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Evaluation of L-Pyrrolidonyl Peptidase Paper Strip Test for Differentiation of Members of the Family *Enterobacteriaceae*, Particularly *Salmonella* spp.

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The L-pyrrolidonyl peptidase activities of 1,033 strains of the family *Enterobacteriaceae* were investigated by the paper strip method to evaluate their usefulness for screening those organisms, especially *Salmonella* cultures. We also evaluated the usefulness of indole and tryptophan deaminase paper strip tests as supplements to the L-pyrrolidonyl peptidase test for the rapid identification of *Salmonella* cultures. The paper strip tests are simple, and the results are obtainable within 10 min.

Many methods are available for the identification of microorganisms. In recent years, enzymatic tests that use chromogenic or fluorogenic substrates have been developed for a rapid screening of bacterial species (1, 3, 5). Mulczyk and Szewczuk (4) suggested that the test for L-pyrrolidonyl peptidase (PYR) activity is of great value in differentiating between *Salmonella* and *Citrobacter freundii* complex strains. Chagla et al. (2), who studied approximately 800 cultures of strains of the family *Enterobacteriaceae*, indicated that the PYR test is particularly useful for separating *Salmonella* and *Escherichia coli* strains from *Citrobacter* spp.

In the study described in this report, we evaluated the usefulness of the tests as an aid in differentiating members of the family *Enterobacteriaceae*, with special reference to *Salmonella* and *Citrobacter* spp. Also, simple and rapid methods for the detection of indole (ID) and tryptophan deaminase (TDA) as supplements to the PYR test are described.

All tests were carried out by the paper strip method. The paper strips (10 by 40 mm) were cut from Whatman no. 3 filter paper and were then soaked in a solution of substrate. These strips were dried overnight in an incubator at 37°C and were stored in a tightly capped brown bottle at −20°C. A 0.2% solution of L-pyrrolidonyl-β-naphthylamide (Sigma) in 95% ethanol was used for the PYR strip. For the ID and TDA tests, paper strips soaked in a solution (0.2%) of L-tryptophan in 1/15 M phosphate buffer (pH 7.2) were prepared in the same manner. These strips were stable for at least 6 months at −20°C.

For each test, the paper strips were wetted with 1/15 M phosphate buffer (pH 7.2) just before use, and then one or two colonies grown overnight on isolation media such as MacConkey, XLD, and Hektoen enteric agar plates were streaked onto the strip. For the PYR test, after 10 min of incubation at 37°C, one drop of a 1% solution of *p*-dimethylaminocinnamaldehyde in 1 N HCl was applied to the inoculated area on the strip. The development of a red or a violet-red color within 1 min was interpreted as a positive result, and when the area remained straw colored, the result of the test was read as negative. For the ID and TDA strips, after 10 min of incubation at 37°C in a moist chamber, one drop of Kovac's indole reagent for the ID test or a 10% aqueous solution of $FeCl_3$ for the TDA test was applied to the inoculum. The development of a red color for the ID test and a brown color for the TDA test was regarded as a positive reaction.

A total of 1,033 strains representing 84 species of 28 genera belonging to the family *Enterobacteriaceae* were studied. The majority of these strains were stock cultures from several institutions, including the Centers for Disease Control and Prevention, Atlanta, Ga.; Institut Pasteur, Paris, France; Institut Pasteur de Lille, Villeneuve d'Ascq, France; Statens Seruminstitut, Copenhagen, Denmark; National Institute of Health, Tokyo, Japan; American Type Culture Collection, Rockville, Md.; and National Collection of Type Cultures, London, England. Approximately 300 strains were clinical isolates.

Each strain was tested independently at three institutions. If there was a discrepancy in the test results between the laboratories, a repeat test of the strain was performed. The results of the PYR test are provided in Table 1. By the PYR test, all strains of the genera *Buttiauxella*, *Citrobacter*, *Ewingella*, *Klebsiella*, *Leclercia*, *Pantoea* (*Pantoea agglomerans*), *Pragia*, *Rahnella*, *Serratia*, and *Yersinia* gave uniformly positive reactions, and all strains of the genera *Budvicia*, *Cedecea*, *Edwardsiella*, *Hafnia*, *Kluyvera*, *Leminorella*, *Moellerella*, *Morganella*, *Obesumbacterium*, *Proteus*, *Providencia*, *Salmonella*, *Shigella*, *Tatumella*, *Trabulsiella*, and *Yokenella* were negative. Within the genus *Enterobacter*, 10 of 11 species were PYR test positive, but *Enterobacter intermedius* was negative by the PYR test. Among five species of the genus *Escherichia*, *Escherichia coli* and *Escherichia blattae* were PYR test negative, while the other three species were positive. In general, the results obtained by the PYR test were similar to those obtained by previous investigators (2).

In evaluating the PYR test for the screening of *Salmonella* spp., 376 strains were examined. They comprised 235 strains of biogroup I (*S. enterica* subsp. *enterica*) including 110 serovars, 141 of the other six biogroups of *Salmonella* spp., and 137

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TABLE 1. Results of the PYR paper strip test for species belonging to the family *Enterobacteriaceae*

Species	No. of strains tested	PYR test result (no. of strains)	
		Positive	Negative
Budvicia aquatica	2	0	2
Buttiauxella agrestis	5	5	0
Cedecea spp., 3′ species	12	0	12
Citrobacter spp., 6 species	157	157	0
Edwardsiella spp., 3 species	22	0	22
Enterobacter spp., 10 species	47	47	0
Enterobacter intermedius	8	0	8
Escherichia blattae	1	0	1
Escherichia coli	20	0	20
Escherichia spp., 3 other species	22	22	0
Ewingella americana	6	6	0
Hafnia alvei	9	0	9
Klebsiella spp., 5 species	55	55	0
Kluyvera spp. 2 species	9	0	9
Leclercia adecarboxylata	10	10	0
Leminorella spp., 2 species	7	0	7
Moellerella wisconsensis	3	0	3
Morganella morganii	10	0	10
Obesumbacterium proteus	1	0	1
Pantoea agglomerans	9	9	0
Pragia fontium	4	4	0
Proteus spp., 4 species	26	0	26
Providencia spp., 4 species	34	0	34
Rahnella aquatilis	4	4	0
Salmonella spp., 7 biogroups	376	0	376
Serratia spp., 9 species	74	74	0
Shigella spp., 4 species	29	0	29
Tatumella ptyseos	3	0	3
Trabulsiella guamensis	4	0	4
Yersinia spp., 10 species	60	60	0
Yokenella regensburgei	4	0	4
Total	1,033		

strains of the *Citrobacter freundii* complex (H_2S-positive *Citrobacter* spp.). All strains of *Salmonella* were found to be PYR test negative, and those of the *C. freundii* complex were all positive. This finding suggests that if H_2S-positive colonies on isolation agar plates are found to be PYR test negative, they could be regarded as *Salmonella* spp. H_2S-nonproducing biovars or serovars of *Salmonella* such as serovars choleraesuis, paratyphi A, and typhi may sometimes be misidentified as lactose nonfermenters such as *E. coli*, *Proteus* spp., and *Providencia* spp. However, if the ID and TDA paper strip tests are used as supplementary tests, such organisms may easily be distinguished from *Salmonella* spp. within a few minutes. The results of these tests with *Salmonella*, *Edwardsiella*, lactose-nonfermenting *E. coli*, *Proteus*, and *Providencia* spp. are provided in Table 2.

TABLE 2. Results of ID and TDA paper strip tests as supplements to PYR test

Species (biogroup)	No. of strains tested	No. of strains positive by		
		PYR test	ID test	TDA test
Salmonella spp. (biogroup 1)	235	0	0	0
Edwardsiella tarda	10	0	10	0
Escherichia coli (lactose negative)	20	0	20	0
Proteus mirabilis	10	0	0	10
Proteus vulgaris	10	0	10	10
Providencia spp.	34	0	34	34

In conclusion, we have confirmed that the PYR paper strip method is useful as a supplementary test for the identification of species or genera belonging to the family *Enterobacteriaceae*. In particular, it is useful for distinguishing *Salmonella* from *Citrobacter* spp. and, if the ID and TDA strip tests are also used, from *Edwardsiella*, lactose-nonfermenting *E. coli*, *Proteus*, and *Providencia* spp. These tests are simple, and the results are obtainable within 10 min.

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