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Identification of *Yersinia pestis* by BBL Crystal Enteric/Nonfermenter Identification System

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The BBL Crystal Enteric/Nonfermenter System (Crystal) was used to test 25 archived isolates of *Yersinia pestis* to obtain a unique biochemical profile code for *Y. pestis*. The revised Crystal system and the API 20E system were compared by using 12 clinical human isolates of *Y. pestis*. Crystal correctly identified 11 of the 12 isolates, while API correctly identified 7 of the 12 isolates.

Yersinia pestis, the bacterial agent of plague, has been commonly misidentified as *Y. pseudotuberculosis* and less frequently as other members of the family *Enterobacteriaceae* by miniaturized rapid biochemical test systems (1). This is partially due to the slow growth and relative biochemical inactivity of *Y. pestis*. Rapid commercial identification systems favor the faster-growing and more biochemically active *Enterobacteriaceae.*

As new miniaturized biochemical test systems are created for commercial use, problems associated with their use continue to occur. *Y. pestis* is not included in some of the taxonomic databases of systems used to identify other *Enterobacteriaceae*. One high-passage laboratory strain of *Y. pestis* is generally used by manufacturers to generate a single biochemical profile as typical for all isolates, but this practice does not consider the biochemical diversity possible with lower-passage field strains. The use and acceptance of these new systems by diagnostic laboratories result in a greater probability of misidentifications (4). Laboratorians who are unfamiliar with the reactivities of *Y. pestis* may accept an incorrect identification without challenging their results.

This study evaluated the ability of BBL Crystal Enteric/Nonfermenter System (Crystal; Becton Dickinson Microbiological Systems, Cockeysville, Md.) versions 1.1 and 3.0, as a rapid miniaturized biochemical identification system, to identify *Y. pestis* uniquely. Evaluations of Crystal have been done with other *Enterobacteriaceae* (2, 7).

Twenty-five isolates of *Y. pestis* from the archived collection at the Centers for Disease Control and Prevention, Fort Collins, Colo., were chosen to represent geographical and host diversity (Table 1). The biochemical diversity of these isolates was determined with the API 20E system (API; bioMérieux Vitek, Hazelwood, Mo.). An additional 12 clinical human isolates of *Y. pestis*, from 1994, were used to test the accuracy of the revised version of Crystal (version 3.0) and were comparatively tested with a traditional rapid miniaturized biochemical identification system, API.

The manufacturer's instructions were modified as follows: sterile wooden applicator sticks were used to pick colonies, and the number of colonies picked was increased threefold. Supplemental oxidase and indole tests were done as required by the systems with BBL dropper reagents (Becton Dickinson Microbiological Systems). Crystal results were obtained by using a software package supplied by the manufacturer (versions 1.1 and 3.0). API results were obtained by using the manufacturer's identification book and call-in phone system (version 10).

Crystal version 1.1 generated 12 different biochemical profile codes from the 25 archived isolates. The most frequently generated profile codes were 0700004000 and 0700004040. Less frequently generated codes were 0700044000, 0700044040, 0600004000, 0600004040, 0600044000, and 0600044040. With these data, a revised version of Crystal (version 3.0) was created.

Comparison of the Crystal version 3.0 and API results obtained with the 12 clinical isolates of *Y. pestis* produced the biochemical profile codes in Table 2. Crystal uniquely identified 6 of the 12 isolates as *Y. pestis*, dually identified 5 of the 12 isolates with a note to test further for both *Y. pseudotuberculosis* and *Y. pestis*, and incorrectly identified 1 of the 12 isolates as *Y. pseudotuberculosis*. However, in the last instance, when the statistics window of the software codebook was queried, the second choice of Crystal, after *Y. pseudotuberculosis*, was *Y. pestis*. Misidentifications by Crystal were due primarily to the sorbitol and p-n-p-β-galactoside reactivities. API correctly identified 7 of the 12 clinical isolates, incorrectly identified 4 of the 12 as *Shigella boydii*, and incorrectly identified 1 of the 12 as *Enterobacter agglomerans*. These misidentifications by API reflect the failure of the system to incorporate the fact that *Y. pestis* can ferment arabinose and sorbitol. The newest version of API (version 10.1, 1996) has updated profile codes to include various arabinose fermentation rates for *Y. pestis.*

Misidentification rates by miniaturized systems, while usually low, can become a problem with less biochemically active or rarely encountered bacteria (2, 3, 5, 7, 10). *Y. pestis* grows slowly on standard media; often, only pinpoint colonies are visible at 24 h. In rapid miniaturized systems, the advantage goes to the faster-growing bacteria. An inoculum 10-fold larger than the manufacturers' recommendation was tried to simulate the more rapidly growing bacteria but produced many false-positive reactions. An inoculum threefold greater than the manufacturer's suggestion produced more reliable results with isolates of *Y. pestis* for Crystal and API. The inoculum size is limited by the amount of a reactive substrate.

The first phase of this evaluation assessed the possibility of generating a unique biochemical profile code that could be incorporated into the Becton Dickinson Microbiological Systems database for the identification of *Y. pestis*. The code for *Y. pestis* had to be distinct enough (in statistical distribution by mathematical matrix) to separate this bacterium from its relatives, especially the less biochemically reactive bacteria. As

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TABLE 1. Twenty-five archived isolates of *Y. pestis*

Strain	Place of origin	Yr of isolation	Source of strain
P EXU 2	Brazil	1966	Mouse
P EXU 16	Brazil	1966	Human
HARBIN 35	Manchuria, People's Republic of China	1940	Human
ECUADOR 7	Ecuador	1965	Human
PB6	Barabank, India	1955	Fleas
JAVA 5	Java, Indonesia	1957	Fleas
H 21/66	Ovamboland, Namibia	1966	Human
F 361/66	Ovamboland, Namibia	1966	Fleas
NEPAL 516	Nepal	?	?
RHAMGIRI	Nepal	?	Human
15-91	Russia	1965	Human
15-39	Russia	?	?
15-36	Russia	?	?
CS617	California	1966	Squirrel[a]
70-259-6f[b]	California	1970	Fleas
A1122	California	1943	Laboratory
742790/JB	New Mexico	1974	Human
77NM-538	New Mexico	1977	Human
742328A/VJ	New Mexico	1979	Human
TX932321	Texas	1993	Squirrel[c]
TX931278	Texas	1993	Rat
13-18 (Urea+)	Vietnam	?	Laboratory
13-18 (Urea−)	Vietnam	?	Laboratory
16-34	Vietnam	?	?
16-53	Vietnam	?	?

[a] Golden-mantled ground squirrel.
[b] This strain, used for Crystal, is the same strain used for the API 20E system. However, the source was fleas from the rodent that was the source of strain 70-259-6.
[c] Fox squirrel.

reactions with increased sensitivity are designed, the likelihood that low biochemical reactivities will be detected will increase. *Y. pestis* was found to display more diverse biochemical reactivity, despite the geographic location, than previously thought. Crystal's design of 30 sensitive miniaturized biochemical reactions demonstrated the heterogeneity of *Y. pestis* easily while still distinguishing it from its close relatives, in most cases. The more tests, the greater the likelihood of discovering differences (2).

The second phase of this evaluation was to determine the usefulness of the newly incorporated codes with Crystal version 3.0 for clinical isolates of *Y. pestis*. API was compared to Crystal because API had been used at the Centers for Disease Control and Prevention since 1974 to provide biochemical profile codes for *Y. pestis*. Crystal was easy and safe to use. Enclosing the inoculated system with a snap-on lid greatly decreased the possibility of hazardous spills. Since no reagents were added after the primary inoculation, the possibility of operator error was also decreased. While reading weakly positive reactions was difficult at first, this task became easier with familiarization. The medium for the bacterial suspension was included in Crystal, which again reduced the chance for operator error. Revised version 3.0 of Crystal should be helpful as a supplemental diagnostic microbiological method of identification.

TABLE 2. Biochemical profile codes and identifications[a] obtained by API 20E version 10.0 and Crystal version 3.0 for 12 human clinical isolates of *Y. pestis*

Isolate	API 20E code (identification)	Crystal code (identification)
AZ940445	1004100 (1)	0720004000 (2)
AZ940666	1004100 (1)	0720044000 (1)
AZ940670	1004100 (1)	0720044000 (1)
CA940045	1004100 (1)	2700004000 (1)
CO940719	1004100 (1)	0720004000 (2)
CO941428	1004503 (3)	0720004000 (2)
NM940501	1004102 (3)	0700044000 (1)
NM940838	1004100 (1)	0720004000 (2)
NM941139	1004102 (3)	4720044000 (2)
OK940827	1004102 (3)	0720224100 (3)
UT941201	1004100 (1)	0700004000 (1)
ZE942122[b]	1004102 (3)	0700044000 (1)

[a] Identifications: 1, correctly identified as *Y. pestis*; 2, dual identification as *Y. pestis* and *Y. pseudotuberculosis*; 3, incorrect identification.
[b] From Matabeleland, Zimbabwe (only clinical isolate from outside the United States).

API version 10 did not perform as expected for identification of *Y. pestis*. A recent update of API (version 10.1, 1996), which now includes the variations in arabinose fermentation so often seen with isolates of *Y. pestis*, should increase the accuracy of this system. In previous studies, API correctly identified 88 to 99.4% of the enteric, nonfermentative bacteria tested (3, 5–9), and API 20NE correctly identified 75.3% of these bacteria (10). These older systems clearly misidentify the less reactive bacteria, which could lead to misdiagnosis.

No rapid biochemical identification system should stand alone as a diagnostic tool. Whether or not bacterial isolates have clinical significance, their correct identification can help rule out potential pathogens in a patient sample (9). Conventional bacteriology is still needed in the laboratory diagnosis of plague. Further, if currently available rapid biochemical identification systems do not incorporate *Y. pestis* into their databases, then laboratorians and physicians must be aware of this omission (1).

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