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Proferrioxamine profiles of *Erwinia herbicola* and related bacteria

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Two strains of *Erwinia herbicola* effective in the biocontrol of *E. amylovora*, the etiological agent of fire blight, were screened for proferrioxamine siderophores by on-line liquid chromatography–electrospray mass spectrometry (LC-MS). Type strains of *E. herbicola* and *Pantoea* species were included in this study for taxonomic comparisons. Proferrioxamine profiles similar to that previously described for *E. amylovora*, including tri- and tetrameric hydroxamates and diaminopropane-containing proferrioxamines, were observed for *P. agglomerans*, but not for other *E. herbicola*-like species. Biocontrol activity was not correlated with proferrioxamine synthesis. The results of this study are consistent with the notion that some, but not all, biocontrol strains may inhibit *E. amylovora* via competition for iron. Further studies into the link between biocontrol of fire blight and siderophores are thus warranted. This study also revealed limitations of standard nutrient utilization and fatty acid profile analyses for the differentiation of *P. agglomerans*, *P. dispersa* and other *E. herbicola*-like species from each other. Given these limitations, LC-MS may become a much needed additional diagnostic tool for the identification of *E. herbicola*-like strains at the species level.

Keywords: enterobactins, fire blight control, metabolic profiling, *Pantoea* spp., siderophores

Introduction

New control agents for *Erwinia amylovora*, the causal agent of the devastating fire blight disease of apple and pear, are urgently needed because currently available antibacterial agents have become ineffective due to resistance of the pathogen (Burr *et al.* 1993, Chiou & Jones 1993, Johnson *et al.* 1993, McManus & Jones 1994). Restrictions on the use of antibiotics in agriculture also limit our ability to prevent fire blight. The recent serendipitous finding that 5-hydroxylysine inhibits growth as well as siderophore production of *E. amylovora* (Feistner 1994, 1995a) has led to the working hypothesis that it may be possible to prevent fire blight via interference with iron acquisition in *E. amylovora*. This in turn has raised the question of whether the inhibitory activity of known biological control strains is due, at least in part, to competition for iron. While it is known that *E. amylovora* produces proferrioxamines (pFOs) (Feistner *et al.* 1993a,b), there is no report yet on the siderophores of fire-blight-inhibitory strains.

Biological control strains can readily be isolated from plants and many have tentatively been identified as *Erwinia herbicola* (Ishimaru *et al.* 1988, Wilson *et al.* 1990, El-Goorani *et al.* 1992, Vanneste *et al.* 1992, Johnson *et al.* 1993, Obrien 1993, Wodzinski & Paulin 1994, Wodzinski *et al.* 1994). However, the classification of *E. herbicola* and *E. herbicola*-like strains from plants or clinical sources has long been debated. Plant pathologists historically use the name *E. herbicola*, while clinical microbiologists use the synonym *Enterobacter agglomerans*. More recently, Gavini *et al.* (1989) proposed reclassification of strains within the Herbicola–Agglomerans complex based on DNA homology and protein profiles. A new genus and two species,

Part 14 in the series 'Metabolites of Erwinia', for Part 13 see Feistner (1995c).

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Pantoea agglomerans and *P. dispersa*, were proposed. Some strains previously identified as *E. herbicola* were reclassified as *P. dispersa*, and others as *P. agglomerans*. While the siderophores of some *E. herbicola (E. agglomerans)* strains have been reported to be proferrioxamines (Berner *et al.* 1988, Berner & Winkelmann 1990), it is not known whether proferrioxamine synthesis is a general phenotype of all strains of *E. herbicola (Pantoea* spp.). It is conceivable that some biocontrol agents produce siderophores other than proferrioxamines and that these may provide a competitive edge for the biocontrol strains over *E. amylovora*.

The initial purpose of this study was to determine whether Eh 198/1, a potent biocontrol agent for *E. amylovora* that was recently identified in the Czech Republic (Kokošková 1991, 1996), would produce proferrioxamines. When Eh 198/1 turned out to be pFO^-, an attempt was made to taxonomically classify this bacterium by extending the study to several type strains of *E. herbicola* and *Pantoea* spp., and by complementing the proferrioxamine analyses with established taxonomic methods, i.e. classical biochemical (Biolog™) and gas chromatography–fatty acid methyl ester (GC-FAME) analyses. Furthermore, in order to evaluate whether the pFO^- phenotype was a general characteristic of fire blight control strains, a second biological control strain, C9-1, was also included in this study.

Materials and methods

Bacterial strains and growth conditions

Bacterial strains used in this study are listed and characterized in Table 1. All strains were maintained at –78°C in LB medium containing 15% glycerol. Siderophore production was stimulated on MMB minimal medium consisting of 2 g l^{-1} $K_2HPO_4{\cdot}3H_2O$, 0.1 g l^{-1} $MgSO_4{\cdot}7H_2O$, 0.4 g l^{-1} $(NH_4)_2SO_4$, 2 g l^{-1} sucrose, 100 µg l^{-1} nicotinic acid, and ferric citrate in a concentration of 10^{-8} M. Cultivation was in batches of 50 ml in 250 ml Erlenmeyer flasks at room temperature (18–25°C) and under moderate stirring using teflon-coated magnetic stir bars.

Proferrioxamine screening

Bacterial culture supernatants were screened for proferrioxamines following the method previously developed for *E. amylovora* (Feistner *et al.* 1993b, Feistner 1995a). Briefly, the culture supernatants were dried and extracted with methanol, and 5–10 µl of each extract were analyzed by a microgradient reversed-phase chromatography system that was coupled to a Finnigan electrospray triple sector quadrupole mass spectrometer. Full scan mass spectra (*m/z* 50–2000) were continuously acquired over the 60 min chromatographic run and the data analyzed for the protonated molecular ions of the various expected proferrioxamines.

Iron-binding compounds of Eh 198/1

To obtain preliminary evidence for the presence of siderophores in Eh 198/1, the corresponding extract was analyzed by RP-HPLC and diode array absorbance detection before and after addition of ferric citrate. A Hewlett-Packard 1090M workstation, an ABI C_{18} column (2.1 × 220 mm) plus a 3 mm precolumn, a linear gradient of 0–45% acetonitrile in 0.1% TFA over 40 min followed by a plateau, and an injection volume of 50 µl were used. Four LC peaks (with retention times of approximately 19, 30, 37 and 43 min) which, following the addition of 150 nmol of ferric citrate, were shifted to apparently shorter retention times, were considered to be due to iron-binding compounds.

Arnow assay

This test for catechols was performed as described (Ishimaru & Loper 1993), except that only 20 µl each of sample and reagents were used. A control test with water as sample was negative.

Enterobactin screening

When the Arnow assays turned out to be positive, the LC-MS data that had been acquired with the intention to screen for proferrioxamines, were also screened for $[M+H]^+$ ions of cyclic (*m/z* 670) and linear enterobactins (trimer *m/z* 688; dimer *m/z* 465; monomer *m/z* 242). Peaks for cyclic enterobactin and its linear trimer, but not the mono- and dimer, were indeed found at elution times longer than those of the proferrioxamines. The iron-binding ability of the compound with *m/z* 688 was corroborated by the fact that a corresponding strong $[M-2H+Fe]^+$ ion at *m/z* 741 could be observed for an extract of ATCC 14589 (100 µl) only after treatment with ferric citrate (775 nmol). The $[M-2H+Fe]^+$ ion was detected in connection with the residual free siderophore LC peak, rather than in connection with a new LC peak for ferric enterobactin. An LC peak for ferric enterobactin could not be observed, presumably because it was washed out in the void volume (Berner *et al.* 1991).

Test for pectolytic activity

Pectolytic activity was determined on modified crystal violet-pectate (CVP) medium (Schaad 1988). Pitting of the medium surrounding individual colonies indicated the presence of pectolytic activity.

Biolog™ analyses

Initial Biolog™ analyses (identified by extension -1) were performed on Eh 198/1 and the two *P. dispersa* ATCC

Table 1. Biochemical and taxonomic characterization of the *E. herbicola*-like strains used in this study

Strain	Source	EA inh	pFO	Arnow Test	Entero-bactins	Primary ID by Biolog 1	Sim coef	Dist coef	Primary ID by Biolog 2	Sim coef	Dis coef	Primary ID by GC-FAME	Sim coef	Dis coef
Eh 198/1	Kokošková	–	–	+	+	*Cedecea lapagei*	0.40	2.2	*Klebsiella pneumoniae*	0.50	4.7	*E. carotovora carotovora*	0.80	2.4
Eh 2406	Kokošková CCM 2406	–	–	–	+	ND			ND			ND		
Eh 1810	Kokošková	–	+	+	·	ND			ND			ND		
P. dispersa-type strain opaque; previously: *E. herbicola*	ATCC 14589	ND	–	++	–	*P. dispersa*	0.85	2.0	*P. dispersa*	0.31	6.0	*P. ananas*	0.83	2.2
P. dispersa translucent	ATCC 14589	ND	ND	ND	ND	*P. agglomerans*	0.63	5.6	*E. agglomerans* biogroup 2A	0.51	7.8	*P. ananas*	0.76	2.7
P. agglomerans: old *E. herbicola* type strain	Beer ATCC 33234	ND	+	+	+	ND			*P. agglomerans*	0.87	2.0	*P. agglomerans* group 1 Clin	0.73	3.0
P. agglomerans-type strain: old *E. agglomerans* neotype	ATCC 27155	ND	+	+	+	ND			*P. agglomerans*	0.82	2.7	*P. agglomerans*	0.89	1.8
C9-1	Ishimaru	+	+	+	+	ND			*P. agglomerans*	0.77	3.4	*P. agglomerans*	0.77	2.6

ND, not determined; inh, inhibitory; ID, identification; Simcoef, similarity coefficient; Dist coef, distance coefficient.

14589 strains by Analytical Services (Essex Junction, VT) using Micrologᵀᴹ System version 3.5 and well-by-well (operator) evaluation of the 96-well plates. After growth on trypticase soy agar (TSA), the cultures were suspended in sterile saline and aliquots loaded into the appropriate microtiter plates (Gram-negative). When it became clear that Eh 198/1 most likely was not *P. dispersa*, the comparison was extended to other *E. herbicola*-type strains. The *P. dispersa* strains were again included in order to have all data acquired under identical conditions. The second set of Biologᵀᴹ analyses (identified by extension –2) was performed by Microbe Inotech Laboratories (St. Louis, MO), using the same culture treatment as above and also Micrologᵀᴹ System version 3.5 but employing an automated microplate reader.

GC-FAME

GC-FAME analyses were performed by Microbe Inotech Laboratories using Method 1 Standard GC-FAME after 24 h incubation on TSA. The results were compared against the Aerobe (TSBA [revision 3.90]) and the Clinical Aerobe (CLIN [revision 3.90]) databases.

Results and discussion

Proferrioxamine profiles

As expected, production of proferrioxamines among strains of the Herbicola–Agglomerans complex varied, with Eh 198/1 and *P. dispersa* being pFO^-, whereas all other strains investigated were pFO^+ including the type strains for *E. agglomerans* (*P. agglomerans*) and *E. herbicola* (Table 1). The pFO^+ strains produced a proferrioxamine profile very similar to that of *E. amylovora* (Feistner *et al.* 1993a,b). The profile for the type strain of *P. agglomerans* (ATCC 27155) is shown as an example

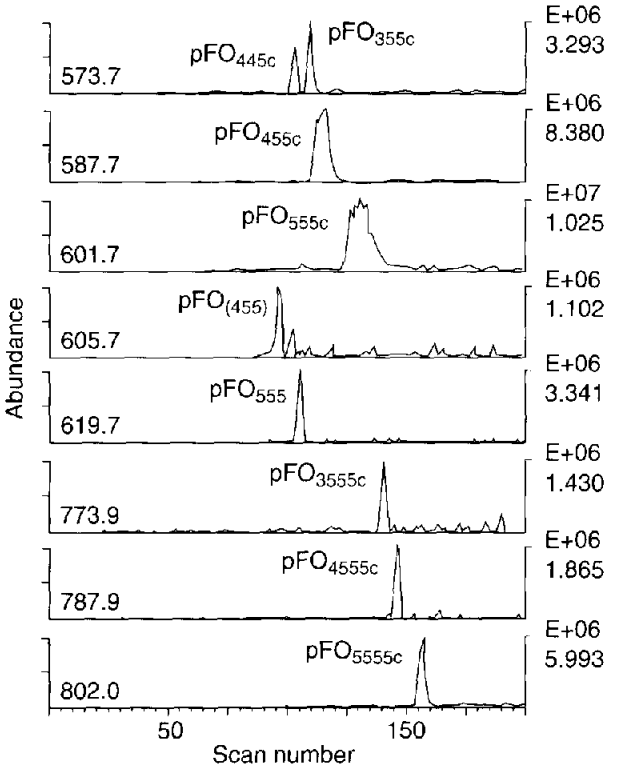

Figure 1. Proferrioxamine profile for the type strain of *P. agglomerans* (ATCC 27155). Shown are the respective molecular ion chromatograms, acquired by on-line reversed phase LC-MS. The numbers at the left side of the panels refer to the respective masses of the protonated proferrioxamines. The numbers to the right side of the panels indicate the relative abundance of each proferrioxamine. The peaks are labeled using the new proferrioxamine nomenclature (Feistner 1995b).

in Figure 1. Our data confirm previous reports by Berner (1988, 1990) in that the major siderophore was found to be pFO_{555c} (pFO E; regarding the new pFO nomenclature, see Feistner 1995b). Under our growth conditions, pFO_{455c} (pFO D_2) also was a major siderophore. In addition, we identified tetrahydroxamates and diaminopropane-containing proferrioxamines that were not previously known to be metabolites of *E. herbicola*. On the other hand, we found no evidence for pFO_{555Ac} (pFO B) in any of our cultures. The qualitative differences between this and the previous reports may be attributed to the sensitivity and precision of the on-line LC-MS method employed in our study.

Siderophores other than proferrioxamines

Our hypothesis that some strains of *E. herbicola* (*Pantoea* spp.) rely on siderophores other than proferrioxamines for their iron nutrition was supported not only indirectly through the absence of proferrioxamines but also more directly by a positive Arnow test, which is indicative of catechol (and potentially of catechol siderophores), for all strains investigated (Table 1). The Arnow reaction was particularly strong for *P. dispersa* and this is consistent with the notion that *P. dispersa* may compensate for the lack of proferrioxamines by overproducing catechol siderophores. A retrospective analysis of the previously acquired LC-MS data showed that these catechols are, at least in part, identical to the enterobactin siderophores previously reported for *E. herbicola* by Berner *et al.* (1991). Molecular ions for both cyclic enterobactin and its linear trimer were observed, but evidence for enterobactin mono- and dimers was lacking. Since our LC-MS system was not thoroughly evaluated for the analysis of enterobactins, we do not wish to make quantitative statements except for the comment that there appeared to be only traces of enterobactins for the strains obtained from the Czech Republic, including Eh 198/1.

In contrast to what was found for *P. dispersa*, the Arnow test did not indicate that Eh 198/1 was compensating for the lack of proferrioxamines with the overproduction of catechol siderophores. In this case, evidence for major siderophores other than proferrioxamines was obtained through differential RP-HPLC analysis of the supernatant of Eh 198/1 before and after the addition of ferric iron. Four chromatographic peaks were found to shift their position upon the addition of ferric citrate and presumably were due to iron-binding compounds (not shown). Studies to identify these atypical siderophores now seem to be warranted. We also propose extension of our LC-MS screening to the siderophores of other biocontrol strains. The corresponding siderophores may find use as novel control agents for fire blight and/or serve as lead compounds for the synthesis of such.

Proferrioxamines and biocontrol

Our study provides evidence for (Eh 198/1) and against (C9-1) the notion that biocontrol of fire blight may be due to incompatible siderophores. C9-1 produces a pattern of proferrioxamines *in vitro* that is nearly identical to that of the pathogen. This may mean that for C9-1, and presumably other biocontrol strains, iron competition is not an important mechanism, since organisms with similar iron acquisition systems would be expected to compete equally for iron. However, biocontrol with live organisms is complex, and even for C9-1, iron

competition cannot be entirely ruled out. Other factors affecting siderophore production and uptake can contribute to the overall outcome of iron competition. For example, regulation of siderophore gene expression might be controlled differently in the pathogen and in the biocontrol agent. Relative growth rates, location and distribution in the host plant, and nutrient availability in colonized tissues also may influence availability of iron and thus expression of siderophores (Loper & Ishimaru 1991).

On the other hand, even when organisms produce different siderophores, as seems to be the case for *E. amylovora* and Eh 198/1, iron competition may or may not be important to biocontrol. For example, biocontrol of *Pythium* damping-off of cotton was found to be independent of the production of aerobactin or enterobactin by the biocontrol agent *Enterobacter cloacae* (Costa & Loper 1994). A combination of genetic and biochemical approaches will be required to conclusively define the role of iron competition in any biocontrol of fire blight. The results of our study encourage such more ambitious investigations. Provided fire blight control through interference with iron acquisition is principally feasible, the complications arising from control with live organisms may be avoided by applying the siderophores (produced through synthesis or fermentation) directly.

Taxonomic studies

To direct future searches for biocontrol strains that produce siderophores other than proferrioxamines, it was of interest to taxonomically classify Eh 198/1. The results from the proferrioxamine study (Table 1) suggested that Eh 198/1 might be a strain of *P. dispersa*. The LC-MS studies were therefore complemented with fatty acid profiling (GC-FAME) and carbon source utilization (Biolog™) studies. In connection with these studies, our stock culture of *P. dispersa* was found to consist of two strains that were distinguishable on grounds of their colony type: opaque and translucent, and hence they were analyzed separately. As Table 1 shows, the Biolog™ and the GC-FAME systems unanimously corroborated the identity of the *E. agglomerans* (*P. agglomerans*) reference strains (ATCC 33234 and 27155). The *E. amylovora*-inhibitory strain C9-1 also was unambiguously identified as *P. agglomerans*. However, the GC-FAME and Biolog™ primary identifications were unfortunately inconsistent with each other for Eh 198/1 and the two *P. dispersa* strains. To better understand the underlying reasons for these inconsistencies, the data were analyzed in more detail.

GC-FAME

Table 2 presents a summary of the fatty acid profiles for the two *E. amylovora*-inhibitory strains, Eh 198/1 and C9-1, the two *P. agglomerans* ATCC reference strains, and the two *P. dispersa* strains. Considering the fact that there are more than 200 fatty acids and related lipids known, the fatty acid profiles for all strains in our study are quite similar. Consequently, the differentiation of *Pantoea* spp. is not straightforward. The last column of Table 2 lists previous summary results by Wells *et al.* (1994) obtained on various *E. herbicola* strains. Retrospectively it is clear that their selection of *E. herbicola* strains comprised both *P. agglomerans* and *P. dispersa*; this may explain the wide percentage range given for some fatty acids. In contrast, Table 2 suggests that the *E. herbicola* strains investigated in this study fall into several clusters with narrower fatty acid distributions, and thus differentiation of *Erwinia* species beyond the stage achieved by Wells *et al.* may be possible. In particular, it appears that *P. agglomerans* and *P. dispersa* might be distinguishable on grounds of the ratio of the GC peaks at 9.7 (16:1 + others) and 13.1 min (18:1 + others). In our study, the ratio of the first to the second peak turned out to be >1 for *P. agglomerans* and <1 for *P. dispersa*. This is a tentative conclusion, which will have to be confirmed with a larger number of strains. Also, the specific fatty acid giving rise to this difference will have to be identified by GC-MS studies.

Two other conclusions could be drawn. First, the two strains derived from ATCC 14589 are obviously closely related to each other and thus we believe that one is a mutant of the other. Of the two strains, the one with the opaque colony type most closely resembles the Biolog™ *P. dispersa* reference data (see below). The reason why neither strain was identified as *P. dispersa* turned out to be a trivial one: Having only recently been introduced as a new species, *P. dispersa* was not yet in the GC-FAME database; *P. ananas* obviously was the next closest match. Our second conclusion is that, in contrast to our expectation, Eh 198/1 is not closely related to *P. dispersa*, and also differs from *P. agglomerans*, and this suggests to us that the Herbicola–Agglomerans complex is more diverse than previously thought. Apparently, the species that Eh 198/1 belongs to is also not in the GC-FAME data base; Eh 198/1 was ruled out to be *E. carotovora* on grounds of it being non-pectolytic.

Table 2. GC-FAME analyses of the *E. herbicola*-like strains investigated in this study

RT (min)	Fatty acid	33234	27155	C-91	14589-t	14589-o	198/1	Wells
4.5	12:0	3.3	3.2	4.3	3.8	3.8	6.9	3.5–4.5
6.9	14:0	5.8	4.9	5.7	3.1	3.5	1.7	3.6–4.3
7.6	unknown	0.7	0.7	0.6	0.7	0.6	0.5	
8.4	15:0	0.5		0.3	0.4	0.4	0.5	
8.7	14:0 2-OH				1.9	1.5		
9.2	14:0 3-OH + others	8.3	7.6	8.2	8.1	7.6	7.4	4.6–7.2
9.7	16:1 ω7c + others	33.7	33.8	29.4	**18.1**	**19.8**	31.8	17.5–24.2
10.0	16:0	32.6	30.5	29.7	33.8	33.8	29.3	28.1–35.7
11.5	17:0 cyclo	4.3	8.5	3.3	3.9	2.9	1.6	5.0–6.7
11.7	17:0	0.4		0.3	0.4	0.3	0.9	
13.1	18:1 ω7c + others	8.7	13.6	11.9	**23.6**	**23.7**	17.4	13.9–31.9
13.4	18:0			0.3	0.4	0.4	0.3	0.3
15.0	19:0 cyclo + others				**0.3**	0.3		

Table 3. Differences in the Biolog™ patterns between the strains analyzed

Carbon source	Pa_{exp} (%)	33234	27155	C9-1	Pd-t-1	Pd-t-2	Pd-o-1	Pd-o-2	Pd_{exp} (%)	198/1-1	198/1-2
A7	22	–	–	–	–	–	–	–	0	–	+
A9	6	–	–	–	–	–	–	–	0	+/–	+
A11	90	+	+		+	+	+	+	100	–/+	+
A12	30	–	–	–	+	+	+	+	100	+	+
B1	0	–	–	–	+	+	+	+	100	–	+/–
B3	7	–	–	+	–	–	+/–	+	0	+/–	+
B5	72	+	–	+	+	+	+	+	100	+	+
B8	51	–	–	+/–	–	–	–	–	100	+	+
B9	30	–	–	–/–	–	–	–	–	0	–/–	+
C1	30	+/–	–	+	–	+/–	+	–	20	+	+
C4	14	–	–	–	–	–	+/–	–	0	+	+
C6	100	+/–	+/–	+	–	–	+	+	100	+	+
C9	72	–	–	–/–	–	–	–	–	100	+/–	+
D5	7	–	–	–	+	+	+	+	100	+	+
D8	45	–	–	+/–	+	+	+	+	100	+	+
E1	0	–	–	–	–	–	+/–	–	0	–	–
E4	64	–	–		–	–	+	+	100	+	–
E7	68	+	–	+	–	–	–	–	0	+	–
E9	66	+	+	+	–	+	+	+	100	–	–
F2	100	+	–	–	–	–	+	+	50	+	–
F4	0	–	–	–	–	–	+	+	100	+	–
F11	56	+	–	+	+	+	+	–	100	+	–
G4	70	+	+	+	–	–	+	+/–	40	+	–
G8	57	–	–	–	–	–	+	–	100	+/–	–
G12	52	+	+	+	–	+	+	+	100	–	–
H1	100	+	+	+	+	+	+	+	100	+	–

Pa_{exp} and Pd_{exp}: % of strains of *P. agglomerans* and *P. dispersa*, respectively, expected to be positive for a particular biochemical characteristic; Pd-t-# and Pd-o-#: results from two different assays for the translucent and opaque strains, respectively, which were derived from ATCC 14589; 198/1-#: results from two different assays for the *E. amylovora*-inhibitory strain Eh 198/1 obtained from B. Kokošková; A7: *N*-acetyl-D-galactosamine; A9: adonitol; A11: D-arabitol; A12: cellobiose; B1: i-erythitol; B3: L-fucose; B5: gentiobiose; B8: α-D-lactose; B9: lactulose; C1: D-melibiose; C4: D-raffinose; C6: D-sorbitol; C9: turanose; D5: D-galactonic acid lactone; D8: D-glucosaminic acid; E1: *p*-hydroxy-phenylacetic acid; E4: α-keto glutaric acid; E7: malonic acid; E9: quinic acid; F2: succinamic acid; F4: alaninamide; F11: glycyl-L-aspartic acid; G4 :L-ornithine; G8: D-serine; G12: γ-amino butyric acid; H1: urocanic acid.

Biochemical analyses

As could be expected, the metabolic activities of the strains in this study were also quite similar. The Biolog™ system monitors 95 metabolic activities and with regard to 69 of these, all strains, except the translucent mutant of ATCC 14589, which had lost several metabolic activities compared to the parent strain, were indistinguishable. The other 26 metabolic activities are listed in Table 3 together with the Biolog™ expectations (in %) for *P. agglomerans* (Pa_{exp}) and *P. dispersa* (Pd_{exp}). The Biolog™ expectations are based on information about several strains for each species (exact number proprietary knowledge). Out of these 26 activities, we found many to be variable even between different strains of a given species. There seemed to be only one clear-cut difference between *P. agglomerans* and *P. dispersa*, i.e. with regard to the utilization of i-erythritol (B1). This carbon source can obviously be utilized by *P. dispersa* but apparently not by *P. agglomerans*. As with the differentiation on grounds of the fatty acid profiles (see above), this seemingly discriminating feature will have to be confirmed with a larger number of strains, but is already corroborated by the previous report of Gavini *et al.* (1989). The latter report also suggested further differences, but these are either not tested for in the Biolog™ system for Gram-negative bacteria [salicin, amygdalin (may be evaluated, however, by using the microplates for Gram-positive bacteria)] or could not be confirmed (gentiobiose, B5).

The Biolog™ characterization for Eh 198/1 corroborates our conclusion from the GC-FAME analyses, i.e. that Eh 198/1 most likely is neither a strain of *P. dispersa* nor of *P. agglomerans*. However, Table 3 also indicates that even for a given strain, independent Biolog™ analyses may give rise to different patterns and this certainly does not contribute to the robustness of the method. In addition to differences arising from manual versus automatic readout, pattern variations may also be due to inducible metabolic activities that are responsive to subtle differences in culture conditions. One pertinent example would be that of basic amino acid decarboxylases, which are known to be induced in response to iron-deficiency (Feistner 1994) and low pH (Meng & Bennett 1992). In light of the information from Table 3 (G4) and the knowledge that basic amino acid decarboxylase activity is part of the proferrioxamine biosynthetic pathways (Schupp *et al.* 1987, Feistner 1994, 1995a), previous descriptions of *P. agglomerans* (*E. agglomerans*) being ornithine and lysine decarboxylase negative (Lindh *et al.* 1991) cannot be supported.

Conclusion

Our overall conclusion from the above results is that a broader and more in depth study of the siderophores of *E. herbicola*-like strains is warranted both in terms of the potential to find siderophores that may lead to new biocontrol agents for fire blight, and in terms of the potential for improving the taxonomic differentiation of the various members of the heterogenous Herbicola–Agglomerans group. Since the structural genes for proferrioxamines in *Erwinia* have not yet been identified, the possibility exists that the corresponding cistrons are plasmid encoded or flanked by IS elements. If so, then the feasibility of horizontal transfer would greatly diminish the value of proferrioxamines for taxonomic typing. However, the usefulness of LC-MS for taxonomic studies is not limited to just the characterization of siderophores. It might as well be extended to the monitoring of other microbial metabolites.

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