Clinical Studies on a Transformation Test for Identification of *Acinetobacter* (Mima and Herellea)

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Deoxyribonucleic acid (DNA) from 250 strains of aerobic, nonfermentative, gram-negative coccobacilli and rods were tested for the ability to transform a stable competent auxotroph of *Acinetobacter* (strain *trp E 27*) to prototrophy by using the method established by Juni. Several modifications of Juni's original procedure were made to adapt it for use in a clinical diagnostic laboratory. These modifications were directed primarily towards shortening the procedure to allow completion in a time framework consistent with current procedures. The modifications included changes in sterilization temperature, incubation time and temperature of the competent auxotroph and DNA preparation, overnight incubation temperature, and variations in the age of the auxotroph culture when used. Under these conditions, the transformation can easily be performed in 24 h, the final 16 to 18 h being an overnight uninterrupted incubation period. When used in conjunction with the glucose oxidative fermentative basal metabolism test, it provided a rapid highly efficient means for grouping and identifying acinetobacters which is far superior to a biochemical schema. Without exception, the 141 strains of DNA from *Acinetobacter* species were able to transform the auxotroph to prototrophy. None of the 105 oxidase-positive nonfermenters possessed DNA which was able to transform the *Acinetobacter* auxotroph to prototrophy.

In recent years there have been reports in the literature linking aerobic, nonfermentative, nonmotile, gram-negative rods and coccobacilli with disease (1–4, 6–9, 11–14). Clinically, therefore, it is becoming increasingly more important that these organisms be identified rapidly and accurately. In the clinical laboratory phenotypic characters are generally used to identify bacteria because of the ease with which they can be detected by morphological study and biochemical testing. Genetic analysis has long been thought of as slow and cumbersome for use by a routine clinical diagnostic laboratory. However, recently Juni (10) described a simple test for interspecies transformation of *Acinetobacter*. The method, as outlined by Juni, can be performed in a minimum of 26 h, including two 12-h incubation periods. With this test it is possible to separate the *Acinetobacter* group (Mima and Herellea) with a high degree of accuracy from other nonfermentative organisms. Acinetobacters can further be broken down into two distinct groups with the aid of the glucose oxidative fermentative basal metabolism test—carbohydrate oxidizers (Herellea) and nonoxidizers (Mima). Since these two groups have not yet been given species names, in this paper we shall refer to them as *Acinetobacter* oxidizer and *Acinetobacter* nonoxidizer, respectively.

It was the purpose of this study to determine whether the transformation test would be a suitable assay for use in the clinical laboratory. Initial experiments confirmed that Juni's procedure does distinguish acinetobacters from other gram-negative bacilli; however, it could not be completed within a 24-h period because of the incubation periods. To be advantageous as a diagnostic tool, it was felt that the initial steps in the transformation test must be performed within the limits of an 8-h day, followed by a final incubation period overnight. This would allow completion of the entire test within a 24-h
period, consistent with current biochemical methods.

This study was undertaken to determine whether modifications in the temperature used for sterilization, in the incubation time and temperature of the competent auxotroph and deoxyribonucleic acid (DNA) preparation, in the overnight incubation temperature, and variations in the age of the auxotroph culture would allow completion of the test within 24 h with sufficient transformation of the competent Acinetobacter auxotroph trp E 27 to prototrophy.

MATERIALS AND METHODS

Two hundred and fifty aerobic, nonfermentative, gram-negative coccobacilli and rods were used in this study. Two hundred and twenty-six were fresh clinical isolates and 24 were stock cultures (Table 1). All organisms were isolated in pure culture on either MacConkey agar (BBL), 5.0% sheep blood agar (BBL), or chocolate agar (BBL), depending on the nutritional requirements of the isolate. If on the basis of distinct colonial morphology a clinical isolate appeared to be a nonfermentative coccobacillus or rod, it was identified according to the following biochemical tests: (i) oxidase; (ii) motility; (iii) Simmons citrate; (iv) P agar; (v) nitrate; (vi) growth at 42 C; (vii) 1% oxidative-fermentative (OF) glucose; (viii) 1% OF maltose; (ix) 1% OF xylose; (x) lecithinase; (xi) deoxyribonuclease; and (xii) brain heart infusion broth, slide motility. If the organism cannot be identified with the initial eight biochemical reactions, then tests (ix) through (xii) are inoculated. If an identification still cannot be made, additional biochemical tests may be employed. For information on interpretation of results, the reader is referred to Gilardi (11). Fifty-five strains of Pseudomonas aeruginosa and all other nonfermentative coccobacilli and rods isolated during a 4-month period were tested for their ability to transform the competent strain of Acinetobacter.

The method for transformation is essentially identical to Juni’s (10). The competent auxotrophic mutant (trp E 27) used in this study was obtained from E. Juni. This auxotrophic mutant was maintained on brain heart infusion agar (BBL; dehydrated) at room temperature.

A crude transforming DNA suspension was prepared as outlined by Juni (10) with the following modifications. The suspension was contained in a 10-mm disposable polystyrene culture tube. Organisms were dispersed in the solution by continued agitation of the inoculating loop. The suspended cells were heated in a 70-C water bath for 2 h with occasional manual shaking. Two hours at 70 C was found to effect sterile DNA preparations, whereas 1 h at 60 C left some cells viable in cases where large clumps of organisms could not be dispersed.

An 18- to 24-h culture of trp E 27 auxotroph and DNA preparation were mixed and incubated at 31 C for 3 to 4 h. At the same time the sterility of the DNA preparation was confirmed. About ten DNA samples were tested on each plate.

After incubation, growth in each of the inoculated areas was streaked on a sector of a lactate mineral agar plate, and a loopful of the stable auxotroph was streaked on another sector of the plate to serve as the non-DNA-treated control as outlined by Juni (10). These plates were incubated at 31 C overnight (16 to 18 h) (Fig. 1a and b). Small colonies visible to the naked eye appeared after incubation if the auxotroph had been transformed to prototrophy. Plates were read and reincubated for an additional 24 h, at which time prototrophic colonies were much larger. This was done solely to insure that no strain of DNA which had converted the auxotroph to prototrophy was read as negative for transformation after 16 h of incubation simply because the prototrophic colonies were too small to be seen at that time.

RESULTS AND DISCUSSION

According to Juni (10), transformation of strain trp E 27 with DNA from various acinetobacters results in more prototrophic recombinant colonies than when strain lv1-10 is used. Therefore, the former strain was selected for our study.

Juni indicated (personal communication) that a culture of the auxotroph trp E 27 could be several days old and still give a positive transformation test. Our findings confirmed this, but we also noted that an 18- to 24-h culture of the competent auxotroph trp E 27 showed visible prototrophic colonies sooner and in greater numbers after being treated with Acinetobacter DNA than a 3- or 4-day-old culture. This is probably related to the growth phase of the younger cultures. It must be emphasized that for the transformation test to be successfully completed in 24 h from the time it is begun, the auxotroph must be transferred daily to a fresh brain heart infusion agar plate so that an 18- to 24-h culture is available. Bacterial cultures 24 h to 2 weeks old, as well as nonviable organisms,

| Organism                           | Fresh isolates | Stock cultures | Total |
|------------------------------------|----------------|----------------|-------|
| Acinetobacter oxidizer             | 128            | 1              | 129   |
| Acinetobacter nonoxidizer          | 11             | 1              | 12    |
| Oxidase-positive organisms         | 83             | 22             | 105   |
| Pseudomonas maltophilia           | 4              | 0              | 4     |
| Totals                             | 226            | 24             | 250   |

*This category includes the following: Alcaligenes species (6), Flavobacterium species (2), Moraxella species (13), Pseudomonas aeruginosa (55), P. alcaligenes (3), P. cepacia (2), P. diminuta (5), P. fluorescens (3), P. kingii (3), P. pseudoalcaligenes (1), P. putida (7), and P. stutzeri (5).
Transformed prototrophic colonies after 16 (1a) and 40 (1b) h of incubation at 31 C, respectively. A, Acinetobacter oxidizer; B, Acinetobacter nonoxidizer; C, trp E 27 control; D, P. aeruginosa; and E, Moraxella. After 16-h incubation at 31 C, prototrophic colonies in Fig. 1a, sections A and B, are extremely small and are barely visible in this figure due to photographic limitations. They are, however, easily visible to the naked eye. The colonies are present, as evidenced by the same plate photographed at 40 h (1b). No strain of DNA from these various organisms is positive for transformation after 40 h of incubation that was not positive after 16 h of incubation. DNA from strains of Moraxella and P. aeruginosa did not transform the competent auxotroph, whereas DNA from a strain of an Acinetobacter oxidizer and an Acinetobacter nonoxidizer did transform the auxotroph to prototrophy.

were used to make transforming DNA. In support of Juni’s findings, the age and viability of cultures was not found to be critical.

Juni has stated that acinetobacters have an optimal temperature near 35 C and recommended that the transformation test be performed at 35 C. Experiments were performed at 25, 31, 35, and 36.5 C. At 25 and 36.5 C, prototrophic colonies were not visible to the naked eye after 16-h incubation on lactate mineral agar, but were visible after 40-h incubation. Parallel tests at 31 and 35 C showed no significant difference in detection of growth after 16 to 18 h. Colonies do, however, appear slightly larger at 35 C after 20 h of incubation than those grown at 31 C. Either temperature may be used and will allow completion of the transformation test within a 24-h period. The transformation test in this study was performed at 31 C because of the availability of an incubator at this temperature.

It was found that the crude DNA and the competent auxotroph needed to be mixed and incubated at 31 C for a minimum of 1 h in order for some cells to be transformed to prototrophy. We selected 3 to 4 h of incubation at 31 C rather than 1 h because a larger number of cells were transformed to prototrophy, thus producing more prototrophic colonies and earlier detection.

Of the 141 strains tested, not one was found to transform the competent auxotroph to prototrophy after 40 h of incubation that had not shown small colonies which were read as positive transformation after 16 h of incubation.

The DNA samples from 141 strains of Acinetobacter oxidizers and nonoxidizers used in this study were able to transform the competent auxotroph trp E 27 to prototrophy. These strains were uniformly gram-negative, oxidase-negative, and nonmotile. The remaining 105 gram-negative, oxidase-positive strains representing various genera (Alcaligenes, Flavobacterium, Moraxella, and Pseudomonas) and four oxidase-negative strains of P. maltophilia were not able to transform trp E 27 to prototrophy. On the basis of biochemical identification, nine out of 250 strains gave transformation results contrary to those anticipated from biochemical reactions. Seven strains gave a positive transformation test after having been identified as P. maltophilia (5), P. cepacia (1), and Moraxella (1) by biochemical methods. Two strains identified as acinetobacters failed to give a positive transformation test and when reidentified were Alcaligenes sp. Subsequent testing revealed that all of these nine strains were misidentified.
due to an incorrectly interpreted biochemical characteristic.

This study confirms Juni's findings (10) that oxidase-positive organisms thus far studied are unable to transform the competent Acinetobacter auxotroph to prototrophy. All 141 Acinetobacter oxidizers and nonoxidizers used in this study were able to transform Juni's competent Acinetobacter trp E 27 strain to prototrophy.

On the basis of the results of this study, we feel that the method outlined for the interspecies transformation of Acinetobacter is a quick and reliable procedure. The original procedure as established by Juni remains the accepted method. The modifications of the procedure presented here are intended for use in the diagnostic clinical laboratory to allow completion of the transformation test rapidly with the best visualization of colonies. The authors recommend that all aerobic, nonfermentative, gram-negative, oxidase-negative, nonmotile rods and coccobacilli be tested by this method. The transformation tests run simultaneously with a triple sugar iron agar slant, motility test, glucose oxidative fermentative basal metabolism test, and oxidase test will be sufficient to identify all Acinetobacter oxidizers and nonoxidizers. The transformation test will not necessarily speed up the identification of Acinetobacters (neither will it cause delay) when run concurrently with the biochemical tests necessary for identification, but it provides an undisputed degree of accuracy in identification while at the same time eliminating the need for extensive biochemical testing.

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