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Serratia ficaria: a Misidentified or Unidentified Rare Cause of Human Infections in Fig Tree Culture Zones

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***Serratia ficaria*, an enterobacterium involved in the fig tree ecosystem, has been isolated from human clinical samples in rare instances, and its role as a pathogen is unclear. In 7 years, we have isolated *S. ficaria* from seven patients; it was the only pathogen in 4 patients, including a patient with septicemia described previously and three patients with gallbladder empyemas described in the present report. From March 1995 to July 1997, the incidence of biliary infections due to *S. ficaria* was 0.7%. We discuss the digestive carriage of this bacterium and its epidemiology with respect to the fig tree life cycle. Since fig trees grow around the Mediterranean as well as in the United States (California, Louisiana, Hawaii), *S. ficaria* should be more frequently isolated. In our experience, various strains have been misidentified or unidentified by commercial systems. Incorrect identification could be an additional explanation for the paucity of reported cases. *S. ficaria* produces nonpigmented, lactose-negative colonies which give off a potatolike odor. This odor is the primary feature of *S. ficaria* and must prompt reexamination of the identifications proposed by commercial systems. We tested 42 novel strains using three commercial systems: Vitek gram-negative identification (GNI) cards and API 20E and ID 32E strips (bioMérieux, Marcy-l'Etoile, France). The percentages of positivity that we have obtained were lower than those published previously for the following characteristics: lipase, gelatinase, DNase, and rhamnose. The best system for the recognition of *S. ficaria* is ID 32E, which correctly identified 27 of 42 strains. The API 20E system gave correct identifications for only two strains. *S. ficaria* was not present in the Vitek GNI card system database.**

Serratia ficaria was first described in 1979 by Grimont et al. (9) as part of the fig tree ecosystem. Since then, this bacterium has been isolated from human clinical samples in relatively few instances (1, 2, 7, 14, 15), and its role as a pathogen was always questionable. Since 1990, in Montpellier, France, a city located in the Mediterranean area, we have isolated *S. ficaria* from seven patients. In four patients, three patients with gallbladder empyemas and one patient with septicemia originating from the gut, its pathogenic role was clear (3, 4). To date, no other reports of such infections have been published, even though the fig tree grows throughout the Mediterranean area and in the United States (California [7], Louisiana [7], and Hawaii [14]). Is *S. ficaria* misidentified? As yet, the available data on the biochemical characteristics of this species concern fewer than 20 strains (5, 9). Are all *S. ficaria* biotypes known? The two principal publications (5, 9) report characteristics which were studied by conventional methods. Are these methods still used in routine laboratories? As far as we are concerned, we use API 20E strips for the identification of enterobacteria, and in our work we have encountered some problems with the identification of *S. ficaria*. Consequently, we have tested three commercial systems, gram-negative identification cards (Vitek GNI cards) and API 20E and ID 32E strips (bioMérieux, Marcy-l'Etoile, France), for their abilities to identify 42 novel strains of *S. ficaria*. We report on the biochemical characteristics that we obtained and discuss the main deficiencies in the three systems for the identification of this species.

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CASE REPORTS

Patient 1. On 7 October 1990, a 70-year-old man was admitted to the visceral surgery service for acute cholecystitis. His body temperature was 37.8°C. The leukocyte count was 18,000/mm^3. The patient was given ceftriaxone (2 g per day) and underwent cholecystectomy on the next day. The operation revealed an important local inflammation: the gallbladder was full of multiple stones, was large and purplish, and was covered with numerous false membranes; its wall was exceptionally thick; and an associated odditis was found. On 11 October, the persistence of a subfebrile state (37.9°C) led to a change from ceftriaxone to amoxicillin plus clavulanic acid (1 g twice daily). Apyrexia was achieved 4 days later. The patient returned home on 17 October.

This man had eaten figs in season, but his fig consumption was poorly related to the timing of the beginning of clinical signs.

Patient 2. An 81-year-old man suffering from an acute hydrocholecystis with fever (39°C) and leukocytosis (29,000 polymophonuclear leukocytes/mm^3) was admitted on 20 September 1993 to the visceral surgery service. On the previous day, he had been given amoxicillin plus clavulanic acid (2 g per day) by perfusion. An operation was performed on 21 September. The operation showed a necrotic gallbladder full of pus and infected mud, a sample of which was sent to our laboratory.

Under antibiotic treatment, the body temperature and the leukocyte count (9,800/mm^3) returned to normal on 27 September. From 29 September, the antibiotic was administered per os and the patient was discharged.

It was not possible to ask the patient about his fig consumption.

Patient 3. On 22 May 1996, a 59-year-old woman was referred to the emergency service for acute cholecystitis revealed

TABLE 1. Sources of human strains

Sex[a]	Age (yr)	Geographic location	Isolation date	Source	Reference
M	62	Belgium[b]	January 1982	Expectoration	2
M	62	Belgium[b]	January 1982	Tracheal secretions	2
M	?	France[c]	July 1988	Extra-articular knee wound	10
M	?	France[c]	October 1992	Bronchial secretions	
M	70	France	October 1990	Bile	3
F	42	France	May 1991	Stools	
M		France	June 1993	Drainage tube of ankle wound	
M	83	France	July 1993	Four blood cultures	4
M	81	France	September 1993	Bile	
F	56	France	September 1993	One blood culture	
F	59	France	May 1996	Bile	

[a] M, male; F, female.
[b] From J. A. Brouillard, Department of Bacteriology, Institut Provincial d'Hygiène et de Bactériologie, Mons, Belgium.
[c] From C. Suc, Laboratoire de Bactériologie, Hôpital Rangueil, Toulouse, France.

by pain in the right hypochondrium and biliary vomiting. On admission, the patient was febrile (38°C). Abdominal palpation detected a "guard" reaction at the right side. The leukocyte count was 15,380/mm^3. Therapy with intravenous amoxicillin plus clavulanic acid (1 g three times daily) was started. On the next day, an operation revealed gangrenous cholecystitis with a purulent perivesicular discharge. The gallbladder had a thick wall and contained one calculus of cholesterol.

A couple of days after surgery, oral antibiotic treatment (1 g twice daily) replaced administration by perfusion. The body temperature returned to normal on 26 May. The patient was discharged 1 day later.

In May, edible fresh figs do not grow locally. We asked the patient about the presence of a fig tree in her garden or neighborhood. She said that no fig trees were in proximity to her home but that perhaps there were fig trees in the countryside where she frequently walked.

Laboratory data for patients 1 to 3. Microscopic examination of biliary fluid from the three patients showed numerous polymorphonuclear cells but no bacterium. After incubation for 24 h at 37°C under aerobic and anaerobic conditions, *S. ficaria* was isolated, in pure culture, on MacConkey medium and chocolate agar (bioMérieux) from intraoperative samples from the three patients. The antibiotic susceptibilities of the isolates were tested by disk diffusion and the results were read according to the standards of the French Antibiogram Committee. Except for tetracycline, to which one isolate was resistant, all strains had similar susceptibility patterns: resistance to cephalothin and susceptibility to all other beta-lactams, aminoglycosides, chloramphenicol, colistin, trimethoprim-sulfamethoxazole, nalidixic acid, and fluoroquinolones.

MATERIALS AND METHODS

Strains. The 42 strains studied comprised 11 clinical isolates (the sources of which are given in Table 1) and 31 strains from the fig tree ecosystem. Between February and June 1994, we collected figs, buds, and insects from fig trees growing within or around the precinct in which our hospital is located. This sampling allowed us to recover 11 *S. ficaria* isolates from male figs, isolate 1 from a pollinated female fig, 14 isolates from *Blastophaga psenes* (a fig tree-specific pollinator that breeds in male figs), and 5 isolates from *Philotrypesis caricae* (a parasitic insect which also breeds in figs but which is not implicated in their pollination). Because all of the environmental strains were isolated from fig trees located in an area covering 2.5 km^2, it seemed possible that these strains had the same clonal origin. By means of pulsed-field gel electrophoresis (the restriction enzyme *Xba*I was obtained from Appligene Oncor, Illkirch, France), we looked for clonal strains. Among human strains, the two Belgian isolates constituted a clone. Among the environmental strains we detected four clones, and these clones were essentially among strains isolated from insects collected from the same fig. However, three or four different clones could cohabitate in the same fruit. The isolates from different figs and, a fortiori, from different fig trees were different. Thus, of 11 human strains, 10 were genetically different, and of 31 environmental strains, 23 had different genomes.

At the time of their isolation, our strains were identified as follows. Biochemical characteristics were obtained on API 20E strips with, if necessary, control of the utilization of citrate as the sole carbon source by culture on Simmons citrate agar and examination for a potatolike odor. For environmental strains, isolation from figs or insects that breed in figs was also considered. All human isolates and doubtful environmental strains were sent to P. A. D. Grimont (Unité des Entérobactéries, Institut Pasteur, Paris, France), who confirmed our identification by means of a carbon source utilization study with Biotype 99 carbon source strips (bioMérieux).

Identification. In the opinion of Grimont and Grimont (8), the methodology of carbon source utilization tests is essential to *Serratia* identification. Consequently, they have set up the special gallery of tests mentioned above (Biotype 99; bioMérieux). The supplier of this system presents it as a dedicated tool for research laboratories. Furthermore, the interpretation of results requires special software sold by another group (Institut Pasteur Taxolab, Paris, France), and this program can be loaded only on Macintosh computers. These reasons excluded the use of Biotype 99 since we wished to work under the same routine conditions used by nonspecialized laboratories in order to point out the difficulties encountered in *S. ficaria* identification.

After overnight incubation at 37°C on MacConkey agar, cultures were tested for oxidase, catalase, and DNase reactions and were used to inoculate three systems for the identification of gram-negative organisms: Vitek GNI cards and API 20E and ID 32E strips. These systems were used according to the recommendations of the supplier.

The Vitek GNI cards were introduced into a reader-incubator. The incubation cycle is 4 to 18 h. At the completion of the incubation cycle, the biochemical pattern was printed for each card in the reader-incubator.

The ID 32E system requires incubation at 37°C for 24 h under aerobic conditions. Before strip reading, the indole reaction was revealed by the addition of one drop of James reagent. The automatic reading uses the ATB Expression instrument; the reader records the color of each tube and transmits the data to the computer.

Also, the API 20E strips require incubation at 37°C for 24 h in a humid atmosphere under aerobic conditions. Then, reagents were added as appropriate to the tryptophan desaminase (TDA), Voges-Proskauer, and indole (IND) tubes. Nitrate reduction was revealed in a glucose tube with Griess reagents. All reaction tubes were read visually. When the color interpretation was not clear, the reactions were noted as doubtful.

For DNase detection, a sample of each cultured strain was streaked onto DNA-toluidine blue agar (Sanofi-Diagnostics Pasteur, Marnes la Coquette, France). This medium was incubated for 24 h at room temperature (about 22°C). If DNase is present, the agar shows a pink halo extending several millimeters around the streak.

Catalase reactions were studied with hydrogen peroxide.

The cytochrome oxidase test was performed with cultures from trypto-casein soy agar and disks impregnated with dimethyl-paraphenylenediamine oxalate (Sanofi-Diagnostics Pasteur).

RESULTS

Frequency of biliary infections due to *S. ficaria*. The frequency of occurrence of biliary infections due to *S. ficaria* was estimated from March 1995 to July 1997 (inclusive). During this period, 385 biliary samples were seeded under aerobic and anaerobic conditions. Growth was obtained from 166 of the

cultured samples from 142 infected patients. Eighty-two infections were monomicrobial (the infections were due to an anaerobic bacterium in three patients). Among the 60 remaining mixed infections, 15 were caused by flora that included anaerobes. One enterobacterium or a diverse combination of enterobacteria were responsible for 84 infections, *Serratia* spp. were responsible for 3 infections, and *S. ficaria* was responsible for 1 infection (0.7% of infected patients).

Identification. Table 2 presents our results expressed as the percentages of strains positive by the various tests. The three identification systems do not include the same tests. When a biochemical test exists only in one identification system, the result in Table 2 is the result for that system. In adverse situations, the same strain may give different or concordant results in the various systems, but this depends on the specific characteristic. Tests for H_2S production, urease, and indole and other tests for enzymes implicated in the metabolism of amino acids were constantly negative, whereas tests for β-galactosidase expression and carbohydrate acidifications were much more variable. For both β-galactosidase and carbohydrate acidifications, the percentages reported in Table 2 were established from the number of strains showing positive reactions in all three systems.

Table 2 also presents the characteristics of bacteria isolated from patients and strains obtained from local fig trees and the relatively significant difference as determined by the chi-square test ($P < 0.05$). Human strains reduced nitrate only to nitrite, whereas about half of the environmental strains reduced nitrate to nitrogen. β-Galactosidase was present in about twice as many environmental strains as human strains, and environmental strains were more often glucidolytic for adonitol, inositol, and palatinose. Human strains were more often proteolytic.

With ID 32E strips, 27 strains were correctly identified. The biochemical profiles of other strains were considered unacceptable. Among these strains, *S. ficaria* was proposed as the only choice (2 strains), as the first choice (8 strains), and as the second choice (3 strains) or was never proposed (2 strains); *Serratia rubidaea* also appeared among the proposed identifications for 10 strains.

API 20E strips gave correct identifications for two *S. ficaria* strains. Sixteen strains were identified to the *Serratia* genus level: *S. ficaria* appeared as the first choice for three strains and as the second choice behind *Serratia plymuthica* for 13 strains. One strain was identified as *S. plymuthica*. Twenty-three strains had unacceptable profiles. Among these, *S. ficaria* and/or *S. plymuthica* appeared with various other possible identifications (20 strains); the genus *Serratia* never appeared (3 strains).

Vitek GNI cards did not allow us to identify *S. ficaria* because this species was not included in the system's database. For 27 strains, the proposed identification agreed with *Serratia* genus, for 14 other strains the proposed identification was *Klebsiella ozaenae*, and 1 strain remained unidentified.

DISCUSSION

Pathogenicity. All three intraoperative biliary samples yielded pure cultures of *S. ficaria*, and this bacterium was responsible for the local and general infectious state. Fever and leukocytosis were always present. Pus was observed macroscopically, and microscopic examination showed numerous polymorphonuclear cells. Thus, *S. ficaria* is able to cause severe infections such as these deep suppurations or septicemia as reported previously (4) and can clearly play a pathogenic role. However, the level of this pathogenicity seems to be low because in the patients with septicemia and gallbladder empyema, the course to recovery was both uncomplicated and speedy, even though the patients were elderly and even though the patient with septicemia was suffering from cancer.

Epidemiology. Gallbladder contamination is brought about by the bacteria from the small gut. On the other hand, the septicemia occurred after an antrectomy and anastomosis between the remaining stomach and the first jejunal loop. Both cases of infection imply that *S. ficaria* must be a part (at least transiently) of the human intestinal flora. Consequently, using selective caprylate medium (16), we have looked for its presence in feces, although that effort was in vain (13). Several things may explain this failure. (i) The clinical cases reflect *S. ficaria* carriage at the duodenojejunal level of the intestine, whereas stool culture investigates the colic flora. (ii) The screening method that we used was based on DNase, and we may have missed *S. ficaria* in stools. Indeed, among the 11 clinical strains of *S. ficaria* from our own collection, all except 4 isolated from bile and stools were DNase positive. (iii) Finally, *S. ficaria* is very rarely isolated (in our laboratory, seven isolates in 7 years), and digestive carriage must also be rare and likely depends on both environmental and climatic factors which are difficult to determine considering our present state of knowledge about the epidemiology of *S. ficaria*. All these reasons make the intestinal carriage of *S. ficaria* difficult to probe and prove.

For some investigators it seems logical to explain *S. ficaria* digestive carriage by fresh fig consumption (1, 2, 7, 14, 15). We believe the explanation to be less simple, particularly when some of the details of fig tree biology are considered. The fig tree (*Ficus carica*) is a dioecious species. The male tree yields inedible figs, sometimes called caprifigs, in which breeds a specific pollinator (*B. psenes*), a hymenopteran the size of a midge. In the course of a year, there are usually two generations of *B. psenes*, in May and in July, August, and September. This second generation pollinates female figs, which turn ripe and edible in October. This is the case for both wild *F. carica* and a kind of cultured *F. carica* (for example, the Smyrna variety called Calimyrna in California). Another kind of cultured fig tree gives, in July and October, two crops of parthenocarpic figs which ripen and become edible without pollination. These cultivars are cultured in southern France both in home gardens and for commercial production. The parthenocarpic figs harvested in July do not contain *S. ficaria*; thus, the only edible figs able to harbor *S. ficaria* are those that are pollinated in July and that ripen in October (unpublished observations). Then, if fig consumption was the only cause of human contamination, all clinical isolates should occur in October, like in our patients 1 and 2 and the patient reported by Gill et al. (7). However, how is the infection in the third patient, which occurred in May when no figs were ripe, explained? This month is the time when the first generation of *B. psenes* is flying. In the same way, the case of septicemia (4) occurred in July, when the second generation of hymenopterans leaves male trees to pollinate the female figs on the female trees. These flies can cover several kilometers and therefore can contribute to the spread of *S. ficaria* over a wide area. Grimont and colleagues (10, 11) isolated *S. ficaria* from figs, a fig leaf, and *B. psenes* and also from common grass, scilla, market mushrooms, and an ant. We were unable to consider the isolation of *S. ficaria* from market mushrooms because the season and geographic origin were not indicated. However, the scilla collected in Bordeaux grew 5 m away from a fig tree from which *S. ficaria* was isolated from a fig and a leaf, and all three isolated strains belonged to the same serovar (O2:H1) (11). The picking time was not mentioned, but the isolation of *S. ficaria* from a leaf and from around a tree which bore figs could

TABLE 2. Biochemical characteristics of *S. ficaria*: comparison between human and environmental strains

Test	% Positivity			
	Total (n = 42 strains)	Human strains (n = 11 strains)	Strains from environment (n = 31 strains)	P^a
Catalase	100	100	100	
Cytochrome oxidase	0	0	0	
Nitrate reduction to nitrite	69	100	52	0.01
Nitrate reduction to N_2	31	0	48	0.01
H_2S production	0	0	0	
Lipase	0	0	0	
Gelatinase	57	91	45	0.01
DNase at 25°C	43	64	35	NS
Urease	0	0	0	
Indole production	0	0	0	
Enzyme tests				
Arginine dihydrolase	0	0	0	
Lysine decarboxylase	0	0	0	
Ornithine decarboxylase	0	0	0	
Tryptophan desaminase	0	0	0	
L-Aspartic acid arylamidase	0[b]			
Voges-Proskauer test	71	91	65	NS
Phenol red acidification	38	9	48	0.03
Esculin hydrolysis	100	100	100	
Oxidation of:				
Glucose	100	100	100	
Lactose	67	55	71	NS
Maltose	100	100	100	
Mannitol	100	100	100	
Xylose	100	100	100	
Acid from:				
Adonitol	79	55	87	0.03
Amygdalin	95	91	97	NS
L-Arabinose	86	82	87	NS
D-Arabitol	98	100	97	NS
L-Arabitol	55	36	61	NS
Cellobiose	60	45	65	NS
Galacturonate	64	73	61	NS
Glucose	100	100	100	
Inositol	62	91	52	0.03
5-Ketogluconate	100	100	100	
Maltose	86	82	87	NS
Mannitol	95	91	97	NS
Melibiose	55	64	52	NS
Palatinose	48	18	58	0.03
Raffinose	95	91	97	NS
Rhamnose	2	9	0	NS
Sorbitol	60	82	52	NS
Sucrose	93	91	94	NS
Trehalose	100	100	100	
Glucose fermentation in the presence of:				
2,4,4′-Trichloro-2′-hydroxy-diphenylether (0.3 g/liter)	64	91	55	0.04
P-Coumaric (2 g/liter)	100	100	100	

Continued on following page

TABLE 2—*Continued*

Test	% Positivity			
	Total (n = 42 strains)	Human strains (n = 11 strains)	Strains from environment (n = 31 strains)	P^a
Enzyme tests				
α-Galactosidase	93	100	90	NS
β-Galactosidase	71	45	81	0.03
α-Glucosidase	60	82	52	NS
β-Glucosidase	100	100	100	
β-Glucuronidase	0	0	0	
α-Maltosidase	43	18	52	0.05
N-Acetyl-β-glucosaminidase	0	0	0	
Utilization of:				
Acetamide	0	0	0	
Citrate[c]	100	100	100	
Malonate	0	0	0	
Growth in the presence of polymyxin B (0.3 g/liter)	0	0	0	

[a] P values were determined by the χ^2 test. NS, not significant.
[b] Doubtful for 12% of strains.
[c] A rate of positivity of 100% on Simmons citrate agar and also with Vitek GNI cards. This characteristic is very difficult to read when API 20E galleries are used (positive, 27%; negative, 19%; doubtful, 17%).

indicate whether the month was that of the first generation of *B. psenes* fly (if a male tree is involved) or the season of pollination, since Bordeaux is located in an area of *B. psenes* activity. The *S. ficaria*-carrying ant was also collected in Bordeaux (11), but when? As far as we are concerned, we have not isolated any *S. ficaria* strains from cochineals or ants recovered from fig trees during a period that was not during the period of activity of *B. psenes*. Because a majority of clinical isolates occurred between May and October (corresponding to the activity period of *B. psenes*) and were related to the geographic area of *Blastophaga* activity (California, Louisiana, Hawaii for U.S. cases and below the 46th parallel of northern latitude for French cases) (1, 4, 7, 14, 15), we think that *Blastophaga* plays a part, perhaps the major part, in *S. ficaria* epidemiology. This hypothesis is further supported by reports from Grimont and colleagues (8, 11). Californian figs could mature only after *B. psenes* from male trees imported from Greece and Algeria established its life cycle. Strains of *S. ficaria* from the Mediterranean region were not antigenically uniform, while all U.S. strains studied (isolated from the fig wasp or from human patients) belonged to the same serotype (O1:H1) and were likely taken to the United States by the Mediterranean *B. psenes*. Arguing against a role for *B. psenes*, we must cite the two unexplainable Belgian cases of *S. ficaria* infection that occurred in January (2) and the isolation of *S. ficaria* from common grass picked up in Saint-Remy-lès-Chevreuse, France (11). These isolates are situated well above the 46th parallel of northern latitude. January is the time of fig pollination in the austral hemisphere.

Identification. Table 3 compares the results obtained with 3 commercial systems (from Table 2) with those obtained by Farmer et al. (5) and Grimont et al. (9) by conventional methods. In their princeps publication, Grimont et al. (9) report on the characteristics of 14 original strains issued from the environment (figs and *B. psenes*). Farmer et al. (5) studied 13 strains, including 10 strains supplied by Grimont and 3 clinical isolates. Thus, the distribution of strains (clinical and environmental) in the series of Farmer et al. (5) is similar to ours (3 of 13 and 11 of 42, respectively). We tested 42 other strains by three commercial systems. However, all results are consistent with respect to the following characteristics: catalase; cytochrome oxidase; nitrate reduction; H_2S production; urease production; indole production; decarboxylases and tryptophan desaminase production; Voges-Proskauer test; esculin hydrolysis; acidification of L-arabinose, D-arabitol, cellobiose, glucose, inositol, maltose, mannitol, melibiose, raffinose, sorbitol, sucrose, and trehalose; β-galactosidase production; and utilization of malonate and citrate. Citrate utilization was always positive when it was tested with Simmons citrate medium or by the Vitek GNI card, but the API 20E system did not allow the correct expression of this characteristic.

Farmer et al. (5) describe the genus *Serratia* as usually being colistin resistant and producing the extracellular enzymes DNase, gelatinase, and lipase. The resistance to the polymyxin group concerns all the molecules of this group, but, unexpectedly, none of our *S. ficaria* strains tested with the GNI Vitek card grew in the presence of polymyxin B (300 μg/ml). Is this concentration too high to allow the expression of colistin resistance (the disk for antibiotic susceptibility testing contains 50 μg of colistin)? Is colistin resistance an usual characteristic for members of the genus *Serratia* or only for the species *Serratia marcescens*? Among the 33 strains of *S. ficaria* whose antibiograms have been published, only 8 were resistant (4).

Concerning lipase activity, the use of different substrates may explain the different results. Farmer et al. (5) and Grimont et al. (9) proved the lipase activity using two conventional substrates, corn oil and Tweens, respectively, which revealed the enzyme in 77 and 93% of strains, respectively (not a significant difference). Apparently, *S. ficaria* lipase is not able to lyse 5-bromoindoxyl ester, the substrate selected by bioMérieux for its lipase test (ID 32E).

A total of 57 and 43% of our strains were positive for gelatinase and DNase, respectively. Moreover, clinical strains tend to be more proteolytic than strains recovered from the fig tree ecosystem. The difference is significant for gelatinase but not for DNase. All human strains are DNase positive except the four strains isolated from bile and stools, both of which contain biliary salts (we do not yet have any explanation for this phenomenon). This observation is very surprising if one compares these results with the results obtained by Farmer et

TABLE 3. Biochemical characteristics of *S. ficaria*: comparison with published results

Test	% Positivity		
	This study (n = 42 strains)	Farmer et al. (5) (n = 13 strains)[a]	Grimont et al. (9) (n = 14 strains)
Catalase	100		100
Cytochrome oxidase	0	8	0
Nitrate reduction to nitrite	69	92	100
Nitrate reduction to N_2	31		
H_2S production	0	0	0
Lipase	0[b]	77[c]	93[d]
Gelatinase	57	100[e]	100
DNase at 25°C	43	100	100[f]
Urease	0	0	0
Indole production	0	0	0
Enzyme tests			
Arginine dihydrolase	0	0	0
Lysine decarboxylase	0	0	0
Ornithine decarboxylase	0	0	0
Tryptophan desaminase	0	0[g]	0[g]
Voges-Proskauer test	71	75	79
Esculin hydrolysis	100	100	93
Oxidization of glucose	100		100
Acid from:			
Adonitol	79	0	100
L-Arabinose	86	100	100
D-Arabitol	98	100	79
Cellobiose	60	100	100
Glucose	100	100	100
Inositol	62	55	100
Maltose	86	100	100
Mannitol	95	100	100
Melibiose	55	40	93
Raffinose	95	70	86
Rhamnose	2	35	100
Sorbitol	60	100	100
Sucrose	93	100	100
Trehalose	100	100	100
β-Galactosidase	71	100	100
Utilization of:			
Citrate	100[h]	100[i]	100[i]
Malonate	0	0	0

[a] Including 10 strains supplied by P. A. D. Grimont.
[b] Substrate, 5-bromo-indoxyl ester.
[c] Substrate, corn oil.
[d] Substrate, Tweens 40, 60, and 80.
[e] At 22°C.
[f] Temperature not indicated.
[g] Phenylalanine desaminase.
[h] A rate of positivity of 100% on Simmons citrate agar and also with Vitek GNI cards. This characteristic is very difficult to read when API 20E galleries are used (positive, 27%; negative; 19%; doubtful, 17%).
[i] Simmons citrate.

TABLE 4. Comparison of some results given by the three identification systems

Test	API 20E		ID 32E		GNI Vitek		% Positive	
	No.[a]	%	No.	%	No.	%	Farmer et al. (5)	Grimont et al. (9)
Adonitol			+32 −7 ?2	76	+41 −1	**98**[b]	0	**100**
L-Arabinose	+37 −2 ?3	88	+41 −1	**98**	+42	**100**	**100**	**100**
Inositol	+26 −14 ?2	62	+28 −14	67	+29 −13	69	55	100
Mannitol	+41 ?1	**98**	+41 −1	**98**			**100**	**100**
Rhamnose	+7 −27 ?8	17	+4 −36 ?2	10	+17 −25	**40**	**35**	100
Sorbitol	+35 ?7	83	+26 −14 ?2	62	+42	**100**	**100**	**100**
Sucrose	+41 ?1	**98**	+39 −1 ?2	93	+42	**100**	**100**	**100**

[a] No., number of positive (+), negative (−), or doubtful (?) results.
[b] Boldface numbers are closely related results.

al. (5) or Grimont et al. (9) with environmental strains (100% of strains produce both enzymes) but is not abnormal if one considers that in various bacteria proteolytic enzymes play a part as virulence factors. In the same way, it seems logical that environmental strains show strong nitrate reductase activity up to the nitrogen step and that strains cultured from fruits display strong saccharoclastic metabolism.

With regard to sugar fermentation, the only significant differences between the results of Grimont et al. (9), those of Farmer et al. (5), and those from our present study concern the fermentation of adonitol and rhamnose. Table 4 presents the results provided by the three identification systems compared with those obtained by Farmer et al. (5) and Grimont et al. (9) by conventional methods. The GNI Vitek card gave the best results and results that were nearer the reported ones. With the API 20E and ID 32E strips, most sugar fermentations were difficult to read. Grimont and Grimont (8) noted previously "that utilization tests are preferable to fermentation tests since strains able to utilize a polyalcohol sometimes fail to produce enough acid products to give a positive reaction in fermentation tests." It is a pity that utilization tests useful under routine conditions are not yet available.

Therefore, how should *S. ficaria* be identified? On MacConkey medium, *S. ficaria* looks like a lactose-negative enterobacterium (or a weakly lactose-positive enterobacterium, depending on the supplier of medium). The colonies are smooth, beige, transparent, or opaque and can turn pinkish after several days. The culture gives off a strong potatolike odor. Avoid the GNI Vitek card because it does not include *S. ficaria* in its database. API 20E strips do not differentiate *S. ficaria* from *S.*

plymuthica very well. The biochemical characteristics implicated in this inadequate differentiation are rhamnose acidification and utilization of citrate. In the API 20E database, 100 and 92% of *S. ficaria* strains are expected to be citrate and rhamnose positive, respectively, whereas 65 and 8% of *S. plymuthica* strains, respectively, are expected to be positive. In our experience, few strains of *S. ficaria* can acidify rhamnose. On the other hand, we mentioned above the difficulties encountered with reading the API 20E citrate tubes. The easiest control is to smell the plates. If the culture smells of potato, the bacterium is *S. ficaria*; it is also necessary to control citrate positivity on Simmons citrate agar. With ID 32E strips, a possible misidentification is between *S. ficaria* and *S. rubidaea*. Unfortunately, a few strains of *S. rubidaea* (6) give off a musty or potatolike odor. The latter species, however, appears to be lactose positive on MacConkey medium, and most strains produce prodigiosin. Besides, *S. rubidaea* is "rarely isolated both in the natural environment and in human patients" (12), whereas *S. ficaria* isolates are relatively more frequently isolated (in 7 years, we have isolated *S. ficaria* from seven patients but have isolated *S. rubidaea* from only one patient) and occur in the period and zone of activity of *B. psenes*. Season and geographic area are also very important criteria for consideration in the diagnosis of an *S. ficaria* infection.

Up to now, published reports about characteristics for the identification of *S. ficaria* concerned a maximum of 17 strains. The database of commercial systems was probably established with data for a few strains and must evolve. The aim of the study described in this report, based on 42 strains, is to contribute to such an update.

Conclusion. In the area of activity of *B. psenes*, *S. ficaria* can be responsible for human infections such as gallbladder empyema or even septicemia. In the region surrounding Montpellier (Mediterranean France), the frequency of biliary infections due to *S. ficaria* was estimated to be about 0.7%. However, this bacterium can escape identification by various commercial systems. GNI Vitek cards are inappropriate because *S. ficaria* is not included in the system's database. API 20E strips gave the correct identification for 2 of 42 strains, and ID 32E strips gave correct identifications for 27 of 42 strains. Up to now, reports on the biochemical characteristics of *S. ficaria* have been based on data for 14 or 13 strains, including only 3 clinical isolates and 10 redundant environmental strains. Our results, obtained with data for 42 new strains (11 human strains plus 31 environmental isolates) are in concordance with published data except those for lipase, gelatinase, DNase, and rhamnose. The revision of positivity percentages for these substrates could improve the recognition of *S. ficaria*. In the present state, the best system for *S. ficaria* identification is the ID 32E system.

Our study of 42 novel strains shows interesting differences between human strains, which are the most proteolytic, and environmental strains, which are the most glucidolytic and which are better nitrate reducers.

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