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API *Listeria*, a New and Promising One-Day System To Identify *Listeria* Isolates

J. BILLE,[1]* B. CATIMEL,[2] E. BANNERMAN,[1] C. JACQUET,[2] M.-N. YERSIN,[1] I. CANIAUX,[3] D. MONGET,[3] AND J. ROCOURT[2]

Centre National de Référence des Listeria, World Health Organization Collaborating Center for Foodborne Listeriosis, Centre Hospitalier Universitaire Vaudois, 1011 Lausanne, Switzerland,[1] and Centre National de Référence pour la Lysotypie et le Typage Moléculaire de Listeria, World Health Organization Collaborating Center for Foodborne Listeriosis, Institut Pasteur, 75724 Paris Cedex 15,[2] and BioMérieux, 38390 La Balme-les-Grottes,[3] France

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API *Listeria* is a new 10-test strip for 24-h biochemical identification of *Listeria* isolates. With this commercial system, 85% of 646 *Listeria* strains, including atypical isolates selected for this study, were recognized at the species and subspecies level without a complementary test. A new test differentiates *Listeria monocytogenes* from *L. innocua* on the basis of the absence of arylamidase from the former. With this system, 97.7% (252 of 258) of the *L. monocytogenes* strains tested were correctly identified and differentiated from 99.4% (175 of 176) of the *L. innocua* strains also tested. Gram-positive bacteria other than *Listeria* spp. gave quite different biochemical patterns. This system considerably reduced the time needed for conventional identification, since results were available within 18 to 24 h.

The genus *Listeria* comprises six recognized species: *L. monocytogenes*, *L. ivanovii* subspp. *ivanovii* and *londoniensis* (3), *L. innocua*, *L. welshimeri*, *L. seeligeri*, and *L. grayi* (2, 28). All of these species are psychrotrophic and widely spread in the environment, but only *L. monocytogenes* is a significant human and animal pathogen. During the last decade, *L. monocytogenes* emerged as a foodborne pathogen, and various contaminated foodstuffs (milk and dairy products, meat and meat products, vegetables, and seafood) were implicated in major outbreaks and sporadic cases in North America and Europe (10). The total cost of human listeriosis was estimated to $255 million per year, and food recalls were evaluated at $15 million in 1985 through 1987 (27). This situation led to regular controls of both processed and nonprocessed food and their environment-related sources. Since all *Listeria* species are potential food contaminants, rapid and reliable detection and identification of *L. monocytogenes* appear to be of utmost importance.

Rapid methods such as flow cytometry (9), enzyme-linked immunosorbent assay techniques (21), DNA hybridization (6, 16, 24), and the polymerase chain reaction (1, 33) have been recently developed to specifically detect *Listeria* spp. and, in some cases, more precisely *L. monocytogenes*. Nevertheless, in many laboratories, isolation followed by identification of the microorganism remains the method used to detect *Listeria* spp. (19, 22). At the present time, *Listeria* isolates are mainly recognized on the basis of morphological and biochemical characteristics (2). Among these characteristics, hemolysis is the only marker that distinguishes *L. monocytogenes* (hemolytic and pathogenic) from *L. innocua* (nonhemolytic and nonpathogenic), the two species most frequently isolated from foodstuffs (4, 25).

The purpose of this study was to evaluate a new API system, specifically designed to identify *Listeria* isolates at the genus, species, and subspecies levels. The strip consists of 10 tests and allows complete identification in 24 h without additional tests such as hemolysis.

* Corresponding author.

MATERIALS AND METHODS

Bacterial strains. The following 646 *Listeria* strains were tested in Paris and Lausanne: *L. monocytogenes*, 258 strains; *L. ivanovii* subsp. *ivanovii*, 37 strains; *L. ivanovii* subsp. *londoniensis*, 38 strains; *L. innocua*, 176 strains; *L. welshimeri*, 47 strains; *L. seeligeri*, 76 strains; *L. grayi*, 14 strains. Type strains of each species, reference strains for serotyping, and some *L. ivanovii* subsp. *londoniensis* strains were tested in both laboratories.

In addition, the following 31 bacteria belonging to other gram-positive genera were included: *Jonesia denitrificans*, 1 strain; *Brochothrix thermosphacta*, 6 strains; *B. campestris*, 1 strain; *Kurthia zopfii*, 1 strain; *Enterococcus avium*, 2 strains; *E. durans*, 2 strains; *E. faecalis*, 2 strains; *E. faecium*, 2 strains; *Lactobacillus rhamnosus*, 2 strains; *L. paracasei* subsp. *casei*, 2 strains; *L. ofilis*, 1 strain; *L. plantarum*, 1 strain; *Carnobacterium mobile*, 1 strain; *C. piscicola*, 1 strain; *C. divergens*, 1 strain; *Erysipelothrix rhusiopathiae*, 1 strain; *Oerskovia turbata*, 1 strain; *Corynebacterium xerosis*, 1 strain; *Rhodococcus equi*, 1 strain.

Identification by conventional methods. Before the strains were identified with the API *Listeria* system, they were identified by using morphological and biochemical characteristics in Lausanne (2) and the API 50 CH and hemolysis tests in Paris (29, 30).

API test procedure. The API *Listeria* system (BioMérieux, La Balme-les-Grottes, France) consists of the following 10 tests: differentiation between *L. innocua* and *L. monocytogenes*, based on the presence or the absence of arylamidase (DIM test), hydrolysis of esculin, presence of α-mannosidase, and acid production from D-arabitol, D-xylose, L-rhamnose, α-methyl-D-glucoside, D-ribose, glucose-1-phosphate, and D-tagatose. Bacteria were removed from a 24-h culture on nutrient agar with a pipette and suspended in 2 ml of sterile distilled water (BioMérieux) to an opacity of the MacFarland 0.5 standard. Sterile water (5 ml) was poured into the tray to create a moist atmosphere, and then the reaction strip was removed from its packaging and placed in the tray. The bacterial suspension was then distributed into the 10 microtubes (100 μl for the DIM test and

TABLE 1. Identification scheme of *Listeria* species on the API *Listeria* system as provided by the manufacturer

Test	Test results[a] with:						
	L. monocytogenes	*L. innocua*	*L. seeligeri*	*L. ivanovii* subsp. *ivanovii*	*L. ivanovii* subsp. *londoniensis*	*L. welshimeri*	*L. grayi*
DIM	−	+	+	V	V	V	+
Esculin hydrolysis	+	+	+	+	+	+	+
α-Mannosidase	+	+	−	−	−	+	V
Acidification of:							
D-Arabitol	+	+	+	+	+	+	+
D-Xylose	−	−	+	+	+	+	−
L-Rhamnose	+	V	−	−	−	V	−
α-Methyl-D-glucoside	+	+	+	+	+	+	V
D-Ribose	−	−	−	+	−	−	+
Glucose-1-phosphate	−	−	−	+	V	−	−
D-Tagatose	−	−	−	−	−	+	−

[a] +, positive reaction; −, negative reaction; V, variable reaction.

50 μl for the other cupules). Afterward, the strip box was closed and incubated at 37°C for 18 to 24 h. After incubation, one drop of ZYM B (supplied by the manufacturer) was added to the DIM test and allowed to react for 3 min; then all of the reactions were read. Reactions were determined according to color changes as indicated in the manufacturer's instructions. Typical *Listeria* profiles on the API *Listeria* strip are reported in Table 1.

RESULTS

Results from the API *Listeria* test were interpretable after incubation for 24 h. In particular, the DIM results for all strains tested were unequivocal. Occasionally, acid production from some carbohydrate fermentations was difficult to interpret. Tests were repeated with these strains to facilitate the reading of the pH indicator color change.

The data obtained with the 646 *Listeria* strains are shown in Tables 2 and 3. These patterns were quite different from those obtained with the other gram-positive bacteria frequently encountered in medical and food microbiology included in this study, thus allowing to exclusively identify *Listeria* isolates in a short time. Three biochemical characteristics were positive (>95% of the strains) for *Listeria* isolates: hydrolysis of esculin and acid production from D-arabitol and α-methyl-D-glucoside (except for *L. grayi*). These three characteristics, combined with species and subspecies markers, easily enable the elimination of non-*Listeria* subcultures. The combination of the three tests (esculin hydrolysis, D-arabitol acidification, and α-methyl-D-glucoside acidification) intended to be positive within the genus *Listeria*, was never positive in the 31 non-*Listeria* strains tested. In particular, all enterococci were esculin positive, α-methyl-D-glucoside negative, and (except for *E. avium*) D-arabitol negative.

The presence of arylamidase (DIM test) and α-mannosidase and acid production from D-xylose, L-rhamnose, D-ribose, glucose-1-phosphate, and D-tagatose were used for species and subspecies identification. With this strip, 548 (84.8%) of the 646 *Listeria* strains were correctly identified at the species and eventually subspecies level (Table 2). It is noteworthy that 97.7% of the *L. monocytogenes* strains and 99.4% of the *L. innocua* strains were identified with this system. Confirmatory tests (acid production from a carbohydrate by conventional methods) allowed further identification of 94 strains (including some strains with known atypical results for hemolysis, α-mannosidase, and acid production from L-rhamnose). Three strains could not be allocated to any known species, and one was misidentified at the species level. With the exception of acid production from glucose-1-phosphate for *L. ivanovii* and acid production from D-tagatose for *L. welshimeri* (81 and 85%, respectively, of positive strains), positive and negative reactions as re-

TABLE 2. Identification of 646 *Listeria* strains with the API *Listeria* system

Test	No. (%) of strains giving a positive reaction						
	L. monocytogenes (*n* = 258)	*L. innocua* (*n* = 176)	*L. seeligeri* (*n* = 76)	*L. ivanovii* subsp. *ivanovii* (*n* = 37)	*L. ivanovii* subsp. *londoniensis* (*n* = 38)	*L. welshimeri* (*n* = 47)	*L. grayi* (*n* = 14)
DIM	0 (0)	176 (100)	76 (100)	32 (86)	37 (97)	45 (96)	14 (100)
Esculin	258 (100)	176 (100)	76 (100)	37 (100)	38 (100)	47 (100)	14 (100)
α-Mannosidase	253 (98)	176 (100)	4 (5)	0 (0)	0 (0)	45 (96)	13 (93)
Acidification of:							
D-Arabitol	257 (100)	176 (100)	76 (100)	37 (100)	37 (97)	45 (96)	14 (100)
D-Xylose	2 (1)	1 (1)	76 (100)	34 (92)	37 (97)	43 (91)	0 (0)
L-Rhamnose	254 (98)	123 (70)	0 (0)	0 (0)	0 (0)	40 (85)	0 (0)
α-Methyl-D-glucoside	258 (100)	176 (100)	76 (100)	37 (100)	35 (92)	46 (98)	2 (14)
D-Ribose	0 (0)	0 (0)	0 (0)	37 (100)	0 (0)	0 (0)	14 (100)
Glucose-1-phosphate	1 (0)	0 (0)	0 (0)	34 (92)	30 (79)	0 (0)	0 (0)
D-Tagatose	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	42 (89)	0 (0)

TABLE 3. Identification of 646 *Listeria* strains with the API *Listeria* system

Species	No. of strains tested	No. (%) with the indicated overall result				
		Correct identification			No identification	Misidentification
		Without additional tests	With additional tests	Total		
L. monocytogenes	258	252 (97.7)	6 (2.3)	258 (100)	0 (0)	0 (0)
L. innocua	176	175 (99.4)	1 (0.6)	176 (100)	0 (0)	0 (0)
L. seeligeri	76	0 (0)	76 (100)	76 (100)	0 (0)	0 (0)
L. ivanovii	75	66 (88.0)	8 (10.7)	74 (98.7)	1 (1.3)	0 (0)
L. welshimeri	47	41 (87.2)	3 (6.4)	44 (93.6)	2 (4.3)	1 (2.1)
L. grayi	14	14 (100)	0 (0)	14 (100)	0 (0)	0 (0)
Total	646	548 (84.8)	94 (14.6)	642 (99.4)	3 (0.4)	1 (0.2)

corded by the manufacturer were observed for 91 to 100% of the strains (Table 3).

DISCUSSION

Recent epidemiological investigations of foodborne outbreaks of human listeriosis and the subsequent regular control of various foods have clearly established an acute need for effective methodology for a rapid, simple, and reliable system of identification of *Listeria* isolates that is easily applicable to large sample numbers.

Because of the time-consuming nature of traditional identification procedures, some commercial identification systems, such as Mast ID (15), API 20E (29), API 20 STREP (20), API 50 CH (15, 23, 29), API ZYM (7, 23, 29), and LRA ZYM (23), have been evaluated in this view. These strips often yield good results regarding genus identification, but they were not fully adequate at the species level, especially regarding the differentiation between *L. monocytogenes* and *L. innocua*, the species that are most frequently encountered in laboratory practice. Even the API 50 CH system, which has been successfully used for the identification of *Listeria* species by selecting for some of the presently used markers (D-xylose, L-rhamnose, and α-methyl-D-mannoside [30] and D-arabitol and D-tagatose of API *Listeria* strip), is not appropriate because it includes a large number of carbohydrates of which only a few are relevant for this genus and because it requires the hemolysis test. A rapid and inexpensive identification scheme has been proposed by Lachica (17). It relies on the detection of hemolysis and on two carbohydrate (L-rhamnose and D-xylose) fermentation tests done on agar plates with one isolated colony growing on selective lithium chloride-ceftazidime agar plate after 40 h of incubation. Hemolysis can be difficult to assess, and various atypical *L. monocytogenes* strains (nonhemolytic, catalase negative, rhamnose negative, nonmotile) would not be identified.

The identification of *Listeria* species has long been hampered by the small number of tests allowing the differentiation between these closely related species; numerical phenetic studies did not help to solve this crucial problem (14, 31). With the conditions described here, the API *Listeria* test provided three new markers: fermentation of D-tagatose for *L. welshimeri* and glucose-1-phosphate for *L. ivanovii* (replacing the CAMP test with *R. equi*) and DIM (avoiding the need for the CAMP test with *Staphylococcus aureus*). DIM is a new test based on the detection of arylamidase, which is present in *L. innocua* strains and in the majority of the other non-*L. monocytogenes* listerial strains but is absent in *L. monocytogenes* strains. Spontaneous hemolysis, a major characteristic for *Listeria* species identification, may, in some cases (and especially for environmental and food isolates), be difficult to read on blood agar plates when differentiating *L. monocytogenes* from *L. innocua* (26, 32). To circumvent this important problem, more sensitive methods like the use of microtiter plates (8), a CAMP test with *S. aureus* (12), or tests for pathogenicity (19) have been suggested. Nevertheless, even with these tests, some true nonhemolytic *L. monocytogenes* isolates might be misidentified as *L. innocua* on the basis of their phenotypical behavior. To date, only genomic methods can firmly identify such isolates (5, 11, 13, 18). With the DIM test, all of the *L. monocytogenes* strains so far studied, even including these atypical nonhemolytic isolates, were easily and clearly separated from *L. innocua*. Therefore, this test unambiguously improves the speed and accuracy of species identification. In accordance with the manufacturer's instructions, the API *Listeria* system should be used for the identification of catalase-positive, gram-positive bacilli or coccobacilli. The kit is all that is required for identification to the species level, except on the rare occasion when an *L. seeligeri* isolate and a glucose-1-phosphate-negative *L. ivanovii* subsp. *londoniensis* strain could be confounded.

API *Listeria* system is easy to use (only one strip), tests are not numerous (10 tests) and are simple to interpret, and the system does not require use of additional tests like hemolysis on blood agar. It allows genus identification and species determination of large numbers of microorganisms with minimal amounts of materials and labor, yielding reliable results after a 24 h of incubation. Furthermore, because of its structure, this API strip is quick and inexpensive. It therefore appears to be a particularly promising tool for the routine practice of many laboratories, especially those concerned with food and environment microbiology.

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