeruginosa ATCC10145	A. calco-aceticus ATCC23055	P. fluorescens ATCC13525	X. campestris ATCC6402	P. stutzeri ATCC17588	Ac. xyloseoxi-dans ATCC27061
TSI [a]	KK—[b]	KK—	KK—	KK—	KK—	KK—	KK—	KK—
Urease	+	-	+	-	+	-	-	-
Indole	-	+	-	-	-	-	-	-
Citrate	+	-	+	-	+	-	+	+
ONPG	+	+	-	-	-	-	-	-
Oxidase	-	+	+	-	+	+	+	+
Catalase	3+[c]	3+	3+	1+	2+	3+	1+	3+
Lysine Decarb.	+	-	-	-	-	-	-	-
Ornithine De-carb.	-	-	-	-	-	-	-	-
PHENYL	-	-	-	-	-	-	+	-
Motility	+	-	+	-	+	+	+	+
PBHB	-	-	-	-	-	-	-	+
$NO_3 \rightarrow NO_2$	+	-	-	-	-	-	-	-
DNAse	+	+	-	-	-	-	-	-
OF Dextrose	A	A	A	A	A	A	A	-
OF Maltose	A	A	K	A	A	A	A	K
OF Sucrose	-	K	K	K	A	A	K	K
OF Lactose	-	A	K	A	K	K	K	K
OF Xylose	K	K	A	A	A	A	A	A
OF Mannitol	K	A	A	K	A	-	A	K
OF Rhamnose	K	K	-	A	A	-	K	K
OF Control	K	K	K	K	K	K	K	K
Auto-Fluor.	-	-	+	-	+	-	-	-
$NO_3 \rightarrow N_2$ gas	-	-	+	-	-	-	+	+
$NO_2 \rightarrow N_2$ gas	-	-	+	-	-	-	+	+
42°C BHI/TSB	-/-	-/-	+/+	-/-	-/-	-/-	+/+	+/+
Gluconate	-	-	+	-	+	-	+	+
Esculin	+	+	-	-	-	+	-	-
Gelatin	+	+	+	+	+	-	-	-
MacConkey pl/sl	+/+	-/+	+/+	-/-	+/+	-/-	+/+	+/+
Hemolysis (β)	-	-	+	-	-	-	-	-
Caseinase	+	+	+	-	-	-	-	-
Lipase	+	-	+	-	-	-	+	-
Lecithinase	-	-	+	-	-	-	+	-
Pigment	-	-	Green	-	-	Yellow	-	-
Growth on SS	-	-	+	-	+	-	+	+

[a] TSI reaction is read as follows: slant/butt/gas/H_2S

[b] KK— = alkaline/alkaline/no gas/ no H_2S

[c] The following gradations were used for the catalase test: 1+, weak or delayed bubble production; 2+, moderate bubble production; and 3+, vigorous bubble production (71).

Table 6.

Biochemical Properties of Control Organisms As Determined With the API 20E System

BIOCHEMICALS TESTED[a]	STRAINS TESTED							
	P. maltophilia TCC13637	F. meningosepticum ATCC13253	P. aeruginosa ATCC10145	A. calcoaceticus ATCC23055	P. fluorescens ATCC13525	X. campestris ATCC6402	P. stutzeri ATCC17588	Ac. xyloseoxidans ATCC27061
ONPG	+	+	–	–	–	+	–	–
Arginine Dihydrolase	–	–	+	–	+	–	–	–
Lysine Decarboxylase	+	–	–	–	–	–	–	–
Ornitnini Decarboxylase	–	–	–	–	–	–	–	–
Citrate	+	–	+	–	+	–	–	+
H_2S	–	–	–	–	–	–	–	–
Urease	–	–	+	–	–	–	–	–
TDA	–	–	–	–	–	–	–	–
Indole	–	+	–	–	–	–	–	–
Voges-Proskauer	–	–	–	–	+	–	–	–
Gelatin	+	+	+	–	–	+	–	–
Glucose	–	–	+	–	–	–	–	–
Mannitol	–	–	–	–	–	–	–	–
Inositol	–	–	–	–	–	–	–	–
Sorbitol	–	–	–	–	–	–	–	–
Rhamnose	–	–	–	–	–	–	–	–
Sucrose	–	–	–	–	–	–	–	–
Melibiose	–	–	–	–	–	–	–	–
Amygdalin	–	–	–	–	–	–	–	–
Arabinose	–	–	+	–	–	–	–	–
$NO_3 \rightarrow N_2$ gas	–	–	+	–	–	–	+	+
$NO_3 \rightarrow$	+	–	–	–	–	–	–	–

[a]The following additional conventional tests, which are not included on the API strip, are recommended by the manufacturers for use in the identification of the nonfermenters: OF-Oxidation, OF-Fermentation, Oxidase, Motility and Growth on MacConkey agar.

C. Antibiotic Susceptibility Patterns of the *P. cepacia* Strains

The Kirby-Bauer antibiotic susceptibility results of the 38 *P. cepacia* strains showed the organisms to be very resistant, especially to the antibiotics commonly used for *Pseudomonas* infections, such as gentamicin, carbenicillin, colistin, amikacin and tobramycin. Tables 7 and 8 summarize these sensitivity patterns. All of the 38 strains of *P. cepacia* tested were resistant to the following nine drugs: ampicillin, cephalothin, clindamycin, colistin, erythromycin, nitrofurantoin, oxacillin, penicillin G and vancomycin. Ninety-seven percent (37/38) of the strains were resistant to gentamicin, tobramycin, tetracycline and amikacin (Table 8). Ninety-five percent (36/38) of the strains were resistant to carbenicillin; one strain showed an indeterminate zone size and one a sensitive zone size. Eighty-two percent (31/38) were resistant to kanamycin; five strains had indeterminate zone sizes and two strains were sensitive. Eighty-seven percent (33/38) were found to be resistant to ticarcillin; 3 strains showed indeterminate zone sizes and 2 strains were sensitive. Forty-two percent (16/38) of the strains were resistant, 3 strains were indeterminate and 19 strains were sensitive to nalidixic acid. Thirty-two percent (12/38) were resistant, 1 strain indeterminate and 25 strains (65%)

Table 7.

Antibiotic Susceptibility Testing Results on 38 Strains of P. cepacia Using the Kirby-Bauer Method [a]

Strains Tested	Gentamicin (10 mcg)	Tobramycin (10 mcg)	Tetracycline (30 mcg)	Amikacin (10 mcg)	Ticarcillin (75 mcg)	Carbenicillin (100 mcg)	Kanamycin (30 mcg)	Nalidixic Acid (30 mcg)	Chloramphenicol (30 mcg)	Trimethoprim-Sulfa (1.25 mcg/23.75 mcg)
2331	R	R	R	R	R	R	R	R	R	R
K360	R	R	R	R	R	R	R	R	R	S
75-2013	R	R	R	R	R	R	R	R	R	S
2106	R	R	R	R	R	R	R	R	R	S
ATCC25416	R	R	R	R	R	R	R	R	R	S
CJ62	R	R	R	R	R	R	R	R	R	S
FB143	R	R	R	R	R	R	R	R	R	S
K808	R	R	R	R	R	R	R	R	S	S
K358	R	R	R	R	R	R	R	R	S	S
LU2800	R	R	R	R	R	R	R	R	S	S
75-3351	R	R	R	R	R	R	R	R	S	S
2368	R	R	R	R	R	R	R	R	S	S
2369	R	R	R	R	R	R	R	R	S	S
ATCC10856	R	R	R	R	R	R	R	S	R	S
K998	R	R	R	R	R	R	R	I	I	S
2332	R	R	R	R	R	R	R	S	I	S
B4134	R	R	R	R	R	R	R	S	S	S
B5910	R	R	R	R	R	R	R	S	S	S
B5912	R	R	R	R	R	R	R	S	S	S
B6566	R	R	R	R	R	R	R	S	S	S
2077	R	R	R	R	R	R	R	S	S	S
2416	R	R	R	R	R	R	R	S	S	S
K1036	R	R	R	R	R	R	R	S	S	S
K981	R	R	R	R	R	R	R	S	S	S
K1075	R	R	R	R	R	R	R	S	S	S
FB96	R	R	R	R	R	R	I	S	S	S
2160	R	R	R	R	R	R	I	S	R	S
LU3468	R	R	R	R	R	R	I	S	R	S
K1021	R	R	R	R	R	R	I	S	R	S
D6460	R	R	R	R	R	R	S	S	R	S
B-2	R	R	R	R	S	R	R	R	S	S
B4648	R	R	R	R	R	S	R	R	S	S
2371	R	R	S	R	R	R	S	R	S	S
2078	R	R	R	R	I	R	R	S	S	S
2075	R	R	R	R	I	R	R	S	S	S
2076	R	R	R	R	I	R	R	S	S	S
B-1	R	R	R	R	S	I	R	S	S	S
K359	S	S	R	S	R	R	S	S	S	S

[a]All 38 strains of P. cepacia were resistant to the following nine drugs: ampicillin, cephalothin, colistin, nitrofurantoin, vancoymcin, oxacillin, erythromycin, penicillin G, and clindamycin.

Table 8.

Summary of Antibiotic Susceptibility Testing Results

on 38 Strains of *Pseudomonas cepacia*

Using the Kirby-Bauer Method

Antimicrobial Tested	# of Strains Sensitive	# of Strains Indeterminate	# of Strains Resistant	% of Strains Resistant
Ampicillin	0	0	38	100
Cephalothin	0	0	38	100
Colistin	0	0	38	100
Nitrofurantoin	0	0	38	100
Vancomycin	0	0	38	100
Oxacillin	0	0	38	100
Erythromycin	0	0	38	100
Penicillin G	0	0	38	100
Clindamycin	0	0	38	100
Gentamicin	1	0	37	97
Tobramycin	1	0	37	97
Tetracycline	1	0	37	97
Amikacin	1	0	37	97
Carbenicillin	1	1	36	95
Ticarcillin	2	3	33	87
Kanamycin	2	5	31	82
Nalidixic Acid	19	3	16	42
Chloramphenicol	25	1	12	32
Trimethoprim-Sulfamethoxazole	37	0	1	3

sensitive to chloramphenicol. Only one strain was found to be resistant to trimethoprim-sulfamethoxazole by this method.

The control organisms used with Kirby-Bauer antibiotic susceptibility testing, E. coli ATCC #25922, S. aureus ATCC #25923, and P. aeruginosa ATCC #27853, were tested with each batch of susceptibilities run. Zone sizes in the acceptable range (45) were consistently achieved for the control organisms against the nineteen drugs tested with the P. cepacia strains.

The minimum inhibitory concentrations (MIC's) of nine drugs against the 38 strains of P. cepacia as determined by the Micro-Media microtiter antibiotic dilution method are listed in Tables 9 and 10. Data on the relationship between the MIC values obtained and Kirby-Bauer zones was taken from studies by Matsen and Barry (45). All 38 strains were found to have MIC's in the sensitive range (0.5-2/9.5-38 mcg/ml) to trimethoprim-sulfamethoxazole. All 38 strains tested against nitrofurantoin (at a concentration of 64 mcg/ml) and colistin (at a concentration of 4 mcg/ml) with this method were found to be resistant to both of these drugs. Most of the strains tested were also found to be resistant to ampicillin, cephalothin, gentamicin, and tobramycin. Eighty-two percent (32/38) of the strains had MIC's ≥ 16 mcg/ml for tetra-

Table 9.
Minimum Inhibitory Concentrations
of Selected Antibiotics for 38 Strains of P.cepacia

STRAINS TESTED	ANTIBIOTICS										
	TM-SXT	FD	Cs	Am	Cl	Gm	NN	Te	Cb	C	K
K1021	<.5/9.5[a]	>64	>4	>16	>64	16	8	>16	512	32	16
D6460	<.5/9.5	>64	>4	>16	>64	16	8	16	128	32	8
K808	.5/9.5	>64	>4	>16	>64	>16	>16	>16	>512	8	16
B4134	.5/9.5	>64	>4	>16	>64	>16	>16	>16	>512	8	16
B-2	2/38	>64	>4	>16	>64	>16	>16	>16	256	8	32
2076	.5/9.5	>64	>4	>16	>64	>16	>16	16	512	2	32
K1075	.5/9.5	>64	>4	>16	>64	>16	>16	>16	256	8	64
K1036	.5/9.5	>64	>4	>16	>64	>16	>16	>16	128	8	64
B4648	1/19	>64	>4	>16	>64	>16	>16	>16	128	8	32
2077	.5/9.5	>64	>4	>16	>64	>16	8	8	512	2	16
2416	.5/9.5	>64	>4	>16	>64	>16	16	8	512	2	16
2078	.5/9.5	>64	>4	>16	>64	16	16	8	512	2	16
2075	.5/9.5	>64	>4	>16	>64	16	16	8	512	4	16
K359	.5/9.5	>64	>4	>16	>64	0.5	0.5	16	>512	32	<1
2371	.5/9.5	>64	>4	8	32	16	16	1	>512	8	8
K981	<.5/9.5	>64	>4	>16	64	>16	>16	>16	>512	8	>64
B-1	.5/9.5	>64	>4	>16	>64	>16	>16	4	64	8	64
K358	1/19	>64	>4	>16	>64	>16	>16	>16	>512	16	>64
2369	.5/9.5	>64	>4	>16	>64	>16	>16	>16	>512	8	>64
2368	.5/9.5	>64	>4	>16	>64	>16	>16	>16	>512	8	>64
75-3351	.5/9.5	>64	>4	>16	>64	>16	>16	>16	>512	8	>64
2332	1/19	>64	>4	>16	>64	>16	>16	>16	>512	8	>64
2331	2/38	>64	>4	>16	>64	>16	>16	>16	>512	>32	>64
K360	.5/9.5	>64	>4	>16	>64	>16	>16	>16	512	32	>64
B6566	.5/9.5	>64	>4	>16	>64	>16	>16	>16	256	4	>64
75-2013	2/38	>64	>4	>16	>64	>16	16	>16	>512	16	16
2160	2/38	>64	>4	>16	>64	>16	>16	>16	>512	32	16
LU3468	1/19	>64	>4	>16	>64	>16	16	>16	>512	32	16
CJ62	1/19	>64	>4	>16	>64	>16	16	>16	>512	16	32
ATCC25416	1/19	>64	>4	>16	>64	>16	16	>16	>512	16	16
2106	1/19	>64	>4	>16	>64	>16	16	>16	>512	16	16
ATCC10856	.5/9.5	>64	>4	>16	>64	16	16	>16	>512	16	16
B5910	.5/9.5	>64	>4	>16	>64	>16	>16	>16	512	8	64
B5912	.5/9.5	>64	>4	>16	>64	>16	>16	>16	256	4	32
FB96	.5/9.5	>64	>4	>16	>64	>16	16	>16	>512	8	16
FB143	.5/9.5	>64	>4	>16	>64	>16	>16	>16	>512	32	32
K998	1/19	>64	>4	>16	>64	>16	>16	>16	>512	16	32
LU2800	.5/9.5	>64	>4	>16	>64	>16	>16	>16	>512	8	>64

[a]All values reported are in mcg/ml.

Table 10.

Summary of

Microtiter Antibiotic Dilution Susceptibility

Testing Results on 38 Strains of Pseudomonas cepacia

Drugs Tested	MIC value (mcg/ml)	Kirby-Bauer Breakpoints	# of Strains	% of Strains
Colistin	>4	R[a]	38	100
Nitrofurantoin	>64	R	38	100
Trimethoprim-Sulfamethoxazole	0.5-2/ 9.5-38	S	38	100
Ampicillin	8	S	1	3
	>16	R	37	97
Cephalothin	32	R	1	3
	>64	R	37	97
Gentamicin	0.5	S	1	3
	≥16	R	37	97
Tobramycin	0.5-4	S	1	3
	8	R	3	9
	≥16	R	34	88
Carbenicillin	64-128	S	4	12
	256	R	4	12
	≥512	R	30	76
Tetracycline	1-4	S	2	6
	8	I	4	12
	≥16	R	32	82
Kanamycin	<1-8	S	3	9
	16	I	14	38
	32->64	R	21	53
Chloramphenicol	2-8	S	23	61
	16	I	7	18
	≥32	R	8	21

[a]S= Sensitive, I= Indeterminate, R= Resistant.

cycline; 12% were in the indeterminate range with 8 mcg/ml (Table 10). Seventy-six percent (30/38) of the strains showed MIC's ≥ 512 mcg/ml, 4 strains showed MIC's of 256 mcg/ml and 4 strains had MIC's in the sensitive range for carbenicillin. Only 21% of the strains had MIC's in the resistant range (≥ 32 mcg/ml) for chloramphenicol; 18% had MIC's in the indeterminate range and 61% (23/38) showed MIC's in the sensitive range (2-8 mcg/ml). Fifty-three percent (20/38) of the strains were found to be resistant to kanamycin; 38% had MIC's in the indeterminate range and 3 strains were found to be sensitive to kanamycin.

The control organisms, E. coli ATCC #25922 and P. aeruginosa ATCC #27853, were run with each batch of the microtiter MIC determinations, and gave minimum inhibitory concentration values within plus or minus one well of the known values for these organisms (47).

D. Tube Agglutination Studies

The antisera prepared against the twenty-nine strains of P. cepacia and the control P. fluorescens ATCC strain 13525 were reacted in tube agglutination assays with each of the thirty-eight strains of P. cepacia and with the control P. fluorescens organism. The results are shown in Table 11 a,b,c. All of the strains were readily agglutinated by the homologous antiserum. Homologous titers ranged from 80 to 2560. Titers obtained in reciprocal

Table 11a.
Tube Agglutination Assay Titers of 29
Antisera against P.cepacia and 38 P.cepacia Strains Used as Antigens

Strains of P.cepacia Antigens	Possible Serogroups	Antisera Against P.cepacia												
		K1021	D6460	K359	2416	B4134	K808	B4648	2077	K1036	B-2	2076	K1075	2371
K1021	I	1280[a]	1280	-[b]	-	-	-	-	-	-	-	-	-	-
D6460		1280	1280	-	-	-	-	-	-	-	-	-	-	-
K359	II	-	-	80	320	320	80	640	1280	80	320	320	80	-
2416		-	-	320	640	640	640	320	1280	320	640	320	-	80
B4134		-	-	320	320	1280	320	1280	1280	160	640	320	80	160
K808		-	-	80	320	1280	1280	1280	640	160	320	160	-	20
B4648		-	-	40	80	640	320	640	320	20	160	160	20	40
2075		-	-	80	320	640	320	320	640	80	160	320	80	40
2078		-	-	320	640	640	320	320	320	320	160	320	40	40
2077		-	-	640	2560	2560	1280	2560	2560	640	2560	1280	-	80
K1036		-	-	-	40	320	320	320	40	1280	-	-	1280	-
B-2		-	-	640	1280	1280	1280	2560	2560	640	2560	1280	640	320
2076		-	-	640	1280	1280	640	1280	1280	320	640	1280	-	160
K1075		-	-	-	40	80	80	80	40	320	20	20	160	-
2371		-	-	-	-	20	80	160	80	-	160	80	40	640
K981	III	-	-	-	-	-	-	-	-	-	-	-	-	40
B-1	IV	-	-	-	-	-	-	-	-	-	-	-	-	-
B6566	V	-	20	-	-	-	-	-	40	20	640	-	-	20
2331		-	-	-	-	-	-	-	-	-	-	-	-	-
K358		-	-	-	-	-	-	-	-	-	40	640	160	-
2369		-	-	-	-	-	-	-	-	-	-	1280	640	-
2368		-	-	-	-	-	-	-	-	-	-	2560	320	-
K360		-	-	-	-	-	-	-	-	-	-	640	640	-
2332		-	-	-	-	-	-	-	-	-	-	-	-	-
75-3351		-	-	-	-	-	-	-	-	-	-	-	-	-
25416	VI	-	-	-	-	-	-	-	-	-	-	-	-	-
10856		-	-	-	-	-	-	-	-	-	-	-	-	-
2160		-	-	-	-	-	-	-	-	-	-	-	-	-
75-2013		-	-	-	-	-	-	-	-	-	-	-	-	-
LU3468		-	-	-	-	-	-	-	-	-	-	-	-	-
2106		-	-	-	-	-	-	-	-	-	-	-	-	-
CJ62		-	-	-	-	-	-	-	-	40	-	-	-	-
B5910	VII	-	-	-	-	-	-	-	-	-	-	-	-	-
B5912		-	-	-	-	-	-	-	-	-	-	-	-	-
FB96	VIII	160	-	-	80	-	160	80	80	-	80	160	-	-
K998		80	-	-	80	40	80	40	80	-	40	80	-	-
FB143		320	-	-	40	-	80	80	160	-	-	80	-	-
LU2800		40	80	20	-	-	40	-	-	-	-	20	80	10

[a]Highest serum dilution resulting in at least a 2+ (definite clumping, supernate cloudy) agglutination.

[b](-) = No agglutination

Table 11b.
Tube Agglutination Assay Titers

Strains of *P. cepacia* Antigens	Possible Serogroups	Antisera Against P.cepacia						
		K981	B-1	2331	B6566	2369	K358	75-3351
K1021	I	-[b]	-	-	-	-	-	-
D6460		-	-	-	-	-	-	-
K359	II	-	-	-	-	-	-	-
2416		-	-	-	-	-	-	-
B4134		-	-	-	-	-	-	-
K808		-	-	-	-	-	-	-
B4648		-	-	-	-	-	-	-
2075		-	-	-	-	-	-	-
2078		-	-	-	-	-	-	-
2077		-	-	-	-	-	-	-
K1036		80[a]	-	-	-	-	-	-
B-2		-	-	-	-	-	-	-
2076		-	-	-	-	-	-	-
K1075		40	-	-	-	-	-	-
2371		80	320	80	80	80	-	-
K981	III	640	-	-	-	-	-	-
B-1	IV	-	320	-	-	-	-	-
B6566	V	-	-	2560	2560	-	-	160
2331		-	-	640	80	-	-	320
K358		-	-	160	-	-	640	-
2369		-	-	160	-	320	2560	-
2368		-	-	320	-	40	1280	-
K360		-	-	80	-	-	160	-
2332		-	-	80	-	-	-	80
75-3351		-	-	40	-	-	-	80
25416	VI	-	-	-	-	-	-	-
10856		-	-	-	-	-	-	-
2160		40	-	-	-	-	-	-
75-2013		-	-	-	-	-	-	-
LU3468		-	-	-	-	-	-	-
2106		-	-	-	-	-	-	-
CJ62		-	-	-	-	-	-	-
B5910	VII	-	-	-	-	-	-	-
B5912		-	-	-	-	-	-	-
FB96	VIII	-	-	160	20	-	-	-
K998		-	-	80	20	-	-	-
FB143		-	-	160	-	-	-	-
LU2800		-	-	-	20	-	-	-

[a] Highest serum dilution resulting in at least a 2+ agglutination.

[b] (-) = No agglutination.

Table 11c.

Tube Agglutination Assay Titers

Strains of *P. cepacia* Antigens	Possible Serogroups	Antisera Against *P. cepacia*								
		25416	10856	2160	75-2013	LU3468	CJ62	B5910	FB96	LU2800
K1021	I	-[b]	-	-	-	-	-	-	-	-
D6460		-	-	-	-	-	-	-	-	-
K359	II	-	-	-	-	-	-	-	-	-
2416		-	-	-	-	-	-	-	-	-
B4134		-	-	-	-	-	-	-	-	-
K808		-	-	-	-	-	-	-	-	-
B4648		-	-	-	-	-	-	-	-	-
2075		-	-	-	-	-	-	-	-	-
2078		-	-	-	-	-	-	-	-	-
2077		-	-	-	-	-	-	-	-	-
K1036		-	-	-	-	-	-	-	-	-
B-2		-	-	-	-	-	-	-	-	-
2076		-	-	-	-	-	-	-	-	-
K1075		-	-	-	-	-	-	-	-	-
2371		-	20[a]	-	-	-	-	-	-	-
K981	III	-	20	-	-	-	-	-	-	-
B-1	IV	-	-	-	-	-	-	-	-	-
B6566	V	40	-	-	-	-	-	-	-	-
2331		80	-	-	-	-	-	-	-	-
K358		-	-	-	-	-	-	-	-	-
2369		-	-	-	-	-	-	-	-	-
2368		-	-	-	-	-	-	-	-	-
K360		-	-	-	-	-	40	-	-	-
2332		-	-	-	-	-	-	-	-	-
75-3351		-	-	-	-	-	-	-	-	-
25416	VI	1280	640	1280	640	1280	1280	-	-	-
10856		1280	1280	640	640	1280	1280	-	-	-
2160		1280	1280	1280	1280	1280	1280	-	-	-
75-2013		320	320	160	320	320	320	-	-	-
LU3468		80	640	640	320	640	640	-	-	-
2106		320	320	640	160	320	640	-	-	-
CJ62		2560	1280	1280	640	1280	1280	-	-	-
B5910	VII	-	-	-	-	-	-	160	-	-
B5912		-	-	-	-	-	-	1280	20	-
FB96	VIII	-	-	-	-	-	-	160	320	40
K998		40	80	-	-	-	-	160	320	40
FB143		80	80	-	-	-	-	320	640	-
LU2800		40	40	20	40	-	-	-	160	640

[a]Highest serum dilution resulting in at least a 2+ agglutination.

[b](-) = No agglutination

tests for cross-reactivity were usually lower; however, a few strains were agglutinated in heterologous antisera at the same or even higher titers than in homologous antisera (e.g. K359, 2416, K1075, 2369). This phenomenon may be due to the variability of individual rabbits' abilities to respond to various organisms as antigens. The antiserum prepared against the _P. fluorescens_ reference strain reacted at a relatively low titer of 80 with its homologous antigen. This reference strain, in turn, only reacted with its own antiserum; there was no cross-reactivity found between any of the _P. cepacia_ strains and the _P. fluorescens_ reference strain.

The results indicated that the _P. cepacia_ strains vary in their antigenic markers, since no one antiserum agglutinated all of the organisms. Moreover, patterns of reactivity were noted which appeared to distinguish 8 groups as follows: Group I, consisting of strains K1021 and D6460; Group II, consisting of strains K359, 2416, B4134, K808, B4648, 2077, 2078, 2075, B-2, 2076, K1036, K1075 and 2371; Group III, consisting of one strain, K981; Group IV, also consisting on one strain, B-1; Group V, consisting of strains B6566, K358, 2368, K360, 2369, 2331, 2332 and 75-3351; Group VI, consisting of strains 2160, 75-2013, LU3468, 2106, CJ62 and ATCC strains 25416 and 10856; Group VII, consisting of strains B5910 and B5912;

and Group VIII, consisting of the four remaining strains FB96, K998, FB143 and LU2800.

Each rabbit's preimmunization serum was diluted 1:10 and was used as a control against each P. cepacia strain and the ATCC P. fluorescens strain tested in the tube agglutination studies. The normal serum from the rabbit immunized with P. cepacia strain B6566 reacted at a dilution of 1:10 with strains (a) 2332, (b) D6460, (c) K1036, (d) 2371 and (e) P. fluorescens ATCC 13525. This titer was considered to be too low to interfere with any test agglutination titers since these titers were started at a 1:20 dilution. None of the remaining 29 normal sera reacted with any of the strains tested. When incubated in a saline suspension, none of the 29 P. cepacia strains or the ATCC P. fluorescens strain were found to self-agglutinate.

CHAPTER IV

DISCUSSION

With the marked increase in the isolation of gram negative rods as a cause of nosocomial infections in hospitals in recent years, the search for more rapid identification and characterization methods for these organisms has been extensive. Presently used conventional techniques involve assays for biochemical utilization of carbohydrates, amino acids, fatty acids, etc. in order to identify the nonfermenters. The necessity for standardization of production, storage and use of the diverse media and of result interpretation makes these assays cumbersome to perform in the clinical microbiology laboratory. As an answer to this problem, several manufacturers have devised miniaturized, standardized, commercially prepared biochemical methods for the identification of gram negative bacilli (1). One such system, the API 20 Enteric strip, has been used successfully to identify members of the *Enterobacteriaceae* family. However, with the significant increase in isolation from hospitalized patients and the hospital environment of other gram negative bacilli as those belonging to the *Pseudomonadaceae* and *Achromobacteriaceae* families, this system has also been applied to the identification of these

organisms. One purpose of this work was to compare conventional biochemical methods and the API 20E system for the identification of Pseudomonas cepacia.

The results for the 38 strains of P. cepacia with conventional media revealed certain nutritional characteristics common to all or most of the P. cepacia strains. These organisms oxidize a wide variety of carbohydrates in OF media, they are motile and they do not denitrify nitrate or nitrite to nitrogen gas. Most strains produce lipase and oxidase, hydrolyze casein and esculin and produce β-galactosidase and lysine decarboxylase, but not arginine dihydrolase or DNAse. Many strains produce a yellow pigment and accumulate poly-β-hydroxybutyrate in the cells. All of strains grow on MacConkey agar slants but not on SS agar.

Our data based on 38 strains compares very closely with that obtained by Gilardi (33). However, 97% of our strains produced lysine decarboxylase and 61% produced ornithine decarboxylase using the Key Scientific substrate tablets and ninhydrine reagent in chloroform (Table 2), while Gilardi reported 94% of the P. cepacia strains positive for lysine decarboxylase and only 47% for ornithine decarboxylase using Moeller Decarboxylase Base media. In our hands, this Moeller Decarboxylase medium was found to be unsatisfactory for the detection of lysine, ornithine or arginine decarboxylase activity from nonfermenters for the

following reasons: (a) The reaction in the medium is based on a two step process (43), the first of which is glucose fermentation and nonfermenters will not ferment glucose; (b) the media is overlayed with paraffin or oil after inoculation and nonfermenters do not readily grow in an anaerobic environment; and (c) when growth of the organisms does occur, it takes several days to detect any change in the indicator system. In contrast, the Key Scientific method is simple to perform, does not require actual growth of the organism and results can be read in 24 hours. Other minor discrepancies in biochemical patterns were noted. For example, 82% of our strains were positive for urease production but only 5% were positive for lecithinase production (Table 2), whereas Gilardi found that only 34% of his strains of P. cepacia produced urease and 34% produced lecithinase.

Using conventional methods, many different biochemical assays are required to differentiate these strains of P. cepacia from other nonfermentative gram negative rods. Moreover, this method of identification was found to be time consuming and the assays difficult to standardize. Some of the assays required 48 to 72 hours of incubation. Results with this method were cumbersome to compile and many variables had to be considered to arrive at an identification.

The API 20E method was easy to use and many of the nonfermentative gram negative rods could be identified in 24 hours. The identification of less active nonfermenters required 48 hours and inoculation of four additional tests. One advantage of the API 20E identification system is that it is computer-based; that is, a computer is utilized to store and update data on biochemical variations of the different strains being isolated, to sort the data and to come up with possible biochemical reaction combinations (profiles) which could identify a particular species. This "real time" information is available to the user of the system. The API system is capable of differentiating between even relatively inert strains of nonfermenters with the aid of the computer profile.

Two organisms which P. cepacia is often confused with and misidentified as, using conventional methods, are Pseudomonas paucimobilis (King Group IIK-1) and Pseudomonas fluorescens. However, with the profile generated with just a few key positive reactions on the API strip, a differentiation between these three strains can be made. P. fluorescens always shows negative reactions for lysine decarboxylase and amygdalin, whereas P. cepacia is always negative for arginine dihydrolase and the Voges-Proskauer reaction. P. cepacia can be differentiated from P.paucimobilis (IIK-1), which is biochemically quite similar, on the basis

of a "pattern" of biochemicals, which includes lysine decarboxylase, gelatin hydrolysis, and acid production from amygdalin, arabinose and glucose (1).

The conventional and the API 20E methods showed an overall 72% agreement on the 15 biochemicals comparatively tested with the 38 strains of P. cepacia. An examination of Tables 2 and 4 shows slightly higher percentages of positive reactions using the conventional method for the assays comparatively tested. For instance, 97% (37/38) of the strains of P. cepacia produced lysine decarboxylase with the Ninhydrine (Key Scientific) method, whereas only 82% (31/38) of the strains were positive with the API method. Eighty-nine per cent of the strains oxidized sucrose in the OF media, whereas only 66% (25/38) of the strains oxidized the sucrose in the API microtubes. Nitrate reduction was shown in 37% of the strains with the conventional method, whereas only 24% (9/38) of the strains were shown to reduce nitrate to nitrite with the API method. Eighty-four per cent (32/38) of the strains liquefied gelatin with the Key Scientific gelatin strips and 84% of the strains liquefied gelatin with the API method. However, there were ten strains which gave a positive result with one method and a negative result with the other method for this test. Therefore, it is felt that the sensitivity of the two methods is probably comparable and that neither

method is particularly sensitive in detecting strains producing lesser amounts of gelatin hydrolase. Perhaps a longer incubation period (>24-48 hours) for this test would have shown a higher correlation of results for these ten strains. Ornithine decarboxylase was detected in 61% of the 38 strains with the conventional Ninhydrine method, but in only 50% of the strains with the API method. The two methods were in agreement on 32 out of the 38 strains on this reaction and only one strain (LU2800) showed a positive reaction for ornithine decarboxylase with API and a negative reaction with the conventional method. There were only a few other reactions in which a positive result was shown with API and a negative result with conventional media. One such exception was β-galactosidase: 35 out of 38 strains produced β-galactosidase with conventional tube methods, whereas 36 out of 38 strains were positive with the API method. Only two biochemical assays, urease production and mannitol oxidation, showed dramatically differing results between the two methods for the 38 strains of P. cepacia. Eighty-two percent of the strains produced urease and 92% oxidized OF Mannitol using conventional media, whereas none of the strains were positive for these two assays on the API strip. It was noted that the production of urease on the conventional urea agar slant was quite weak for the P. cepacia strains tested, and that the

amount of acid detectable in OF Mannitol for most P. cepacia was lesser than with most of the other sugars oxidized by the strains in OF media. Therefore, the API system must be less sensitive than conventional media in detecting weak reactions in these two assays. The urease reaction is not critical in the identification of P. cepacia strains when using conventional methods; however, the OF Mannitol reaction is of importance in differentiating P. cepacia from King Group IIK organisms. Therefore, a positive reaction in mannitol on the API strip could be helpful in differentiating P. cepacia strains from other nonfermentative gram negative rods. Even though this slight variability in percentages of positive reactions was found between the two methods, it is the overall pattern of reactions that is important in arriving at an identification with the API system. Therefore, a 100% agreement was found between the two methods on the final identification of the 38 strains of P. cepacia.

In general, the API 20E system was found to be easy, rapid and convenient to use. The 22 biochemicals which are read directly from the API strip can easily be uniformly inoculated in a minimal amount of time and then easily be read. The highly satisfactory agreement between results from the API system when compared with conventional methods. definitely shows the API 20E system to be very useful in

the rapid identification of P. cepacia and probably most gram negative nonfermentative rods.

The control ATCC strains of P. maltophilia, P. aeruginosa, P. stutzeri, F. meningosepticum, P. fluorescens and Ac. xyloseoxidans were readily identified with both biochemical systems. X. campestris does not appear in the Weaver scheme but the organism can be identified using conventional methodology and interpreting the results with the aid of Bergey's Manual. The API 20E profile also does not provide for the identification of Xanthomonas so ATCC 6402 was identified as a IIK-1. The Acinetobacter calcoaceticus ATCC strain grew poorly, and was eventually properly identified using conventional methods, but could not be identified on the API 20E strip.

The biochemical patterns of the P. cepacia strains were analyzed to determine whether there might be clusters of organisms showing similar reactions. Three biochemical characteristics appeared to be useful in this regard: (a) the ability to oxidize sucrose, (b) the ability to produce ornithine decarboxylase and (c) the ability to reduce nitrates to nitrites. The reactions of the five biogroups obtained are shown in Table 12. When other selected biochemical characteristics, including pigment production, gluconate and OF mannitol oxidation, and urease, casein hydrolase, β-galactosidase and lecithinase production, were

Table 12.

Biogroupings of the 38 Strains of *P. cepacia* Based on Sucrose Oxidation, Nitrate Reduction and Ornithine Decarboxylase Production

Biogroup	Strains of *P. cepacia*	Sucrose Oxidation	Nitrate Reduction	Ornithine Decarboxylase
1	K1021	-	-	+
	D6460			
2	2075	+		
	2076			
	2077			
	B4134			
	2078			
	2416			
	B4648			
	B-2			
	K808			
3	B5910	+	+	
	B5912			
	2332			
	B6566			
	2368			
	2369			
	2331			
	75-3351			
	K358			
	K359			
	K360			
	LU2800			
4	K1075	+	-	-
	K1036			
	K981			
	75-2013			
	LU3468			
	10856(ATCC)			
	25416(ATCC)			
	2160			
	CJ62			
	2106			
	FB143			
	K998			
	FB96			
5	2371	-	+	
	B-1			

compared for the five biogroups (Table 13), Biogroups 1, 2, 3, and 5 seemed to be quite homogenous, whereas the 13 strains assigned to Biogroup 4 showed many variable reactions. Thus, additional biochemical tests may be needed to differentiate additional subgroups among these Biogroup 4 strains.

Antibiograms have been recognized not only as epidemiological tools in tracing outbreaks or patterns of organism isolation in hospitals (6, 19) but also as a diagnostic aid in the identification of organisms (31). The antibiotic susceptibility testing of the thirty-eight P. cepacia strains revealed a characteristic sensitivity pattern for this organism which is quite distinctive from that of most of the other Pseudomonas sp. Most of the strains of P. cepacia were found to be resistant to tetracycline,colistin. tobramycin, gentamicin, carbenicillin, amikacin and ticarcillin. In contrast, P. fluorescens is usually resistant to carbenicillin, gentamicin and tetracycline (33), but only P. cepacia consistently shows a combined resistance to tobramycin, colistin and amikacin in addition to these three drugs. Therefore, the antibiotic sensitivity pattern is definitely helpful in the differentiation of P. cepacia from other Pseudomonas sp.

Since organisms being isolated from hospitalized patients today are becoming increasingly resistant to the

Table 13.

Analysis of the Five Biogroups of *P. cepacia*

Based on Seven

Additional Biochemical Characteristics

Characteristic	Biogroups 1	2	3	4	5
Yellow pigment	-[a]	+[b]	variable[c]	+	-
Gluconate oxidation	+	-	-	variable	-
Urease	+	+	+	variable	+
Casein hydrolysis	+	+	+	variable	-
ONPG	-	+	+	variable	+
OF Mannitol	K	A	A	variable	A
Lecithinase	-	-	-	-	+

[a](-) = At least 90% of the members of named Biogroup showed a negative reaction.

[b](+) = At least 90% of the members of named Biogroup showed a positive reaction.

[c]variable = Named Biogroup's members did not show the same reaction for this assay.

antibiotics currently available for treatment, there has been an increasing demand on the clinical microbiology lab from physicians for determining minimum inhibitory concentrations of antibiotics against a clinical isolate. The method chosen to determine the MIC's for the 38 strains of *P. cepacia* was a microtiter technique (Micro-Media Systems, Inc.) in which the antibiotics are prepared and frozen in microtiter plates. This method is standardized, FDA approved, simple to perform, easy to read and to interpret results. The only drawback of the method is its expense, yet this is partly compensated by a savings of technician time.

A comparison of the antibiotic susceptibility patterns determined for the 38 strains of *P. cepacia* with the Kirby-Bauer (Table 7) and the MIC (Table 9) methods revealed a high degree of agreement for most of the antibiotics studied. There was complete agreement of results between the two methods 92.5% of the time. Twenty-five antibiotic-organism combinations showed an indeterminate value with the MIC method and either sensitivity or resistance with the Kirby-Bauer method. Generally, this is considered as acceptable agreement (29). Definite differences in results were seen in only six instances, where strain 2331 for TM-SXT, strain B-1 for tetracycline, strain 2371 for ampicillin and strains K1036 and D6460 for carbenicillin showed resistance with Kirby-Bauer and sensitivity with the MIC method,

and where strain K359 showed resistant MIC values for chloramphenicol but was found to be sensitive with the Kirby-Bauer method (Tables 7 and 9). In these cases, the MIC result may be considered more accurate than the Kirby-Bauer result (38). If one also considers the level of antibiotics, which can be achieved in the blood using different routes of drug administration (38), the benefits of MIC determinations are evident. For instance, five strains (75-2013, CJ62, ATCC25416, 2106, ATCC10856)which showed MIC's of 16 mcg/ml for chloramphenicol(Table 7) were interpreted as having resistant zones with the Kirby-Bauer method (Table 9). However, if chloramphenicol were administered intravenously (1 gm, q6h), 20 to 30 mcg/ml levels of this drug could be reached in the blood; therefore, chloramphenicol may still be an effective drug against those strains of _P. cepacia_.

Even though _P. cepacia_ strains are resistant to colistin, Rahal (60) has reported that the combination of this drug with trimethoprim-sulfamethoxazole, to which most _P. cepacia_ strains are very sensitive, is synergistic, bactericidal and more effective than trimethoprim-sulfamethoxazole alone against _P. cepacia_ infections. Although possible synergism between drugs can sometimes be shown using the Kirby-Bauer method (60, 67), performing checkerboard MIC's would be very useful in determining exactly what achievable

levels of these two antibiotics would show most synergism against a particular strain.

Eight serogroups were distinguished from the 38 strains of P. cepacia serologically tested on the basis of the cross-reactivity seen between these strains in agglutination studies with the prepared antisera. The reactions observed were probably due to agglutinins formed against thermostable O antigens, since the thermolabile H antigens would be destroyed by the boiling used in preparing the antigens. However, the possibility that some of these reactions were associated with either heat stable components, possibly even "core" polysaccharide, can not be ruled out (13). To determine whether there are true antigenic similarities between the strains that seem to fall into similar serogroups, adsorption studies of the appropriate antisera with the cross-reacting antigens would have to be carried out.

The eight possible groups proposed for the P. cepacia strains show some cross-reactivity between the groups. For example, strains K1036, K1075 and 2371 of Group II agglutinated with antisera to antigens belonging to Group II and to the antiserum to K981 in Group III (Table 11 a, b). Strains K358, 2369, 2368 and K360 from Group V agglutinated with antisera belonging to Group V but also with antisera against strains 2076 and K1075 which were assigned to Group II. An unusual nonreciprocal cross-reactivity pattern was

observed between strain B-1, belonging to Group IV and strain 2371 of Group II. Anti-B-1 reacted with B-1 and 2371 at a titer of 320; however, anti-2371 only reacted with 2371 and other strains in Group II (Table 11 a, b). Dudman (21) observed a similar occurrence between strains of Rhizobium japonicum and suggested that either cross-reacting antibodies were involved, or the antigenic determinants were present but only in limited amounts. Therefore, a similar phenomenon may be involved with these two strains of P. cepacia, but cross-adsorption studies would be necessary to clarify these points. Strains FB96, K998, FB143 and LU2800 assigned to Group VIII differed from all the other strains in that these strains as a group cross-reacted with 19 out of the 29 antisera prepared against the P. cepacia strains, even though their antisera only reacted with the four members of their group (Table 11 a, b, c). Cross-reactivity with the unadsorbed antisera prepared for typing O antigens of P. aeruginosa has also been reported to be a problem (22). Duncan, et al (22) were able to abolish cross-reactivity in the typing antisera without significantly reducing titers against the homologous strains in two ways, either by adsorbing with the specific O antigens cross-reacting or by adsorbing the antisera with a polyagglutinable strain. Therefore, the preparation of fully specific, non-cross-group-reacting antisera for the P.

cepacia groups may also be possible by either of these methods. The antiserum showing highest reactivity in each group would be adsorbed with cross-reacting antigens outside of the group or possibly with a poly-agglutinable strain as one of those in Group VIII to prepare antisera specific for members of its group.

The results of the biochemical reactions and antibiotic susceptibility and serological studies of these 38 strains of P. cepacia confirm previous observations that strains of P. cepacia share many distinct characteristics, yet the species is not very homogenous internally (49). Since distinct biochemical, antibiogram and tube agglutination patterns were observed, an attempt was made to see if the members of the eight serological groups also shared similar biochemical and antibiotic susceptibility characteristics.

Strains K1021 and D6460 which comprise serological Group I were found to be biochemically identical and unique. They produced ornithine decarboxylase but did not oxidize sucrose or reduce nitrate. They also oxidized gluconate but did not oxidize mannitol, produce β-galactosidase or form a yellow pigment (Tables 12 and 13). The MIC values showed that both strains are resistant to chloramphenicol, show indeterminate values to kanamycin and moderately resistant values for tobramycin (Tables 9 and 10).

The nine out of 13 members of serological Group II (that is, organisms 2077, 2076, 2416, K808, B4648, B4134, B-2, 2075 and 2078) (Table 11a) which were found to react most closely serologically were also found to have virtually identical biochemical reactions (Tables 12 and 13). These nine strains all produced ornithine decarboxylase, oxidized sucrose, produced a yellow pigment, hydrolyzed gelatin (Table 1) and did not reduce nitrate. The other four members of Group II showed quite variable biochemical reactions. Strains K1036 and K1075 did not produce ornithine decarboxylase nor reduce nitrate but they did oxidize sucrose (Table 12). K359 was positive for all three of these reactions. Strain 2371 reduced nitrate but did not produce ornithine decarboxylase or oxidize sucrose. The antibiograms of the organisms in this group were quite similar. Eleven out of the 13 strains had comparable MIC values against the nine antibiotics titered. All eleven of these strains showed low MIC values to chloramphenicol and intermediate or moderately resistant values to kanamycin (Table 9). Nine out of the 11 strains had resistant MIC values to carbenicillin --strains K1036 and B4648 were found to be sensitive. Seven out of these 11 strains had resistant MIC values to tetracycline--strains 2077, 2416, 2078 and 2075 showed intermediate values. The two remaining strains K359 and 2371, both showed much more sensitive drug susceptibility patterns (Table 9).

Strain K981, was serologically distinct and, therefore, was designated Group III. This organism was unique since it was the only one of the 38 P. cepacia strains tested which produced β-hemolysis on a Blood agar plate (Table 1). K981 was found to have similar MIC values to K1036 and K1075 of Group II in that they were only sensitive to chloramphenicol, trimethoprim-sulfamethoxazole and nalidixic acid (Table 7). The antiserum prepared against K981 also reacted with strains K1036 and K1075. Moreover, it was found that K981 biochemically resembles K1036 and K1075 in that all three strains oxidized sucrose but did not produce ornithine decarboxylase or reduce nitrate (Table 12).

Group IV, which consists of one member, B-1, appears to be distinguishable from most P. cepacia strains in that the MIC values showed it to be sensitive to tetracycline and carbenicillin (Table 9). However, B-1 does biochemically resemble organism 2371 of Group II. Both of these strains did not produce ornithine decarboxylase or oxidize sucrose but did reduce nitrate to nitrite (Table 12). These two strains are also similar to each other and different from other P. cepacia strains in that they did not produce a yellow pigment, hydrolyze casein but did produce lecithinase (Table 13). The two strains do not, however, have similar antibiograms.

The eight strains in Group V (Table 11 b) are biochemically very closely related and quite representative of a

"typical" P. cepacia strain. All 8 strains produced ornithine decarboxylase, oxidized sucrose and reduced nitrate to nitrite (Table 12). All 8 strains had very high MIC's (>64 mcg/ml) to kanamycin (Table 9). Seven of the 8 strains also showed very high MIC values to carbenicillin and were resistant to nalidixic acid (Table 7). Six of the 8 strains were sensitive to chloramphenicol.

Group VI, which is comprised of seven serologically closely related strains (Table 11c), was found to be quite homogenous in biochemical characteristics and antibiotic susceptibilities. All eight strains oxidized sucrose but did not produce ornithine decarboxylase or reduce nitrate. All the strains produced a yellow pigment and hydrolyzed esculin. Seven of the 8 strains hydrolyzed gelatin and casein (Tables 1, 12 and 13). This group of strains differed from most other strains of P. cepacia in that their MIC values for chloramphenicol were either intermediate or resistant. These 9 strains also showed very high MIC's (>512 mcg/ml) for carbenicillin but intermediate values for kanamycin (Table 9).

Strains B5910 and B5912, which are included in Group VII, are almost identical in their biochemical characteristics and are very similar in their drug sensitivities. Both strains produced ornithine decarboxylase, oxidized sucrose and reduced nitrate to nitrite (Table 12). Both strains were found to be resistant to all of the drugs tested except

chloramphenicol, trimethoprim-sulfamethoxazole and nalidixic acid (Table 7). They differed only in that B5910 produced a yellow pigment and B5912 did not, on all media on which the two strains were grown.

Strains FB96, FB143, K998 and LU2800 were all assigned to a single serological group (VIII) because of serological relatedness. Biochemically, the first three strains were also found to be quite similar. All three oxidized sucrose but did not produce ornithine decarboxylase or reduce nitrate (Table 12). Strain LU2800 was positive for all three of these reactions. All four strains had similar antimicrobial susceptibility patterns, showing very high MIC values to tetracycline and carbenicillin and resistance to kanamycin and nalidixic acid (Tables 7, 9). All of the strains except FB143 were found to be sensitive to chloramphenicol.

Thus, analysis of the biochemical, antibiotic susceptibility and serological properties of the P. cepacia strains indicates that differences in serological reactivity can be correlated with differences in antibiograms and biochemical reaction patterns. Therefore, it seems plausible that a simple agglutination procedure exists which permits the rapid presumptive identification and determination of the antibiograms of P. cepacia strains isolated from clinical specimens. Further studies of new isolates are required to confirm the full feasibility of this approach.

SUMMARY

A detailed analysis of the biochemical, antibiotic susceptibility and serological characteristics of 38 different clinical isolates of P. cepacia was conducted. Conventional methods for biochemical assays, described by Weaver, Gilardi and Pickett, and the API 20E Identification System were comparatively studied for their use in the identification of P. cepacia strains. Antimicrobial susceptibility patterns of P. cepacia were studied using the Kirby-Bauer single disk diffusion method and the Micro-Media Microtiter method. Serological reactivity of the 38 strains of P. cepacia was examined by preparing antisera against 29 strains of P. cepacia and testing the antisera against heat-killed antigens of P. cepacia by performing tube agglutinations. An attempt was then made to correlate the differences in serological reactivity with differences in antibiotic susceptibility and biochemical reaction patterns determined for the diverse strains of P. cepacia.

Conventional methods of identification of nonfermentative gram negative bacilli based on physiological characteristics were used to differentiate the P. cepacia strains from the other nonfermentative rods. These assays were found to be time-consuming and difficult to standardize. Results were cumbersome to compile and many variables had to

be considered to arrive at an identification of the strains. The API 20E strip was found to be easy to use, could be uniformly inoculated and identifications could be made in 24-48 hours. Some variabilities in the biochemical assay reactions were found between the two methods. However, 100% agreement was reached on the final identification of the 38 strains of P. cepacia. This highly satisfactory agreement between the API system when compared with the conventional methods and the rapidity and ease of identification with the API system definitely shows the API 20E system to be very useful in the rapid identification of P. cepacia.

Antibiotic susceptibility testing with the Kirby-Bauer and the MIC methods of the P. cepacia strains showed that most P. cepacia strains exhibit a combined resistance to tobramycin, colistin, amikacin, carbenicillin, gentamicin and tetracycline, a characteristic which distinguishes them from the other Pseudomonas sp. Therefore, antimicrobial susceptibility testing was found to be helpful in the differentiation of P. cepacia from other Pseudomonas sp. The MIC Microtiter system was studied with the 38 strains of P. cepacia in search for an alternative to the Kirby-Bauer method and for a more accurate system for drug susceptibility testing. The Micro-Media MIC determination method was found to be a highly standardized method which is simple to use and easy to read and interpret results. A high degree of correlation was found between the MIC values and

the Kirby-Bauer method results for the eleven drugs comparatively tested. The determination of MIC values is thought to be beneficial in the treatment of highly-resistant organisms, such as *Pseudomonas cepacia*, because the numerical drug susceptibility results can better be correlated with achievable blood levels and different routes of drug administration.

With the growing need to detect and document the spread of specific strains of organisms through hospitals, a serological analysis of *P. cepacia* was begun. Eight possible serogroups were determined from the 38 *P. cepacia* strains tested, based on the cross-reactivity seen between these strains in tube agglutination studies with unadsorbed antisera. True antigenic similarities between the strains cannot be determined at this point without adsorption studies of the appropriate antisera with cross-reacting antigens.

An analysis of the biochemical, antibiotic susceptibility and serological patterns of the *P. cepacia* strains indicates that differences in serological reactivity can be correlated with differences in antibiograms and biochemical reaction patterns. Thus, it may be possible to develop serotyping antisera which could be used as a diagnostic as well as epidemiological tool, permiting the rapid presumptive identification and determination of the antibiograms of *P. cepacia* strains isolated from clinical specimens.

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The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science in Microbiology.

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