

The Genus *Acinetobacter*

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Introduction

Gram-negative nonfermentative bacteria belonging to the genus *Acinetobacter* have been classified previously under at least 15 different names (Bergogne-Bérézin and Towner, 1996), and it is only relatively recently that rational taxonomic proposals have emerged. Delineation of species is still the subject of ongoing research, and a rapid and reliable method of assigning new isolates to individual species is particularly needed.

Notwithstanding the continuing taxonomic confusion, it is clear that acinetobacters are common, free-living saprophytes found in soil, water, sewage and foods. They are also ubiquitous organisms in the clinical environment, where they can be isolated as commensals from the skin of hospital staff and patients. They have increasingly been recognized as important nosocomial pathogens involved in outbreaks of hospital infection, particularly in high-dependency or intensive care units, where they rapidly develop resistance to even the most potent antimicrobials (Bergogne-Bérézin and Towner, 1996).

Although the significant pathogenic role of *Acinetobacter* is largely confined to nosocomial infections, it is important to note that these organisms are ubiquitous in both the clinical and nonclinical environments. Their metabolic versatility means that they may play an important role in a variety of commercially important industrial processes, as well as in the biodegradation of a wide range of environmental pollutants. While much remains to be learned about the lifestyles of the different members of the genus, it is clear that some facets of their behavior are unique and others provide new insights into prokaryotic behavior in general.

Members of the genus *Acinetobacter* have suffered a long history of extensive taxonomic change which has inhibited a proper appreciation and understanding of their significant biological properties and pathogenic importance. Although the delineation of species within the genus is still the subject of ongoing discussion and research, it appears that the current taxon-

omy should form a rational scientific foundation for the numerous important investigative studies required in the future.

Phylogeny

Members of the genus *Acinetobacter* have historically been classified by various authors under a variety of different names (Bergogne-Bérézin and Towner, 1996) and consequently, much of the early literature concerning this group of organisms is difficult to interpret owing to confusion over phylogeny and the lack of a widely accepted classification scheme. The genus *Acinetobacter*, as originally proposed in 1954, included a heterogeneous collection of nonmotile Gram-negative saprophytes that could be distinguished from other similar bacteria by their lack of pigmentation. The *Subcommittee on the Taxonomy of Moraxella and Allied Bacteria* subsequently proposed in 1971 that the genus *Acinetobacter* should include only the oxidase-negative strains, and this division has been supported by the use of transformation tests (Juni, 1978) that still form a good basis for inclusion of isolates in the genus.

{*Bergey's Manual of Systematic Bacteriology*} classified the genus *Acinetobacter* in the family *Neisseriaceae* (Juni, 1984), but more recent molecular taxonomic studies have resulted in the proposal that *Acinetobacter* should be classified in the new family *Moraxellaceae* (Rossau et al., 1991). This new family includes *Acinetobacter*, *Moraxella*, *Psychrobacter* and related organisms, and constitutes a discrete phyletic branch in superfamily II of the Proteobacteria on the basis of 16S rRNA studies and rDNA-DNA hybridization assays (van Landschoot et al., 1986; Rossau et al., 1989).

Taxonomy

Delineation of species within the genus *Acinetobacter* is still the subject of much research. Phenotypic identification of individual species is

complex and time-consuming (Gerner-Smidt et al., 1991). However, using the formal molecular definition (Wayne et al., 1987) of a microbial species — i.e., that a species should include strains of approximately 70% or greater DNA-DNA relatedness and 5°C or less divergence values (ΔT^m) — more than 20 separate genomic species (DNA-DNA homology groups) have been recognized within the genus by different research groups on the basis of DNA hybridization studies. Based on the taxonomic recommendation that only genomic groups readily distinguishable by phenotypic methods and containing more than 10 strains should be given names, seven *Acinetobacter* genomic species have been given formal species names (Table 1). Genomic species 1, 2, 3 and 13TU are closely related and are sometimes referred to as the *A. calcoaceticus*–*A. baumannii* complex for epidemiological purposes.

Certain genomic species were described independently by Bouvet and Jeanjean (1989) and Tjernberg and Ursing (1989a), and there are some minor discrepancies in the numbering systems. To avoid further confusion, it is current practice to add the suffix BJ or TU to denote the genomic species delineated by the two studies. In addition, many reports in the scientific literature have reported *Acinetobacter* isolates that cannot be identified with any of the formally recognized genomic species. One such isolate from the Venice lagoon has been given the species name *A. venetianus* by its discoverers Di Cello et al. (1997) and has been shown to belong to the same

genomic species as the industrially important strain RAG-1 (Vanechoutte et al., 1999). Because many of the strains studied in DNA-DNA hybridization studies have been derived from hospital sources, and because the most common habitats of these organisms are soil and water, it seems clear that many naturally occurring genomic species of *Acinetobacter* have yet to be delineated and that the current taxonomic listing is incomplete (Nemec et al., 2000). Unfortunately, many reports of *Acinetobacter* in the scientific and medical literature still do not use the latest taxonomy or use inadequate identification methods. Although phenotypic identification is problematical, various molecular methods have been developed in an attempt to provide a rapid identification method suitable for routine taxonomic and epidemiological use.

Habitat

Certain acinetobacters are normal inhabitants of human skin, and members of the genus have increasingly been implicated as a presumed causal or contributory agent in numerous infectious disease processes. In addition to their occurrence in a range of clinical specimens (e.g., tracheal aspirates, blood and urine), acinetobacters can be readily isolated from moist skin areas, such as toe webs, the groin and the axilla. Other reservoirs of these organisms may include a range of both moist and dry surfaces and equipment within the hospital environment as well as

Table 1. Formally recognized genomic species of *Acinetobacter*.^a

Genomic species number	Genomic species name	Type strain
1	<i>Acinetobacter calcoaceticus</i>	ATCC 23055
2	<i>Acinetobacter baumannii</i>	CIP 70.34
3	Not named	ATCC 19004
13TU	Not named	ATCC 17903
4	<i>Acinetobacter haemolyticus</i>	ATCC 17906
5	<i>Acinetobacter junii</i>	ATCC 17908
6	Not named	ATCC 17979
7	<i>Acinetobacter johnsonii</i>	ATCC 17909
8	<i>Acinetobacter lwoffii</i>	ATCC 15309
9	Not named	ATCC 9957
10	Not named	ATCC 17924
11	Not named	ATCC 11171
12	<i>Acinetobacter radioresistens</i>	IAM 13186
13BJ	Not named	ATCC 17905
14	Not named	Bouvet 382
15BJ	Not named	Bouvet 240
15TU	Not named	Tjernberg 151a
16	Not named	ATCC 17988
17	Not named	Bouvet 942

^aNumerous published reports refer to *Acinetobacter* isolates that cannot be identified with any of the genomic species listed above. Such new isolates have not yet been formally grouped or given species names, although a newly named species, *Acinetobacter venetianus*, has been proposed (Di Cello et al., 1997).

the patients and staff (Bergogne-Bérézín and Towner, 1996).

Many studies within the genus *Acinetobacter* have been performed with clinical isolates. However, acinetobacters are also ubiquitous organisms in soil, water and sewage (Towner, 1996). It has been estimated that *Acinetobacter* may constitute as much as 0.001% of the total heterotrophic aerobic population of soil and water (Baumann, 1968). They have been found at densities exceeding 10^4 organisms per 100 ml in freshwater ecosystems and 10^6 organisms per 100 ml in raw sewage (LaCroix and Cabelli, 1982). They can be isolated from heavily polluted water, such as that found in wastewater treatment plants, but are found more frequently near the surface of fresh water and where fresh water flows into the sea (Droop and Jannasch, 1977).

Acinetobacters also are found in a variety of foodstuffs, including eviscerated chicken carcasses, various poultry and other meats, milk products and vegetables. It has been reported that acinetobacters constitute up to 22.7% of the total microflora of chicken carcasses. It is also known that acinetobacters are involved in the economically important spoilage of foods such as bacon, chicken, eggs and fish, even when stored under refrigerated conditions or following irradiation treatment (Towner, 1996).

It is worth noting that there is a significant population difference between the acinetobacters found in clinical and other environments. The vast majority of clinically significant isolates belong to the *A. calcoaceticus*-*A. baumannii* complex, whereas genomic species 7 (*A. johnsonii*), 8 (*A. lwoffii*) and 9 seem to predominate in foods and the environment. Other genomic species appear to comprise only minority components of the different populations investigated, but they may have evolved to acquire a selective advantage in as yet unrecognized specialized ecological niches.

Isolation

Isolation of members of the genus *Acinetobacter* can be accomplished using standard laboratory media such as trypticase soy agar or brain heart infusion agar. A differential medium such as MacConkey agar may be helpful in recognizing colonies of *Acinetobacter* on primary isolation. The optimum growth temperature for most strains is 33–35°C, but many clinically significant isolates will grow well at 37–42°C. In contrast, some environmental and food spoilage isolates have considerably lower optimum growth temperatures and may be unable to grow at 37°C.

Selective Enrichment

Most strains of *Acinetobacter* can grow in a simple mineral medium containing a single carbon and energy source such as acetate, lactate or pyruvate. An enrichment culture procedure for isolating members of the genus from soil and water has been described (Baumann, 1968). Liquid enrichment cultures containing 20 ml of medium (see below) are inoculated with a 5-ml sample of water, or a filtered 10% soil suspension, and vigorously aerated at either 30°C or room temperature. Cultures are examined microscopically after 24 or 48 h and streaked on to suitable isolation media. Strains of *Acinetobacter* have a slightly acid pH optimum for growth, and vigorous aeration at a pH of 5.5–6.0 favors their enrichment.

Baumann's Enrichment Medium

Sodium acetate (trihydrate)	2 g
KNO ₃	2 g
MgSO ₄ · 7H ₂ O	0.2 g

Dissolve in 0.04 M KH₂PO₄.Na₂HPO₄ buffer (pH 6.0), to a total volume of 1 liter.

It should be noted that the original recipe (Baumann, 1968) also contained a complex trace element supplement, but this addition is now rarely considered to be necessary as there are sufficient trace elements in the other medium components.

Differential Isolation on Solid Medium

Selective liquid enrichment is rarely used for isolation of *Acinetobacter* from clinical specimens. For clinical isolation, general purpose media such as blood agar or MacConkey agar are usually preferred because of their broad bacterial coverage, but in certain circumstances, it may be preferable to use a selective medium that suppresses the growth of other bacteria. Such a medium also can be used for plating out liquid enrichment cultures. An antibiotic-containing selective medium, Leeds *Acinetobacter* Medium (see below), has been found useful for the recovery of most *Acinetobacter* genomic species from clinical and environmental sources (Jawad et al., 1994).

Leeds *Acinetobacter* Medium

Bacteriological agar	15 g
Acid casein hydrolysate	15 g
Neutralized soy peptone	5 g
NaCl	5 g
D-(–) Fructose	5 g
Sucrose	5 g
D-Mannitol	5 g
L-Phenylalanine	1 g
Ferric ammonium citrate	0.4 g
Phenol red	0.02 g

Add ingredients to 1 liter of distilled water. Steam to dissolve ingredients. Mix well. Adjust pH of medium to 7.0. Autoclave for 15 min at 121°C and 15 lb/in². Cool to 50–55°C and then add the following ingredients (per liter): 10 mg of vancomycin, 15 mg of cefsulodin and 50 mg of cephadrine.

Mix well. Pour on plates. Pack in plastic bags and store at 4°C for up to 2 weeks.

Identification

Morphology

Short, plump, Gram-negative rods, typically 0.9–1.6 by 1.5–2.5 μm in the logarithmic phase of growth, but often becoming more coccoid in the stationary phase. The cell wall ultrastructure is typical of Gram-negative bacteria in general, but the cells are occasionally difficult to destain. Cells commonly occur in pairs, but also in chains of variable length. No spores are formed and flagellae are absent. Although generally considered to be nonmotile, “twitching” or “gliding” motility has been reported to occur, particularly on semisolid media. Many strains are encapsulated, and the capsule may be readily seen in India wet ink mounts. Colonies are usually nonpigmented, but some strains form white-to cream-colored colonies, which vary in consistency from butyrous to smooth and mucoid, and from 1–2 mm in diameter.

Phenotypic Identification

All members of the genus *Acinetobacter* are strict aerobes and can grow at a wide range of temperatures. Most strains will grow at 33°C, but some environmental isolates prefer incubation temperatures from 20–30°C. Clinical isolates of *Acinetobacter* will normally grow at 37°C and some strains can grow at 42°C. All acinetobacters are oxidase-negative and catalase-positive. The negative oxidase reaction serves to distinguish the genus from other related genera. Most strains of *Acinetobacter* can grow in a simple mineral medium containing a single carbon and energy source. A wide variety of organic compounds can be used as carbon sources by particular strains, although relatively few strains can use glucose. Most strains are unable to reduce nitrate to nitrite in the conventional nitrate reduction assay. A few clinical strains may show hemolysis on blood agar plates owing to the production of phospholipase C. Unlike other members of the *Moraxellaceae*, most strains are resistant to penicillin and many clinical isolates are resistant to cephalosporin antibiotics because of the overproduction of a chromosomal cephalosporinase.

There is no single biochemical test that enables ready differentiation of this genus from similar bacteria, but the nonfastidious nature and wide biochemical activities of the members of the genus makes them readily distinguishable from other bacteria at the genus level by the combination of nutritional tests applied to nonfastidious, nonfermentative organisms in general, including most commercially available diagnostic devices and systems. Phenotypic identification to the genomic species level is more problematic and time-consuming. A scheme of 22 phenotypic tests has been described that differentiates most of the genomic species known at the present time (Kämpfer et al., 1993), but this scheme is laborious and time-consuming. Most commercial rapid identification systems are inadequate for distinguishing the different genomic species, but promising results have been obtained with the automated Biolog system which involves the detection of oxidation with 95 different carbon sources (Dijkshoorn, 1996).

Serological Identification

A large number of capsular types have been identified by means of capsular reactions or immunofluorescence. This has resulted in the delineation of a large number of different serovars in two of the main genomic species (spp. 2 and 3) associated with infection in man (Traub and Leonhard, 1994). Inasmuch as all recently investigated lipopolysaccharide (LPS) molecules from clinical *Acinetobacter* isolates have been shown to be of the smooth phenotype, a serotyping scheme for identification of clinically important members of the genus may be possible (Pantophlet et al., 1998).

Genetic Identification

Members of the genus are themselves only rarely transformable, but can be easily identified by testing the ability of their isolated DNA to transform a nutritional or antibiotic resistance marker to the naturally competent strain BD413 originally isolated by Juni (1972). Transformation of this highly competent strain occurs readily, either on semisolid media or in liquid culture, and has been used as the basis of a test for the identification of *Acinetobacter* in clinical specimens (Brooks and Sodeman, 1974). The DNA samples from unrelated bacteria have consistently failed to transform the auxotrophic strain BD413 to prototrophy (Juni, 1972), and this test is consequently believed to allow unambiguous attribution of unknown strains to the *Acinetobacter* genus. There is currently no genetic test for the identification of individual genomic species.

Molecular Identification

A genus-specific 16S rDNA-targeted oligonucleotide probe has been used to recognize acinetobacters in general (Wagner et al., 1994). However, most work on the development of molecular methods has been dedicated to developing methods for distinguishing the individual genomic species. The "gold standard" method is DNA-DNA hybridization (Tjernberg et al., 1989b), but this technique is rather laborious and is normally used only in special situations in reference laboratories. Consequently, many research groups have concentrated on the development of alternative molecular methods for distinguishing individual genomic species. Unambiguous differences in rDNA sequences have been found in the highly variable regions of 16S rDNA molecules from at least 21 different genomic groups (Ibrahim et al., 1997), although the limited number of strains examined means that these findings cannot be relied upon for absolute identification of genomic species at the present time. It also should be noted that the groupings based on 16S rDNA analysis did not completely correlate with those based on DNA-DNA homology data. This is in contrast with an alternative strategy in which phylogenetic groupings were based on the nucleotide sequences of topoisomerase (*gyrB*) genes (Yamamoto and Harayama, 1996).

As an alternative to direct sequence-based identification, a range of more rapid molecular fingerprinting methods have been developed for distinguishing individual genomic species, with varying degrees of success. These methods can be divided into those based on structural features, such as outer-membrane protein patterns (Ino and Nishimura, 1989; Dijkshoorn et al., 1990), and those based on nucleic acid analysis. The most widely used techniques amongst the latter group include amplified fragment length polymorphism (AFLP) analysis (Janssen and Dijkshoorn, 1996), amplified rDNA restriction analysis (ARDRA; Vaneechoutte et al., 1995; Dijkshoorn et al., 1998), ribotyping (Gerner-Smidt, 1992), tDNA spacer fingerprinting (Ehrenstein et al., 1996) and 16S-23S spacer analysis (Dolzani et al., 1995).

Preservation

Cultures of *Acinetobacter* spp. can be stored for short periods (a few weeks) on nutrient agar slants kept at room temperature. It should be noted that many strains do not seem to survive for more than short periods in the refrigerator at 4°C. For long-term preservation, lyophilization should be used, or a heavy suspension of cells in brain heart infusion broth supplemented with

20% v/v glycerol can be frozen at -80°C. For regrowth, it is usually possible to simply streak out a loopful of the frozen stock without the necessity to thaw the entire suspension.

Physiology

The main identifying biochemical and physiological characteristics of the genus *Acinetobacter* have been discussed in the context of identification and only the most significant aspects are reiterated in this section. Early work on the metabolism of the genus has been comprehensively reviewed previously (Juni, 1978). Numerous subsequent papers describing enzymes, cofactors, metabolic pathways, products and other physiological aspects have confirmed that the *Acinetobacter* are strictly aerobic and broadly typical of other Gram-negative eubacteria, but with a number of distinctive physiological features that support the versatile lifestyle of this genus. It is worth emphasizing that many strains of *Acinetobacter* used in physiological studies were originally isolated in the 1970s or earlier and have never been properly identified to the genomic species level, and that many detailed physiological studies have been based on work with only one or a very limited number of strains.

Although rare strains of *Acinetobacter* showing growth factor requirements have been isolated, the vast majority of strains resemble saprophytic pseudomonads in being able to use any of a large range of organic compounds as a carbon and energy source in an otherwise mineral medium. Although the utilization of carbohydrates is relatively uncommon, the major biochemical feature of the genus is that many strains are able to metabolize a range of compounds including aliphatic alcohols, some amino acids, decarboxylic and fatty acids, unbranched hydrocarbons, sugars, and many relatively recalcitrant aromatic compounds such as benzoate, mandelate, *n*-hexadecane, cyclohexanol and 2,3-butanediol (Juni, 1978). Many such aromatic compounds are converted by acinetobacters to β -ketoacid, which is degraded in turn to succinate and acetyl-CoA (it should, however, be noted that the precise spectrum of compounds degraded is frequently strain-specific). Members of the genus are therefore particularly suitable organisms for studying a variety of unusual biochemical pathways, and they also may have a role to play in degrading a range of pollutants and industrial products.

Central metabolism seems to be based on a Krebs tricarboxylic acid cycle, but with a rather unusual regulatory system in which AMP simultaneously modulates the activities of a number

of the constituent enzymes. This feature may act in a concerted manner to direct metabolic flux through the cycle, and such multipoint regulatory control may be a significant feature of energy metabolism in *Acinetobacter* spp. Numerous complex catabolic pathways feed into the tricarboxylic acid cycle, and various biosynthetic pathways, such as those involved in gluconeogenesis and amino acid synthesis, are subtly influenced by the need to integrate with the overall metabolism of the organism (Towner et al., 1991b). Most strains are unable to utilize glucose as a carbon source, but occasional rare strains are able to do so via the Entner-Doudoroff pathway. Many acinetobacters are, however, able to acidify media containing sugars, including glucose, via an aldose dehydrogenase. This property has previously been considered to be of major taxonomic significance in the subdivision of the genus, but DNA-DNA hybridization studies now suggest that this is not the case unless considered in combination with unrelated biochemical properties. Nevertheless, the property of glucose acidification is still sometimes useful for initial presumptive identification of the main genomic species (the *A. calcoaceticus*-*A. baumannii* complex) involved in human disease.

Although most strains of *Acinetobacter* are unable to reduce nitrate to nitrite in the conventional nitrate reduction assay, both nitrate and nitrite can be used as nitrogen sources by means of an assimilatory nitrate reductase. All acinetobacters are oxidase-negative, since they lack cytochrome *c*, but they do contain cytochromes *b*, *o* and occasionally *d* and *P-450* cytochrome.

One important difference from many organisms is that *Acinetobacter* cannot incorporate extracellular thymine or thymidine into DNA. Enzyme analysis has revealed that *Acinetobacter* lacks the enzymes thymidine phosphorylase, nucleoside deoxyribosyltransferase and thymidine kinase, but does contain enzymes for conversion of thymidine-5'-monophosphate to thymidine-5'-triphosphate. Polyphosphate has been shown to be accumulated by some acinetobacters in wastewater, and it has been suggested that these organisms could possibly remove inorganic phosphate from sewage in activated sludge. A high-affinity phosphate uptake system, inducible in the absence of inorganic phosphate (Kortstee et al., 1994), and a polyphosphate kinase induced by phosphate starvation (Geissdörfer et al., 1998) have both been demonstrated.

For further information on these and other biochemical pathways found in *Acinetobacter*, the interested reader should consult detailed reviews available elsewhere (Juni, 1978; Towner et al., 1991b; Towner et al., 1996).

Genetics

Modes of Gene Transfer

All three of the major modes of gene transfer are known to occur in *Acinetobacter*.

Transformation

The transfer of genetic material by transformation in a strain of *Acinetobacter* was first demonstrated in 1969 and forms the basis of the genetic test for the identification of members of the genus (Juni, 1972). Genetic competence for transformation seems to be a rare trait in *Acinetobacter* (Juni, 1978), and most transformation studies have been performed with the highly competent strain BD413. Studies of the conditions for quantitative transformation in strain BD413 have shown that competence occurs throughout the life cycle, but with a peak early in the exponential growth phase (Cruze et al., 1979). The highest transformation frequencies (0.5–0.7%) were obtained in an aspartate-containing medium which allowed the most rapid growth of BD413. Recipient cell concentrations of $1-6 \times 10^6$ cells per ml were found to give the highest transformation frequencies, regardless of the DNA concentration. Similar findings were reported with strain NCIB 8250 (Ahlquist et al., 1980), which seemed to have two peaks of competence, one during the early phase of batch culture and a second minor peak at the beginning of the stationary phase. The presence of cyclic AMP was found to increase the transformation frequencies obtained. Investigations into the basis for DNA uptake in competent strains of *Acinetobacter* have associated DNA uptake with components involved in assembly of the type IV pilus (Palmen and Hellingwerf, 1997; Link et al., 1998).

Nutritional and other markers (e.g., antibiotic resistance) are all transformed readily to competent strains. Early studies in the 1970s used transformation for preliminary genetic mapping of genes concerned with capsule biosynthesis, tryptophan biosynthesis and proline biosynthesis. More recently, catabolic pathways in *Acinetobacter* have been analyzed by natural transformation (Kloos et al., 1995), and the process has also been used to introduce randomly mutated genes into the chromosome of a competent recipient strain (Kok et al., 1997). Using previously isolated *Acinetobacter* genes modified to incorporate a *NotI* recognition sequence, the process of natural transformation has been exploited to generate a physical and genetic map of more than 40 genes on the circular c.3.8-kb chromosome of strain ADP1 (BD413UE; Galton et al., 1997).

Transduction

A large number of bacteriophages active against specific strains of *Acinetobacter* have been isolated (Ackermann et al., 1994). Most *Acinetobacter* phages are lytic, but one temperate phage (P78) which lysogenizes its host strain and is capable of mediating generalized transduction has been isolated (Herman and Juni, 1974). Phage P78 is specific for its host strain and failed to lysogenize 389 other independently isolated strains of *Acinetobacter*, including the transformation-competent strain BD413. This narrow host specificity may be accounted for by the large number of different surface antigens found in this genus (Marcus et al., 1969). At present, neither P78 nor any other bacteriophage has been used for extensive genetic studies in *Acinetobacter*.

Conjugation

Conjugation in the genus *Acinetobacter* was first reported in 1976 in strain EBF65/65 (Towner and Vivian, 1976a). The broad-host-range plasmid RP4 was used as a vector and was found to be capable of mobilizing chromosomal genes between different mutant derivatives of EBF65/65. Transfer of the chromosome occurred at detectable frequencies only on solid surfaces (not in liquid matings) and mobilization occurred from at least two different chromosomal locations (Towner and Vivian, 1976b). Chromosomal transfer also has been shown to be mediated by the naturally occurring *Acinetobacter* plasmid pAV1 (Hinchliffe and Vivian, 1980). Little is known about the precise nature and events involved in mobilization of the chromosome by RP4 and pAV1, but these systems have been successfully used to map a number of different mutations on a circular chromosomal linkage group in strain EBF65/65 (Towner, 1978; Vivian, 1981; Vakeria et al., 1984). A range of plasmids belonging to different incompatibility groups are capable of transfer by conjugation to *Acinetobacter* from enteric bacteria, although not all are stably maintained or capable of subsequent rounds of transfer (Chopade et al., 1985).

Genetic Organization and Regulation

So far as the gross topological structure of the chromosome is concerned, it is known only that the chromosome of strains EBF65/65 and BD413 is circular. There is some preliminary evidence that *Acinetobacter* is more akin to *Pseudomonas* than enteric bacteria in that functionally related genes can be located at several different positions on the chromosome (Towner, 1978; Gralton et al., 1997).

In contrast, knowledge of the fine structure of certain specific regions of the chromosome is quite well advanced for the highly competent *Acinetobacter* strain BD413. Particular attention has focused on the organization and regulation of genes concerned with the tryptophan biosynthetic and the β -keto adipate degradative pathways. Striking similarities exist between the individual genes from *Acinetobacter* spp. and *Pseudomonas* spp. in these pathways, but considerable gene rearrangements seem to have occurred during the evolutionary process. Although the complete regulatory circuits for the tryptophan biosynthetic pathway have yet to be fully resolved in *Acinetobacter*, studies of the β -keto adipate pathway have indicated that *Acinetobacter* strain BD4 exhibits induction patterns unlike those found in *Pseudomonas* spp. However, an important characteristic shared with *Pseudomonas* is that genes for physiologically interdependent steps in the β -keto adipate degradative pathway tend to be linked together in supra-operonic clusters, a feature that may have important regulatory implications. Mapping and regulatory studies on these and related pathways in strain BD413 continue to be published regularly in the scientific literature. Detailed information on the formative studies can be found in previously published review articles (Haspel et al., 1991; Ornston and Neidle, 1991).

Plasmids

Several studies have reported that more than 80% of *Acinetobacter* isolates carry multiple indigenous plasmids of variable molecular size (Gerner-Smidt et al., 1989; Seifert et al., 1994), although other studies have reported problems in isolating plasmid DNA from *Acinetobacter* spp., often because of unappreciated difficulties in lysing the cell wall of these organisms. Most indigenous plasmids from acinetobacters seem to be relatively small (<23 kb), and therefore probably lack conjugative functions.

As with many other groups of organisms, interest has focused particularly on plasmids associated with resistance to antibiotics. Although many clinical isolates of *Acinetobacter* show widespread and increasing resistance to a whole range of antibiotics, few studies have demonstrated plasmid-mediated transfer of resistance genes. This may partly reflect a lack of conjugative functions on indigenous plasmids, but also may reflect the absence of a suitable test system for detecting such transfer. For historical reasons, attempts to transfer plasmids from clinical isolates of any Gram-negative species have tended to use *Escherichia coli* K12 as a recipient strain. Complex and varied transfer frequencies

of standard plasmids belonging to different incompatibility groups have been observed between *E. coli* K12 and *Acinetobacter* strain EBF 65/65, and a number of these plasmids required an additional mobilizing plasmid for retransfer to occur (Chopade et al., 1985). Accordingly, it is not surprising that most reported cases of indigenous transmissible antibiotic resistance from *Acinetobacter* have been associated with plasmids belonging to broad host-range incompatibility groups (Towner, 1991a).

Apart from antibiotic resistance, genes encoding resistance to heavy metals (Kholodii et al., 1993) and important metabolic steps in the degradation of organic compounds and environmental pollutants, such as polychlorinated biphenyls (PCBs), have been shown to be carried on plasmids in *Acinetobacter* (Towner, 1991a; Fujii et al., 1997). Studies to date indicate clearly that though there is a pool of plasmid-mediated genetic information that is confined largely to *Acinetobacter*, a group of plasmids can cross the boundaries between *Acinetobacter* and other distinct genetic pools.

A range of cloning and shuttle vectors for in-vitro genetic manipulation experiments in *Acinetobacter* have been described (Ditta et al., 1985; Singer et al., 1986; Hunger et al., 1990; Gutnick et al., 1991; Minas and Gutnick, 1993).

Transposons and Integrons

Transposons probably play an important role in ensuring that particular novel genes can become established in a new gene pool, even if the plasmid vectors that transferred them are unstable. There have been several reports of chromosomally located transposons carrying multiple antibiotic resistance genes in clinical isolates of *Acinetobacter* (Towner, 1991a). In general, such transposons closely resemble those found in other Gram-negative bacteria. Transposons also have been used in conjunction with suicide plasmid vectors to introduce mutations to the *Acinetobacter* chromosome (Towner, 1991a; Leahy et al., 1993).

Integrons are conserved genetic elements which encode a site-specific recombination system that enables the insertion, deletion and rearrangement of discrete genetic cassettes within the integron structure (Stokes and Hall, 1989). Most, but not all, cassettes identified to date have been associated with antibiotic resistance, and large numbers of clinical isolates of *Acinetobacter* have been shown to carry integrons incorporated into their chromosome (Gonzalez et al., 1998; Seward and Towner, 1999; Gallego and Towner, 2001). It is clear that clinical isolates of *Acinetobacter* seem to share resis-

tance mechanisms with many other genera, and it has been suggested that integron structures make an important contribution to the dissemination of antibiotic resistance genes in the clinical setting.

Ecology

Species of Clinical Importance

In addition to carriage by patients and staff, various strains of *Acinetobacter* can be isolated from a wide range of both moist and dry inanimate sources in the hospital environment, including formica table tops, dust, ventilatory equipment, humidifiers, mattresses, urinals and wash basins. Numerous studies have now supported the observation that *A. baumannii* and its close relatives are the main genomic species associated with outbreaks of hospital infection. Thus, a typical study of 584 *Acinetobacter* isolates from 420 patients at 12 different hospitals over a 12-month period identified 426 (72.9%) strains as *A. baumannii* (Seifert et al., 1993). This large study also identified 158 isolates as belonging to species other than *A. baumannii*, of which the most common were *Acinetobacter* sp. 3 (55 isolates), *A. johnsonii* (29 isolates) and *A. lwoffii* (21 isolates).

Very little is known about the clinical significance of other *Acinetobacter* genomic species and further detailed investigations are required. The ubiquitous occurrence of acinetobacters in the environment, and as commensals on human skin, means that such isolates in clinical specimens are often considered to be contaminants. Diagnosis of infection with "unusual" *Acinetobacter* genomic species therefore often depends on clinical indications and repeated isolation of the same strain from a single patient. *Acinetobacter* spp. 3 and 13TU have often been implicated in documented outbreaks of infection involving nosocomial spread, whereas *A. junii* and *A. johnsonii* have been associated with point-source outbreaks. It is worth re-emphasising the close relationship between genomic species 1, 2, 3 and 13TU. This "*A. calcoaceticus*-*A. baumannii* complex" contains isolates that are mostly glucose-acidifying. The majority of glucose-negative, nonhemolytic strains found in clinical specimens seem to be identified mainly as *A. lwoffii*, *A. johnsonii* or *Acinetobacter* sp. 12, and it seems that these species are natural inhabitants of human skin. Most of the hemolytic isolates are identified as *A. haemolyticus* or *Acinetobacter* sp. 6. Other groups seem to be implicated only occasionally in human infections (Bergogne-Bérézin and Towner, 1996).

Species from the Environment

Acinetobacters are ubiquitous organisms that can be obtained easily from soil, food, water and sewage with appropriate enrichment techniques, but surprisingly few studies exist in which *Acinetobacter* isolates from environmental sources have been grouped according to the latest taxonomic criteria.

So far as food isolates are concerned, a study of 170 *Acinetobacter* isolates from various food sources (including fresh and spoiled meat and fish, vegetables, raw milk and cheese) demonstrated a clear difference in the distribution of genomic species between food and clinical isolates (Gennari and Lombardi, 1993), with *A. lwoffii* and *A. johnsonii* predominating in foods. The ubiquitous occurrence of *A. johnsonii* in the environment has also been demonstrated in several studies of polyphosphate-accumulating acinetobacters found in wastewater treatment plants (Kämpfer et al., 1992; Knight et al., 1993).

It therefore appears that there is a significant population difference between the acinetobacters found in clinical and other environments. Each population appears to be characterized by predominant groups of genomic species — spp. 2 (*A. baumannii*) and 3 in clinical environments, and spp. 7 (*A. johnsonii*) and 8/9 (*A. lwoffii*) in other environments. Other genomic species appear to constitute only minority components of the different populations. Some genetic interchange between populations is clearly possible for those groups that are capable of growing at both 37°C and lower environmental temperatures, and this fact may have implications for the spread of antibiotic resistance and metabolic genes.

Epidemiology

Human Carriage

At least 25% of healthy individuals may carry acinetobacters as part of their normal skin flora, but carriage of *Acinetobacter* spp. by healthy subjects at other body sites is normally low (Bergogne-Bérézin and Towner, 1996). In contrast, high colonization rates of the skin, throat, respiratory tract or digestive tract of hospitalized patients with clinically significant strains have been reported during outbreaks of infection, with infections involving mechanically ventilated intensive care unit (ICU) patients being associated particularly with a high colonization rate of the respiratory tract. Such patients often have heavy skin colonization, which probably contributes to the spread and persistence of outbreaks via the hands of hospital staff during trivial contacts (Bergogne-Bérézin and Towner, 1996).

From a clinical viewpoint, the isolation of strains belonging to genomic species 1, 2, 3 or 13TU in environmental cross-infection studies should be considered to be of greater epidemiological significance than the detection of genomic species associated mostly with nonhuman sources. The observed discrepancies in carriage rates between hospitalized patients and the normal population suggest that infecting or colonizing organisms in hospitals may be derived more often from cross-transmission or hospital environmental sources than from endogenous sources (e.g., the intestinal and digestive tract of the patients themselves). Differentiation of colonization from infection is not always easy, and many isolates, particularly from the skin, should be considered as colonizing rather than infecting organisms. However, the skin, respiratory tract and superficial wounds also should be considered as potentially important reservoirs of infecting organisms during outbreak situations (Bergogne-Bérézin and Towner, 1996).

Environmental Persistence

Acinetobacter spp. spread easily in the environment of infected or colonized patients, and can persist in that environment for many days or weeks, even in dry conditions on particles and dust. Air contamination is relatively rare, except in the immediate vicinity of infected or colonized patients, but numerous studies have documented the isolation of acinetobacters from various hospital equipment and surfaces (e.g., respirators, air humidifiers, mattresses, pillows, bedside cabinets and cupboards, telephones, door handles, patient charts, floors, washcloths, etc.; Bergogne-Bérézin and Towner, 1996). Survival in the environment is probably helped by the ability of *Acinetobacter* spp. to resist drying (Jawad et al., 1996) and to grow at a range of different temperatures and pH values (Bergogne-Bérézin and Towner, 1996). The population differences found between “clinically significant” and “environmental” isolates means that it is important to identify isolates to the genomic species level and type them (Dijkshoorn, 1996) before any firm epidemiological conclusions can be reached.

Disease

Bacteria belonging to the genus *Acinetobacter* are now recognized as increasingly important opportunistic pathogens causing a wide spectrum of nosocomial infection. Although acinetobacters are generally regarded as relatively low-grade pathogens, they can cause occasional severe infection, predominantly pneumonia,

particularly in immunocompromised patients who have undergone major surgery or trauma, or those with severe underlying disease (e.g., burns, immunosuppression and malignancy). Other opportunistic infections reported include septicemia, endocarditis, meningitis, skin and wound sepsis, and urinary tract infection. Although these organisms are associated primarily with nosocomial infection, some cases of community-acquired infection have also been reported (Bergogne-Bérézin and Towner, 1996).

One of the main reasons for disease caused by *Acinetobacter* is the fact that clinical isolates of these bacteria are frequently multiply resistant to the major antibiotics used to treat nosocomial infection. Widespread resistance to the major groups of antibiotics, particularly following the recent emergence of resistance to the carbapenem antibiotics, means that *Acinetobacter* infections are difficult to treat successfully, even with combination therapy. Indeed, the extensive use of antibiotics in hospitals probably alters the normal flora and actually results in the selection of resistant strains of *Acinetobacter*. Combined with their capacity for long-term survival in the hospital environment, this makes control of outbreaks of hospital infection caused by *Acinetobacter* one of the most difficult challenges faced by infection control teams.

Applications

A number of patent applications incorporating these organisms in various industrial processes have been awarded. An important advantage is that acinetobacters are easy to isolate, cultivate and manipulate genetically in the laboratory.

Pollution Control

The general biochemical versatility of members of the genus has resulted in *Acinetobacter* spp. receiving considerable attention as possible industrial microorganisms. In particular, the normal soil and water habitats of many strains, combined with their ability to degrade a wide range of organic compounds, has suggested that acinetobacters can be used for bioremediation of numerous hazardous and unpleasant waste and residue pollutant compounds produced as by-products of commercial processes. Thus, aromatic compounds which are toxic to most microorganisms, such as salicylate, halogenated aromatics and phenol, are capable of being degraded by acinetobacters (Schirmer et al., 1997). In many cases, total degradation occurs by the synergic action of complex microbial communities in which acinetobacters form an impor-

tant component. Certain acinetobacters are capable of elaborating and excreting polymers (of which the most well-studied is a polyanionic, cell-associated, heteropolysaccharide termed "emulsan" produced by strain RAG-1) that emulsify hydrocarbons, such as oils (Foght et al., 1989), thus making these substrates available for degradation in an aqueous environment (Gutnick et al., 1991; Navon-Venezia et al., 1995; Barkay et al., 1999).

A more controversial area concerns the ability of acinetobacters to remove phosphate from wastewaters. Phosphate removal from wastewater to minimize the problem of eutrophication is an important feature of sewage treatment plants. It can be shown in the laboratory that certain strains of *Acinetobacter* can accumulate polyphosphates, and examination of activated sludge samples from sewage treatment plants has shown that acinetobacters form a significant part of the constituent microflora. It has therefore been postulated that acinetobacters could form the basis of an inexpensive biological method for the direct removal of phosphate from wastewater, but their precise role (and its feasibility) in such a process remains a matter of some debate.

Biopolymers and Biosurfactants

Apart from its use in the degradation of oil, emulsan has a whole range of potential applications in the petroleum industry, including viscosity reduction during pipeline transport following formation of heavy oil:water emulsions, and production of fuel oil:water emulsions for direct combustion. The affinity of purified emulsan for the oil:water interface also has implications for the stability of oil emulsions during transport and storage, and also for their biodegradability following accidental spillage (Gutnick et al., 1991). In addition, U.S. Patent No. 4,619,825, filed on behalf of the Colgate-Palmolive Company, describes the use of emulsan-containing formulations for either mouthwash or toothpaste which have the effect of significantly reducing dental plaque formation and enhancing anti-carries activity.

A second important polysaccharide produced by a strain of *Acinetobacter* has been shown to bind to inorganic materials such as calcium carbonate (limestone). This polysaccharide has been termed "biodispersan" (Rosenberg et al., 1988) and is capable of dispersing limestone in water. Because limestone is used in a wide range of industrial processes, purified biodispersan is considered to have potential applications in manufacturing processes producing common products such as paper, paints and ceramics. A further bioemulsifying polysaccharide, termed "alasan," is produced by *Acinetobacter radiore-*

Table 2. Suggested applications for *Acinetobacter* spp. and their products.

Bioremediation of waste waters and effluents
Phosphate removal
Degradation of petrochemicals
Breakdown of organic pollutants
Production of biopolymers and biosurfactants
For prevention of dental plaque
For use in paper-making and other industries
For efficient emulsification of oil waste pollutants
For incorporation in cosmetics, detergents and shampoos
Biomass production
Single cell protein production
Manganese leaching from ores
Production of immune adjuvants
Clinical uses
Production of glutaminase-asparaginase
Production of L(-) carnitine

From Towner (1996).

sistens strain KA53 (Navon-Venezia et al., 1995; Navon-Venezia et al., 1998; Barkay et al., 1999).

Some other suggested uses for *Acinetobacter* spp. and their products are summarized in Table 2 and have been reviewed elsewhere (Towner, 1996).

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