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The Genome of *Erysipelothrix rhusiopathiae*, the Causative Agent of Swine Erysipelas, Reveals New Insights into the Evolution of *Firmicutes* and the Organism's Intracellular Adaptations▽†

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***Erysipelothrix rhusiopathiae* is a Gram-positive bacterium that represents a new class, *Erysipelotrichia*, in the phylum *Firmicutes*. The organism is a facultative intracellular pathogen that causes swine erysipelas, as well as a variety of diseases in many animals. Here, we report the first complete genome sequence analysis of a member of the class *Erysipelotrichia*. The *E. rhusiopathiae* genome (1,787,941 bp) is one of the smallest genomes in the phylum *Firmicutes*. Phylogenetic analyses based on the 16S rRNA gene and 31 universal protein families suggest that *E. rhusiopathiae* is phylogenetically close to *Mollicutes*, which comprises *Mycoplasma* species. Genome analyses show that the overall features of the *E. rhusiopathiae* genome are similar to those of other Gram-positive bacteria; it possesses a complete set of peptidoglycan biosynthesis genes, two-component regulatory systems, and various cell wall-associated virulence factors, including a capsule and adhesins. However, it lacks many orthologous genes for the biosynthesis of wall teichoic acids (WTA) and lipoteichoic acids (LTA) and the *dltABCD* operon, which is responsible for D-alanine incorporation into WTA and LTA, suggesting that the organism has an atypical cell wall. In addition, like *Mollicutes*, its genome shows a complete loss of fatty acid biosynthesis pathways and lacks the genes for the biosynthesis of many amino acids, cofactors, and vitamins, indicating reductive genome evolution. The genome encodes nine antioxidant factors and nine phospholipases, which facilitate intracellular survival in phagocytes. Thus, the *E. rhusiopathiae* genome represents evolutionary traits of both *Firmicutes* and *Mollicutes* and provides new insights into its evolutionary adaptations for intracellular survival.**

The phylum *Firmicutes* consists of Gram-positive bacteria with low genomic G+C contents. It includes many important pathogens, including *Bacillus*, *Staphylococcus*, and *Streptococcus* species, and bacteria beneficial to humans, including *Lactobacillus* and *Lactococcus* species. *Firmicutes* was previously described as consisting of three classes, *Bacilli*, *Clostridia*, and *Mollicutes* (14). However, *Mollicutes*, which are represented by the genus *Mycoplasma*, have recently been removed from the phylum *Firmicutes* and placed in a newly created phylum, *Tenericutes*, because these species lack rigid cell walls and there is no alternative marker except 16S rRNA sequences to support retention of *Mollicutes* in *Firmicutes* (23). On the other hand, the family *Erysipelotrichaceae*, which consists of Gram-positive walled bacteria that were previously classified as *Mollicutes*, was retained in *Firmicutes* as a member of a newly generated class, *Erysipelotrichia*, which comprises a single order and a single family: the order *Erysipelotrichales* and the family *Erysipelotrichaceae*. The family *Erysipelotrichaceae* is composed of eight genera: *Erysipelothrix*, *Allobaculum* (a newly described genus), *Bulleidia*, *Catenibacterium*, *Coprobacillus*, *Holdemania*, *Solobacterium*, and *Turicibacter*. The last six genera were transferred from other families to *Erysipelotrichaceae* (23).

Erysipelothrix rhusiopathiae, a Gram-positive, non-spore-forming, rod-shaped bacterium, is the standard species of the genus *Erysipelothrix* and comprises the genus, along with *Erysipelothrix tonsillarum* and *Erysipelothrix inopinata* (53). *E. rhusiopathiae* is ubiquitous in nature and has been isolated from many species of wild and domestic mammals, birds, reptiles, amphibians, and fish (58). The organism can grow either aerobically or anaerobically. As has been observed for *Mesoplasma* species in *Mollicutes* (41), it does not grow at all or grows very slowly if the medium is not supplemented with 5 to 10% serum or 0.1% Tween 80 (polyoxyethylene sorbitan), even in a nutrient-rich medium, such as brain heart infusion (BHI) medium (11).

E. rhusiopathiae is generally regarded as an opportunistic animal pathogen that causes a variety of diseases in many species of birds and mammals, including humans. It is best known as a facultative intracellular pathogen that causes swine erysipelas, which may occur as acute septicemia or chronic endocarditis and polyarthritis (58). The organism has a capsule (52). Interestingly, it can survive inside polymorphonuclear leukocytes and macrophages if it is phagocytosed (51). The primary survival strategy in phagocytes appears to be escape

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TABLE 1. General features of the genome of *E. rhusiopathiae* strain Fujisawa

Feature	Value
Genome size (bp)	1,787,941
Overall G+C content (%)	36.6
G+C content of CDS (%)	36.7
No. of CDSs	1704
No. of pseudogenes	7
No. of rRNA operons (16S-23S-5S)	7
No. of tRNA genes	55
No. of prophage	1
No. of plasmids	0

from reactive oxidative metabolites generated by phagocytic cells; however, the precise mechanisms for intracellular survival of the organism are unknown (45, 51).

Here, we report the results of genome sequencing of *E. rhusiopathiae* strain Fujisawa, the first complete genome sequence of a bacterium belonging to the class *Erysipelotrichia*. Fujisawa is a highly virulent strain originally isolated from a diseased pig and has been extensively used in studies of *E. rhusiopathiae* pathogenesis (32, 47, 49–52, 55). This genome analysis provides new insights into how the organism has evolved and adapted as an intracellular pathogen.

MATERIALS AND METHODS

DNA sequencing and annotation, and data analyses. The *E. rhusiopathiae* strain Fujisawa was grown at 37°C in BHI (Becton, Dickinson and Company, Baltimore, MD) supplemented with 0.1% Tween 80 (pH 8.0), and the genomic DNA was prepared as described previously (50). The genome sequence was determined at Dragon Genomics Center Co. Ltd. (Mie, Japan) by a combination of three sequence technologies: the Genome Sequencer 20 (GS20) (Roche), SOLiD (*s*equencing by *o*ligonucleotide *li*gation and *d*etection) (Applied Biosystems), and Genome Analyzer II (GAII) (Illumina) systems. The genomic DNA was first sequenced with GS20 to generate a draft sequence. Then, a total of 140 million SOLiD reads (a 25-bp sequence for each read) produced from the 1.5-kb and 5.5-kb paired-end libraries were mapped to the GS20 sequence to correct sequence errors. At this stage, the depth of coverage from high-quality reads was approximately 23.5-fold. The sequences obtained by GS20 and SOLiD were further compared with data obtained by GAII, and unmatched sequences and gap sequences in the contigs were corrected or closed by PCR amplification followed by Sanger sequencing.

Finally, three gaps, all of which were derived from rRNA (*rrn*) operons, remained to be closed. Because the organization of these *rrn* operons was too complex to be closed by a simple procedure, we constructed a fosmid library. Using the fosmid clones containing each *rrn* locus, we determined the copy number of *rrn* operons and the gene composition (16S-23S-5S rRNA) of each locus by Southern hybridization analysis, PCR, and Sanger sequencing with primer walking. This series of analyses revealed that one locus contains a single *rrn* operon (16S-23S-5S rRNA) and that the other two loci contain two and four operons, respectively, in a tandem organization. Therefore, we were unable to determine the complete nucleotide sequences of these six *rrn* operons. However, by careful inspection of the Sanger sequencing data from these two *rrn* loci, we found no peak patterns suggestive of the presence of sequence heterogeneity between copies. Therefore, we conclude that the *rrn* sequences in each of the two loci are identical to those of the single *rrn* operon.

Potential protein-coding sequences (CDSs) of greater than 150 bp were identified using the gene prediction software MGA (MetaGeneAnnotator) (36). Short CDSs of less than 150 bp in intergenic regions were identified by the IMCGE (*in silico* Molecular Cloning Genomics Edition) software (37) with a BLASTP search (http://blast.ncbi.nlm.nih.gov/Blast.cgi) as a guide. tRNA and rRNA genes were identified by tRNAscan-SE 1.23 (22) and RNAmmer 1.2 (20), respectively. Functional annotation of each CDS was made according to the results from the BLASTP search against the NCBI RefSeq database (release 34) (40). For the metabolic pathway analysis, the KEGG (Kyoto Encyclopedia of Genes and Genomes) database (18; http://www.genome.jp/kegg/) was used.

Phylogenetic analysis. A phylogenetic tree based on 16S rRNA gene sequences was constructed as described previously (56). A genomic phylogenetic tree was constructed from alignments of the concatenated protein sequences of 31 universal genes, which are all involved in translation and have been shown to be conserved in all of the analyzed bacterial species (6). Briefly, multiple-sequence alignments of each COG (cluster of orthologous groups) were created using MUSCLE (8). The sequence alignments were concatenated and poorly aligned, and divergent regions were removed using Gblocks (4). Based on the resulting final sequence alignments, a phylogenic tree was constructed by the maximum-likelihood method using the MEGA 5 program (http://www.megasoftware.net/).

Genome comparison. Genome sequences of other *Erysipelotrichia* strains, including *E. rhusiopathiae* strain ATCC 19414, all of which are incomplete or unfinished, were obtained from the National Center for Biotechnology Information (NCBI database) (http://www.ncbi.nlm.nih.gov/genomes/MICROBES/microbial_taxtree.html) in February 2011. *E. rhusiopathiae* strain-specific CDSs were identified by bidirectional best-hit analysis between the genomes of strains Fujisawa and ATCC 19414 with a threshold of >90% amino acid identity and >60% aligned length coverage of a query sequence. Conservation of the CDSs of Fujisawa in other *Erysipelotrichia* strains (*Solobacterium moorei*, *Coprobacillus* sp., *Holdemania filiformis*, *Catenibacterium mitsuokai*, *Bulleidia extructa*, *Erysipelotrichaceae* bacterium, *Clostridium ramosum*, *Clostridium spiroforme*, *Eubacterium biforme*, and *Eubacterium dolichum*) for which draft genome sequences were available was examined by BLASTP analysis with an E value threshold of 10^{-10}.

Nucleotide sequence accession number. The genome sequence reported in this paper has been deposited in the DDBJ/GenBank/EMBL databases (accession no. AP012027).

RESULTS AND DISCUSSION

General genomic features. The general features of the genome of *E. rhusiopathiae* strain Fujisawa are summarized in Table 1. The origin of replication (nucleotide position 1) was

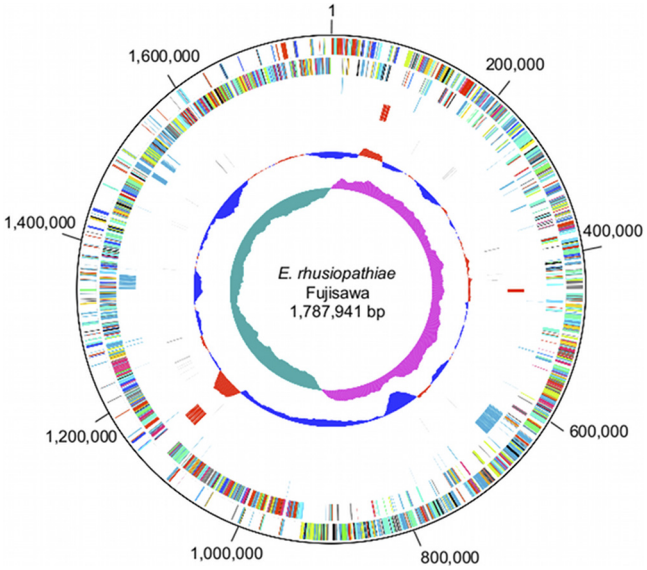

FIG. 1. Circular representation of the genome of *E. rhusiopathiae* strain Fujisawa. Beginning with the outer region, the circle shows (i) nucleotide positions in base pairs, (ii and iii) predicted CDSs transcribed on the forward (clockwise) (ii) and reverse (counterclockwise) (iii) DNA strands, (iv) positions of Fujisawa strain-specific genes not present in the genome of strain ATCC 19414, (v) rRNA operon(s), (vi) tRNA genes, (vii) percent G+C content (red and blue, respectively, represent regions with higher and lower G+C contents compared to the average value for the entire genome), and (viii) the G+C skew curve. The color of each CDS was assigned according to the COG functional grouping (http://www.ncbi.nlm.nih.gov/COG/).

TABLE 2. Insertion sequences identified in *E. rhusiopathiae* strain Fujisawa

Name	No.	IS family	No. of CDSs	Size (bp)	Length of DR (bp)
IS*Erh1*	ERH_IS01	3	2	1,280	3
IS*Erh1*	ERH_IS02	3	2	1,280	3
IS*Erh1*	ERH_IS06	3	2	1,280	3
IS*Erh1*	ERH_IS14	3	2	1,280	4
IS*Erh1*	ERH_IS22	3	2	1,280	3
IS*Erh2*	ERH_IS03	30	1	1,031	–[a]
IS*Erh2*	ERH_IS08	30	1	1,031	27
IS*Erh2*	ERH_IS09	30	1	1,031	29
IS*Erh2*	ERH_IS15	30	1	1,031	32
IS*Erh2*	ERH_IS17	30	1	1,031	23
IS*Erh2*	ERH_IS18	30	1	1,031	24
IS*Erh2*	ERH_IS23	30	1	1,031	30
IS*Erh2*	ERH_IS24	30	1	1,031	31
IS*Erh3*	ERH_IS04	?[b]	3	1,351	6
IS*Erh3*	ERH_IS07[c]	?	3	1,176	6
IS*Erh4*	ERH_IS05	30	1	1,032	30
IS*Erh4*	ERH_IS10	30	1	1,032	26
IS*Erh4*	ERH_IS11	30	1	1,032	37
IS*Erh4*	ERH_IS16	30	1	1,032	26
IS*Erh4*	ERH_IS19	30	1	1,032	41
IS*Erh5*	ERH_IS12	3	2	1,247	–
IS*Erh5*	ERH_IS13	3	2	1,247	–
IS*Erh5*	ERH_IS20[c]	3	2	1,206	–
IS*Erh6*	ERH_IS21	3	2	1,240	–

[a] –, not identified.
[b] ?, unclassified; both copies appear to contain internal deletions.
[c] Truncated IS.

assigned to a region showing a clear G+C skew transition and containing the *dnaA* gene accompanied by several DnaA boxes. The genome contains 1,704 CDSs. Of these, biological functions were assigned to 1,332 CDSs, 327 CDSs showed sequence similarities to proteins of unknown function, and the remaining 45 had no significant database match. The genome contains only seven recognizable pseudogenes (four caused by frameshift and three caused by point mutations).

Like many other members of *Firmicutes* with lower genome G+C contents, the majority (75.6%) of genes are located on the leading strand. The genome contains seven *rrn* operons with the typical order of 16S, 23S, and 5S rRNA genes and 55 tRNA genes, including cognates for all amino acids (Fig. 1). The seven *rrn* operons are located at three loci and show a unique genetic organization: in the two loci (nucleotide positions 78525 to 89100 and 1117642 to 1139038), two and four operons, respectively, which probably have identical sequences (see Materials and Methods), are located in tandem.

We identified a 36.5-kb prophage (named PP_Erh_Fujisawa) including 49 genes (ERH_0581 through ERH_0629) but no CRISPR (clustered regularly interspaced short palindromic repeats). We found 22 intact insertion sequence (IS) elements and two truncated IS elements, none of which were inserted into CDSs of known function. These IS elements were classified into six types (named IS*Erh1* to IS*Erh6*); all types were newly identified, with IS*Erh2* being the predominant type (eight intact copies) (Table 2). Most of the IS elements belong to the IS*30* and IS*3* families, but one type (IS*Erh3*) is unclassified. Notably, the two IS*30* family members, IS*Erh2* and

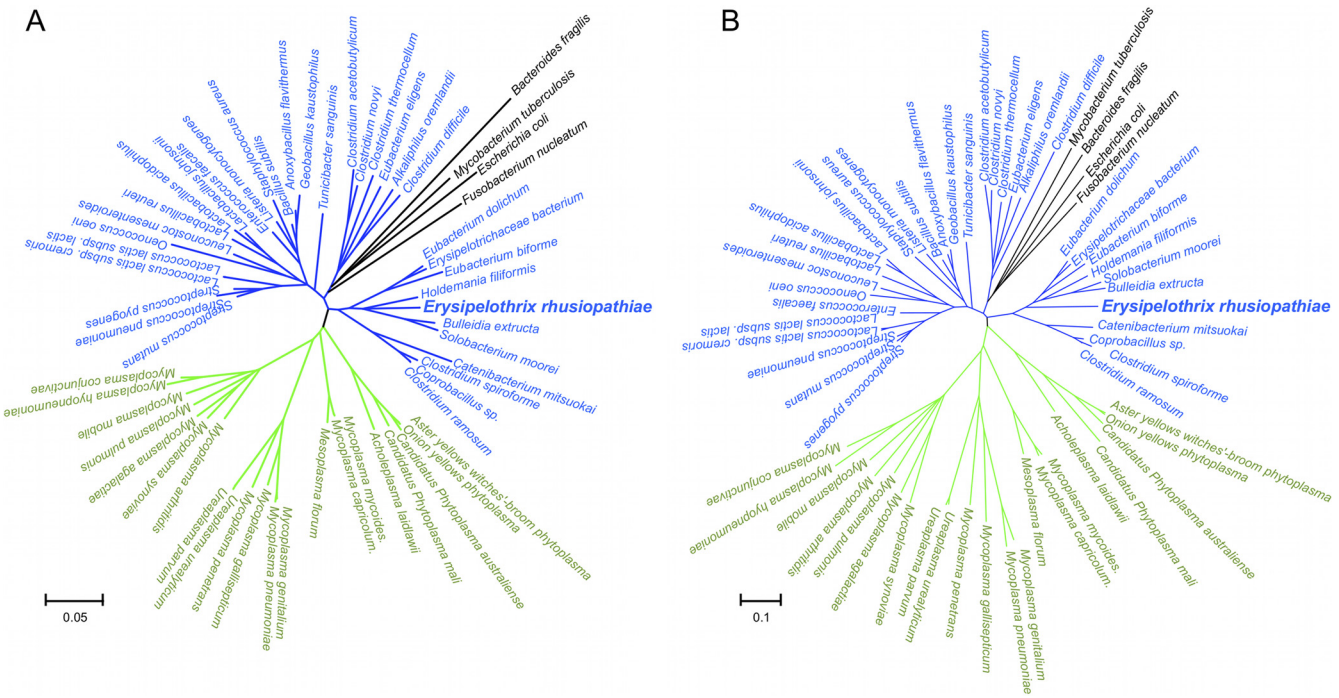

FIG. 2. Phylogenetic position of *E. rhusiopathiae.* Phylogenetic trees based on 16S rRNA gene sequences (A) and on concatenated protein sequence alignments derived from 31 universal protein families (B) are shown. Species are colored according to the current taxonomy: the phylum *Firmicutes*, blue; *Mollicutes*, green; others, black. *E. rhusiopathiae* is indicated by boldface. The scale bar represents the expected number of changes per sequence position. A bootstrap test with 1,000 replicates was used to estimate the confidence of the branching patterns of the trees. Branches corresponding to partitions reproduced in less than 50% of bootstrap replicates are collapsed.

TABLE 3. Numbers of orthologous genes related to DNA repair[a]

Species (strain)	Genome size (Mb)	GC content (%)	Phylum or class	No.															
				Total	*adaA*	*alkA*	*alkB*	*dinP*	*exoA (xth)*	*fpg*	*ligA*	*mfd*	*mpg*	*mutH*	*mutL*	*mutS*	*mutT*	*mutY*	*nfo*	*nth*
Alkaliphilus oremlandii (OhILAs)	3.12	36.3	*Firmicutes*	43	1	2	0	1	1	0	1	1	1	0	1	2	1	1	1	1
Clostridium acetobutylicum (ATCC 824)	3.94	30.9	*Firmicutes*	40	0	1	0	1	1	0	2	1	1	0	1	2	0	1	1	1
Clostridium difficile (630)	4.29	29.1	*Firmicutes*	38	0	1	0	1	1	0	1	1	1	0	1	2	0	0	1	1
Clostridium novyi (NT)	2.55	28.9	*Firmicutes*	35	0	1	0	1	0	0	1	1	1	0	1	2	1	0	1	1
Clostridium thermocellum (ATCC 27405)	3.84	39.0	*Firmicutes*	36	0	1	0	1	1	0	1	1	0	0	1	2	0	0	0	1
Eubacterium eligens (ATCC 27750)	2.14	37.7	*Firmicutes*	39	0	1	0	0	0	0	1	1	0	1	1	2	1	0	1	1
Streptococcus mutans (UA159)	2.03	36.8	*Firmicutes*	37	0	0	0	1	1	1	1	1	1	0	1	2	1	1	0	1
Streptococcus pneumoniae (TIGR4)	2.16	39.7	*Firmicutes*	33	0	0	0	1	1	1	1	1	0	0	1	1	0	1	0	1
Streptococcus pyogenes (SF370)	1.85	38.5	*Firmicutes*	36	0	0	0	1	1	1	1	1	0	0	1	2	1	1	0	1
Anoxybacillus flavithermus (WK1)	2.85	41.8	*Firmicutes*	38	0	1	0	0	0	1	1	1	0	0	1	2	1	1	1	1
Bacillus subtilis (168)	4.22	43.5	*Firmicutes*	49	1	2	0	2	1	1	1	1	1	0	1	2	1	1	1	1
Geobacillus kaustophilus (HTA426)	3.54	52.1	*Firmicutes*	38	0	1	0	0	0	1	1	1	0	0	1	2	2	1	1	1
Listeria monocytogenes (EGD-e)	2.94	38.0	*Firmicutes*	49	2	0	0	1	1	1	1	1	1	0	1	2	1	1	1	1
Staphylococcus aureus (N315)	2.81	32.8	*Firmicutes*	41	0	0	0	1	0	1	1	1	1	0	1	2	0	1	1	1
Enterococcus faecalis (V583)	3.36	37.5	*Firmicutes*	47	1	0	0	1	1	1	1	1	1	0	1	2	0	1	1	1
Lactobacillus acidophilus (NCFM)	1.99	34.7	*Firmicutes*	37	0	0	0	1	2	1	1	1	1	0	1	2	0	0	0	1
Lactobacillus brevis (ATCC 367)	2.29	46.2	*Firmicutes*	41	0	0	0	1	1	1	1	1	1	0	1	2	2	1	1	1
Lactobacillus casei (ATCC 334)	2.90	46.6	*Firmicutes*	45	0	0	0	1	1	1	1	1	1	0	1	2	2	1	1	1
Lactobacillus delbrueckii subsp. *bulgaricus* (ATCC 11842)	1.86	49.7	*Firmicutes*	35	0	0	0	1	1	1	1	1	1	0	1	2	0	0	0	1
Lactobacillus fermentum (IFO 3956)	2.10	51.5	*Firmicutes*	36	0	0	0	0	1	1	1	1	1	0	1	2	1	0	0	0
Lactobacillus gasseri (ATCC 33323)	1.89	35.3	*Firmicutes*	39	0	0	0	1	2	1	1	1	1	0	1	2	0	0	0	1
Lactobacillus helveticus (DPC 4571)	2.08	37.1	*Firmicutes*	36	0	0	0	1	1	1	1	1	1	0	1	2	0	0	0	1
Lactobacillus johnsonii (NCC 533)	1.99	34.6	*Firmicutes*	39	0	0	0	1	2	1	1	1	1	0	1	2	1	0	0	1
Lactobacillus plantarum (WCFS1)	3.35	44.5	*Firmicutes*	42	0	0	0	1	1	1	1	1	1	0	1	2	2	1	1	1
Lactobacillus reuteri (JCM 1112)	2.04	38.9	*Firmicutes*	38	0	0	0	0	2	1	1	1	1	0	1	2	1	0	0	1
Lactobacillus rhamnosus (GG)	3.01	46.7	*Firmicutes*	45	0	0	0	1	1	1	1	1	1	0	1	2	2	1	1	1
Lactobacillus sakei (23K)	1.88	41.3	*Firmicutes*	40	0	0	0	1	1	1	1	1	1	0	1	2	0	1	1	1
Lactobacillus salivarius (UCC118)	1.83	32.9	*Firmicutes*	35	0	0	0	1	1	1	1	1	0	0	1	2	0	0	0	1
Leuconostoc mesenteroides (ATCC 8293)	2.04	37.7	*Firmicutes*	40	0	0	0	1	1	1	1	1	0	0	1	2	2	1	0	0
Lactococcus lactis subsp. *cremoris* (MG1363)	2.53	35.8	*Firmicutes*	40	1	0	0	1	1	1	1	1	0	0	1	2	4	1	0	1
Lactococcus lactis subsp. *lactis* (IL1403)	2.37	35.3	*Firmicutes*	41	1	0	0	1	1	1	1	1	0	0	1	2	3	1	0	1
Oenococcus oeni (PSU-1)	1.78	37.9	*Firmicutes*	33	0	0	0	0	1	1	1	1	0	0	0	1	3	0	0	0
Erysipelothrix rhusiopathiae (Fujisawa)	1.79	36.6	*Firmicutes*	34	0	0	0	0	1	1	1	1	0	0	1	2	1	1	1	1
Acholeplasma laidlawii (PG-8A)	1.50	31.9	*Mollicutes*	33	0	0	0	1	0	1	1	1	0	0	1	2	0	1	1	1
"*Candidatus* Phytoplasma mali" (AT)	0.60	21.4	*Mollicutes*	16	0	0	0	0	0	1	1	0	0	0	0	0	1	0	2	0
"*Candidatus* Phytoplasma australiense"	0.88	27.4	*Mollicutes*	11	0	0	0	0	0	1	1	0	0	0	0	0	1	0	1	0
Aster yellows witches' broom phytoplasma (AYWB)	0.71	26.9	*Mollicutes*	11	0	0	0	0	0	1	1	0	0	0	0	0	1	0	1	0
Onion yellows phytoplasma (OY-M)	0.85	27.8	*Mollicutes*	11	0	0	0	0	0	1	1	0	0	0	0	0	1	0	1	0
Mesoplasma florum (L1; ATCC 33453)	0.79	27.0	*Mollicutes*	17	0	0	0	1	0	1	1	0	0	0	0	0	0	0	1	0
Mycoplasma agalactiae (PG2)	0.88	29.7	*Mollicutes*	16	0	0	0	1	0	1	1	0	0	0	0	0	0	0	1	0
Mycoplasma arthritidis (158L3-1)	0.82	30.7	*Mollicutes*	16	0	0	0	1	0	1	1	0	0	0	0	0	0	0	1	0
Mycoplasma conjunctivae (HRC/581T)	0.85	28.5	*Mollicutes*	16	0	0	0	1	0	1	1	0	0	0	0	0	0	0	1	0
Mycoplasma gallisepticum (R)	1.00	31.5	*Mollicutes*	15	0	0	0	1	0	1	1	0	0	0	0	0	0	0	1	0
Mycoplasma genitalium (G37)	0.58	31.7	*Mollicutes*	13	0	0	0	1	0	1	1	0	0	0	0	0	0	0	1	0
Mycoplasma hyopneumoniae (232)	0.89	28.6	*Mollicutes*	15	0	0	0	1	0	1	1	0	0	0	0	0	0	0	1	0
Mycoplasma mobile (163K)	0.78	25.0	*Mollicutes*	15	0	0	0	1	0	1	1	0	0	0	0	0	0	0	1	0
Mycoplasma capricolum (ATCC 27343)	1.01	23.8	*Mollicutes*	15	0	0	0	1	0	1	1	0	0	0	0	0	0	0	1	0
Mycoplasma mycoides (PG1)	1.21	24.0	*Mollicutes*	16	0	0	0	1	0	1	1	0	0	0	0	0	0	0	1	0
Mycoplasma penetrans (HF-2)	1.36	25.7	*Mollicutes*	17	0	0	0	1	0	1	1	0	0	0	0	0	0	0	1	0
Mycoplasma pneumoniae (M129)	0.82	40.0	*Mollicutes*	14	0	0	0	1	0	1	1	0	0	0	0	0	0	0	1	0
Mycoplasma pulmonis (UAB CTIP)	0.96	26.6	*Mollicutes*	18	0	0	0	1	0	1	2	0	0	0	0	0	0	0	1	0
Mycoplasma synoviae (53)	0.80	28.5	*Mollicutes*	15	0	0	0	1	0	1	1	0	0	0	0	0	0	0	1	0
Ureaplasma parvum (ATCC 700970)	0.75	25.5	*Mollicutes*	15	0	0	0	1	0	1	1	0	0	0	0	0	0	0	1	0
Ureaplasma urealyticum (ATCC 33699)	0.87	25.8	*Mollicutes*	14	0	0	0	1	0	1	1	0	0	0	0	0	0	0	1	0
Esherichia coli (K-12 MG1655)	4.64	50.8	*Proteobacteria*	44	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
Fusobacterium nucleatum (ATCC 25586)	2.17	27.2	*Fusobacteria*	30	0	0	0	1	1	0	1	1	0	0	1	2	0	0	0	1

[a] Orthologous genes were identified using the MBGD (Microbial Genome Database for Comparative Analysis) (http://mbgd.genome.ad.jp/).

IS*Erh4*, are associated with atypically long (23- to 41-bp) direct repeats (DRs). Within the IS*30* family, such long DRs have so far been detected only in IS*1630* (19 to 26 bp) of *Mycoplasma fermentans* (3) and IS*Mbov6* (22 to 36 bp) of *Mycoplasma bovis* (24). The predicted transposases of both IS*Erh2* and IS*Erh4* show the highest homology (32.4% and 32.0% amino acid sequence identity, respectively) to that of IS*1630* of *M. fermentans*.

Phylogenetic position of *E. rhusiopathiae*. We constructed a genomic phylogenetic tree using the 31 universal genes (6) that are conserved among all analyzed bacterial species and compared it with a tree that was constructed using 16S

TABLE 3—*Continued*

No.																											
ogt	*pcrA*	*phr*	*polA*	*priA*	*radA*	*radC*	*recA*	*recD*	*recF*	*recG*	*recJ*	*recN*	*recO*	*recQ*	*recR*	*recU*	*rexA*	*rexB*	*ruvA*	*ruvB*	*ruvC*	*tag1*	*umuC*	*ung*	*uvrA*	*uvrB*	*uvrC*
2	2	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	2	1	1	1	1	1	0	1	1	1	1
1	2	0	1	1	1	1	1	2	1	1	3	1	1	1	1	0	2	1	1	1	0	0	0	0	1	1	1
2	2	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	2	1	1	1	1	0	0	1	1	1	1
0	2	0	1	1	1	1	1	1	1	1	2	1	1	1	1	0	2	1	1	1	0	0	0	0	1	1	1
1	2	0	1	1	1	2	1	2	1	1	2	1	1	0	1	0	2	1	1	1	1	0	0	0	1	1	1
0	3	0	1	1	1	1	2	2	1	1	1	1	1	1	1	1	2	1	1	1	0	0	1	1	1	1	1
1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	2	1	1	1	0	1	0	1	1	1	1
1	1	0	1	1	0	1	1	1	1	1	1	1	1	0	1	1	2	1	1	1	0	1	0	1	1	1	1
0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	2	1	1	1	0	1	0	1	1	1	1
1	2	1	2	1	1	0	1	1	1	1	1	1	1	1	1	1	2	1	1	1	0	0	0	1	1	1	1
2	2	0	2	1	1	1	1	1	1	1	2	1	1	2	1	1	2	1	1	1	0	0	2	1	1	1	1
1	1	0	1	1	1	1	1	1	1	1	2	1	1	1	1	1	2	1	1	1	0	0	0	1	1	1	1
3	1	1	2	1	1	1	1	1	1	1	1	1	1	2	1	1	2	1	1	1	0	1	1	2	1	1	2
1	1	1	2	1	1	1	1	1	1	1	1	1	1	2	1	1	2	1	1	1	0	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	2	2	1	1	1	0	1	5	1	1	1	1
1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	0	1	0	1	1	1	1
1	1	0	1	1	1	1	1	1	1	1	1	1	1	2	1	1	2	1	1	1	0	1	0	1	1	1	1
1	1	0	1	1	1	1	2	2	1	1	1	1	1	2	1	1	2	1	1	1	0	2	1	1	1	1	1
0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	0	0	1	1	1	1	1
1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	0	1	1	1	1	1	1
2	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	0	1	1	1	1	1	1
1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	0	1	0	1	1	1	1
2	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	0	1	0	1	1	1	1
1	1	0	1	1	1	1	1	1	1	1	1	1	1	2	1	1	2	1	1	1	0	1	1	1	1	1	1
1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	0	1	1	1	1	1	1
1	1	0	1	1	1	1	2	2	1	1	1	1	1	2	1	1	2	1	1	1	0	2	1	1	1	1	1
1	1	0	1	1	1	1	1	1	1	1	1	1	1	2	1	1	2	1	1	1	0	1	1	1	1	1	1
1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	0	1	0	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	0	1	2	1	1	1	1
1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	0	0	0	1	1	1	1
1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	0	1	1	1	1	1	1
1	1	0	1	1	1	0	1	1	1	1	1	1	1	0	1	1	2	1	1	1	0	1	1	1	1	1	1
1	1	0	1	1	0	1	1	0	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1
1	1	1	1	1	0	1	1	1	1	1	0	1	1	0	1	1	0	0	1	1	0	1	2	1	1	1	1
0	1	0	1	1	0	0	1	0	0	1	0	0	1	0	1	1	0	0	1	1	0	0	0	1	0	0	0
0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
0	1	0	2	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	1	1	0	1	0	1	1	1	1
0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	1	2	0	0	1	1	0	0	0	1	1	1	1
1	1	0	1	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	1	1	0	0	0	1	1	1	1
0	1	1	1	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	1	1	0	0	0	1	1	1	1
0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	1	1	0	0	0	1	1	1	1
0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	1	1	1	1
0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	1	1	0	0	0	1	1	1	1
0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1	0	0	0	1	1	1	1
0	1	0	2	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	1	0	0	0	0	1	1	1	1
0	1	0	2	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	1	1	0	0	0	1	1	1	1
1	1	0	1	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	1	1	0	1	0	1	1	1	1
0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	1	1	1	1
1	2	0	1	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	1	1	0	0	0	1	1	1	1
0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	1	1	0	0	0	1	1	1	1
0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	1	1	0	0	0	1	1	1	1
0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	1	0	0	0	1	1	1	1
2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
1	1	0	1	1	1	1	1	0	1	1	2	1	1	1	1	0	0	0	1	1	1	0	0	1	1	1	1

rRNA sequences. In this analysis, we included all *Erysipelotrichia* strains, draft sequences of which were available, to better understand the phylogenetic position, not only of *E. rhusiopathiae*, but also of the class *Erysipelotrichia*. In both trees (Fig. 2A and B), *E. rhusiopathiae* and other *Erysipelotrichia* strains, with the exception of *Turicibacter sanguinis*, clustered together, forming a cluster distinct from other *Firmicutes* species and placed at the position closest to *Mollicutes*. These results indicate a very close phylogenetic relationship between *Erysipelotrichia*, including *E. rhusiopathiae*, and *Mollicutes*. Furthermore, our results may raise the possibility that *Erysipelotrichia* should be separated from *Firmicutes* and classified as a distinct phylum, as *Mollicutes* were moved to a separate phylum, *Tenericutes* (23). In con-

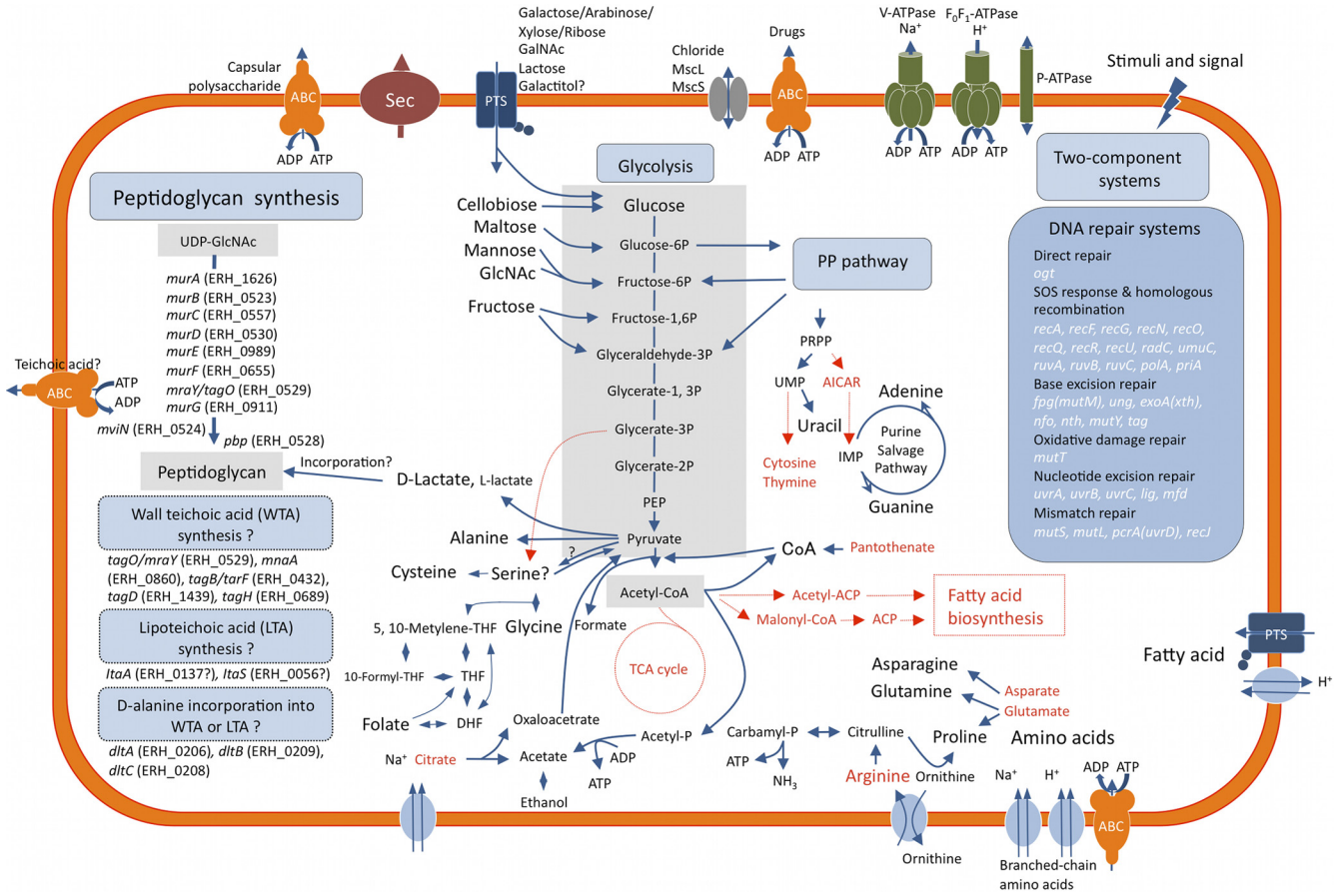

FIG. 3. Overview of the basic metabolic pathways of *E. rhusiopathiae*. Pathways or steps for which no enzymes were identified are indicated in red, as are the compounds for which *de novo* synthetic pathways were not identified. The question marks indicate that particular uncertainties exist. ABC, ATP-binding cassette superfamily; Sec, secretion pathway; PTS, phosphotransferase system; GalNAc, *N*-acetylgalactosamine; GlcNAc, *N*-acetylglucosamine; MscL, large conductance mechanosensitive ion channel family; MscS, small conductance mechanosensitive ion channel family; PP pathway, pentose phosphate pathway; PRPP, phosphoribosyl-pyrophosphate; AICAR, 5′-phosphoribosyl-4-carboxamide-5-aminoimidazole; PEP, phosphoenolpyruvate; ACP, acyl carrier protein; TCA, tricarboxylic acid; THF, tetrahydrofolate; DHF, dihydrofolate.

trast, *T. sanguinis* was placed in both trees at a very distant position from other *Erysipelotrichia* strains and clustered together with other *Firmicutes* species, suggesting that the species should be separated from *Erysipelotrichia*.

Reductive genome evolution and metabolic capabilities. Genome reduction has also taken place in many bacteria living in nutrient-rich environments, including lactic acid bacteria (25) and the members of *Mollicutes* (28). Among the sequenced members of *Firmicutes*, including lactic acid bacteria, the genome of *E. rhusiopathiae* is one of the smallest, indicating that reductive genome evolution occurred in the organism (Table 3).

The loss of DNA repair systems, a genomic feature that is often observed in bacteria showing genome reduction (30, 31), was evaluated and compared with DNA repair system loss in other bacteria in *Firmicutes* and *Mollicutes*. The *E. rhusiopathiae* genome contains 34 genes related to DNA repair functions (Table 3), which is a greater number of DNA repair-related genes than is present in *Mollicutes*, which varies from 11 to 33, and is similar to the number of genes in other *Firmicutes* with reduced genome sizes (Table 3), although these numbers do not always correlate with genome size. It may be noteworthy that, among the DNA repair-related genes that are highly conserved in *Firmicutes* but are absent in *Mollicutes*, four (*radA*, *recD*, *rexA*, and *rexB* homologs) are missing from *E. rhusiopathiae*.

E. rhusiopathiae encodes a full set of enzymes for the glycolysis and pentose phosphate pathways; some enzymes (glucokinase, 6-phosphofructokinase, and phosphoglycerate mutase) are duplicated. However, as expected, the organism lacks numerous genes for many other metabolic pathways (Fig. 3 and Table 4). *E. rhusiopathiae* lacks all of the genes for the tricarboxylic acid cycle, with the exception of fumarate hydratase (Table 4). Importantly, the organism also lacks all of the genes for the biosynthesis of unsaturated and saturated fatty acids. This genomic feature—the complete lack of genes for fatty acid biosynthesis—is observed in the genomes of all *Mollicutes* species (with the exception of *Acholeplasma laidlawii*), but not in other bacteria in the phylum *Firmicutes*, although several gene losses in fatty acid biosynthetic pathways have been observed in some lactobacilli (25).

It appears that the mechanism by which Tween 80 enhances the growth of *E. rhusiopathiae* does not involve oleic acid, which is the major ingredient of Tween 80, because we were

TABLE 4. Enzymes in the biosynthetic pathways of *E. rhusiopathiae*

Category or compound (gene)	Locus tag(s)
Energy metabolism	
Glycolysis	
Glucokinase (*glk*)	ERH_0239, ERH_1009
Glucose-6-phosphate isomerase (*pgi*)	ERH_0099
6-Phosphofructokinase (*pfkA*)	ERH_1011, ERH_1051
Fructose-bisphosphate aldolase (*fba*)	ERH_1632
Triose-phosphate isomerase (*tpiA*)	ERH_1335
Glyceraldehyde 3-phosphate dehydrogenase (*gapA*)	ERH_1534
Phosphoglycerate kinase (*pgk*)	ERH_1336
Phosphoglycerate mutase (*gpmA*)	ERH_0241, ERH_0435, ERH_0457, ERH_1685
Enolase (*eno*)	ERH_1334
Pyruvate kinase (*pyk*)	ERH_1010
Pentose phosphate pathway	
Glucose-6-phosphate 1-dehydrogenase (*zwf*)	ERH_0455
6-Phosphogluconolactonase (*pgl*)	ERH_0398
6-Phosphogluconate dehydrogenase (*gnd*)	ERH_0456
Transketolase (*tkt*)	ERH_1578
Ribose 5-phosphate isomerase (*rpiB*)	ERH_1609
Ribulose-phosphate 3-epimerase (*rpe*)	ERH_1027
TCA cycle	
Fumarate hydratase (*fumC*)	ERH_0730
Amino acid biosynthesis	
Proline	
Glutamate 5-kinase (*proB*)	ERH_0054
Glutamate-5-semialdehyde dehydrogenase (*proA*)	ERH_0055
Pyrroline-5-carboxylate reductase (*proC*)	ERH_0057
Glutamine	
Glutamine synthetase (*glnA*)	ERH_0836
Alanine	
Alanine dehydrogenase (*ald*)	ERH_1073
Asparagine	
Aspartate-ammonia ligase (*asnA*)	ERH_1353
Cysteine	
Serine *O*-acetyltransferase (*cysE*)	ERH_0421
Cysteine synthase A (*cysK*)	ERH_0469
Serine	
L-Serine dehydratase (*sdaA*)	ERH_1322
L-Serine dehydratase (*sdaB*)	ERH_1323
Glycine	
Glycine hydroxymethyltransferase (*glyA*)	ERH_1608
Arginine (incomplete pathway)	
Arginine deiminase (*arcA*)	ERH_0795
Carbamate kinase (*arcC*)	ERH_0797
Ornithine carbamoyltransferase (*argF*)	ERH_0796
Cofactors, vitamins, prosthetic groups and carriers	
Pantothenate (incomplete pathway)	
2-Dehydropantoate 2-reductase (*panE*/*apbA*)	ERH_1317
CoA	
Type III pantothenate kinase (*coaX*)	ERH_0124, ERH_0188
Phosphopantothenate-cysteine ligase (*coaB*)	ERH_0126
Phosphopantothenoylcysteine decarboxylase (*coaC*)	ERH_0125
Pantetheine-phosphate adenylyltransferase (*coaD*)	ERH_1118
Dephospho-CoA kinase (*coaE*)	ERH_1014
Folate (incomplete pathway)	
Folylpolyglutamate synthase (*folC*)	ERH_1520
Dihydrofolate reductase (*folA*)	ERH_0263, ERH_0996
Thiamine (incomplete pathway)	
Cysteine desulfurase	ERH_0498, ERH_0508
Nucleoside-triphosphatase	ERH_0545
Thiamine biosynthesis protein (*thiI*)	ERH_0509

unable to demonstrate growth enhancement of the organism after adding various quantities of oleic acid. Its growth was completely inhibited by concentrations of oleic acid greater than 0.001% (data not shown). This finding suggests that Tween 80 may merely aid in membrane transport or another nutrient utilization process.

E. rhusiopathiae also lacks many genes for amino acid biosynthesis; the organism can synthesize only seven amino acids

TABLE 5. Transporter proteins of *E. rhusiopathiae*

Family (% of proteins)	No. of transport systems (no. of genes involved)	Locus tag(s)
Channels (1.3)		
Chloride channel	1 (1)	ERH_1566
Large conductance mechanosensitive ion channel family	1 (1)	ERH_0678
Small conductance mechanosensitive ion channel family	1 (1)	ERH_0358
Secondary transporters (13.7)		
Major facilitator superfamily	5 (5)	ERH_0034, ERH_0137, ERH_0232, ERH_0502, ERH_0783
Amino acid transporter family	5 (5)	ERH_0305, ERH_0316, ERH_0766, ERH_0842, ERH_1618
Arginine/ornithine antiporter	2 (2)	ERH_0798, ERH_1416
Ethanolamine transporter family	1 (1)	ERH_0866
Cation diffusion facilitator family	1 (1)	ERH_0032
Resistance-nodulation-cell division superfamily	1 (1)	ERH_0839
Small multidrug resistance family	3 (3)	ERH_0155, ERH_1251, ERH_1256
Dicarboxylate/amino acid: cation (Na^+ or H^+) symporter family	1 (1)	ERH_0776
Citrate: cation symporter family	1 (1)	ERH_0754
K^+ transporter (Trk) family	1 (2)	[ERH_0373, ERH_0374]
Nucleobase: cation symporter family	3 (3)	ERH_0078, ERH_0275, ERH_1333
Chromate ion transporter family	2 (4)	[ERH_0158, ERH_0159],[ERH_1595, ERH_1596]
Phosphate: Na^+ symporter family	2 (3)	ERH_0216, [ERH_1127, ERH_1128]
Primary active transporters (68.4)		
ATP-binding cassette superfamily		
Carbohydrate uptake transporter	7 (14)	ERH_0199, [ERH_0226, ERH_0227, ERH_0228], [ERH_0413, ERH_0414], ERH_0417, [ERH_1083, ERH_1084, ERH_1085], [ERH_1244, ERH_1245, ERH_1246], ERH_1523
Amino acid uptake transporter	4 (11)	[ERH_0895, ERH_0896], [ERH_1350, ERH_1351, ERH_1352], [ERH_1517. ERH_1518, ERH_1519], [ERH_1666, ERH_1667, ERH_1668]
Ion uptake transporter	7 (16)	[ERH_0024, ERH_0025], [ERH_0044, ERH_0045, ERH_0046, ERH_0047], [ERH_0202, ERH_0203], [ERH_0945, ERH_0946], [ERH_0977, ERH_0978, ERH_0979, ERH_0980], ERH_1017, ERH_1356
Polyamine uptake transporter	2 (8)	[ERH_0876, ERH_0877, ERH_0878, ERH_0879], [ERH_1495, ERH_1496, ERH_1497, ERH_1498]
Peptide/nickel uptake transporter	1 (5)	[ERH_0442, ERH_0443, ERH_0444, ERH_0445, ERH_0446]
Cobalt/nickel uptake transporter	1 (3)	[ERH_1134, ERH_1135, ERH_1136]
Thiamine uptake transporter	1 (1)	ERH_1061
Ion chelate uptake transporter	2 (9)	[ERH_0496, ERH_0497, ERH_0498, ERH_0499, ERH_0500], [ERH_1367, ERH_1368, ERH_1369, ERH_1370]
Lipoprotein uptake transporter	1 (1)	ERH_0873
Quaternary amine uptake transporter	1 (4)	[ERH_1627, ERH_1628, ERH_1629, ERH_1630]
Nitrate/sulfonate/bicarbonate uptake transporter	1 (3)	[ERH_0294, ERH_0295, ERH_0296]
Drug exporter	18 (27)	[ERH_0095, ERH_0096], [ERH_0113, ERH_0114], [ERH_0322, ERH_0323], [ERH_0426, ERH_0427], [ERH_0449, ERH_0450], [ERH_0689, ERH_0690], ERH_0710, ERH_0714, [ERH_0792, ERH_0793], ERH_0939, ERH_1112, ERH_1372, [ERH_1376, ERH_1377], ERH_1381, ERH_1387, [ERH_1411, ERH_1412], ERH_1508, ERH_1683
Peptide exporter	14 (19)	ERH_0026, [ERH_0465, ERH_0466], ERH_0704, ERH_0733, ERH_0874, ERH_0986, ERH_1182, ERH_1184, [ERH_1186, ERH_1187], ERH_1263, [ERH_1360, ERH_1361], ERH_1383, [ERH_1457, ERH_1458], [ERH_1491, ERH_1492]
F-type and V-type ATPase superfamily	2 (16)	[ERH_0365, ERH_0366, ERH_0367, ERH_0368, ERH_0369, ERH_0370, ERH_0371, ERH_0372], [ERH_1042, ERH_1043, ERH_1044, ERH_1045, ERH_1046, ERH_1047, ERH_1048, ERH_1049]
P-type ATPase superfamily	7 (7)	ERH_0074, ERH_1075, ERH_1200, ERH_1283, ERH_1307, ERH_1338, ERH_1401
Uncharacterized ABC-type transporters	8 (16)	[ERH_0409, ERH_0410, ERH_0411, ERH_0412], ERH_0819, ERH_0846, [ERH_1002, ERH_1003, ERH_1004], ERH_1052, ERH_1125, ERH_1186, [ERH_1477, ERH_1478, ERH_1479, ERH_1480]

Continued on following page

TABLE 5—*Continued*

Family (% of proteins)	No. of transport systems (no. of genes involved)	Locus tag(s)
Group translocations (16.2)		
Phosphotransferase system (PTS)	23 (38)	ERH_0005, ERH_0020, [ERH_0133, ERH_0134, ERH_0135], ERH_0219, ERH_0222, [ERH_0255, ERH_0256], [ERH_0282, ERH_0283, ERH_0284], ERH_0286, [ERH_0348, ERH_0349, ERH_0350, ERH_0351], ERH_0680, [ERH_0722, ERH_0723, ERH_0724], ERH_0814, ERH_0849, ERH_0851, ERH_1041, ERH_1120, ERH_1122, ERH_1145, ERH_1208, [ERH_1219, ERH_1220, ERH_1221], ERH_1327, [ERH_1393, ERH_1394, ERH_1395, ERH_1396], ERH_1399
Unclassified (0.4)		
Metal ion transporter (MIT) family	1 (1)	ERH_1190
Total	132 (234)	

(alanine, asparagine, glutamine, serine, cysteine, glycine, and proline) through *de novo* pathways or using intermediate molecules as derivatives. Moreover, all of the genes for the biosynthesis of biotin, riboflavin, ubiquinone, and menaquinone and some of the genes involved in the biosynthesis of pantothenate, thiamine, and folate are missing from the *E. rhusiopathiae* genome (Table 4).

In agreement with its poor biosynthetic capacities, *E. rhusiopathiae* devotes as much as 13.7% (minimally) of its genes to transport functions (Table 5), a level similar to that observed for lactic acid bacteria (13 to 18%) (21). In particular, *E. rhusiopathiae* contains a remarkably high percentage of primary active transporters (68.4%). This property is also shared with *Mycoplasma* species (39).

Virulence-associated genes. (i) Two-component signal transduction systems. Most bacteria employ two-component signal transduction systems to regulate the expression of many genes in response to various changes in environmental conditions (54). In the *E. rhusiopathiae* genome, we identified a total of 15 genes encoding response regulators, 14 of which were adjacent to genes encoding cognate histidine kinases (Table 6). The numbers of two-component signal transduction systems in the genomes of *Streptococcus pyogenes* (12), *Streptococcus pneumoniae* (57), *Listeria monocytogenes* (15), *Enterococcus faecalis* (16), and *Bacillus subtilis* (10) are 13, 14, 16, 17, and 35, respectively. Compared to these bacteria, lactic acid bacteria contain fewer two-component systems (between five and nine) (9), and *Mycoplasma* species lack this system entirely, with the exception of *Mycoplasma penetrans* (42). A loss of regulatory systems is also an evolutionary pattern observed in many bacteria with reduced genome sizes (31). Considering the presence of many two-component systems in the *E. rhusiopathiae* genome compared to lactic acid bacteria and the important roles of these systems in stress responses, such as oxidative or acid stress responses (9), the two-component systems of *E. rhusiopathiae* may be very closely associated with its virulence.

(ii) Cell wall synthesis. *E. rhusiopathiae* possesses a full set of the peptidoglycan (PG) biosynthesis genes, which are relatively dispersed throughout the genome (Fig. 3). However, it is not clear whether all the genes involved in the complete biosynthetic pathways of wall teichoic acids (WTA) and lipoteichoic acids (LTA) are present. Furthermore, although the

TABLE 6. Two-component systems in *E. rhusiopathiae*

Kinase	Response regulator	Organization[a]	Predicted function	Reference
ERH_0119	ERH_0118	RH	Similar to secretion stress response	17
ERH_0212	ERH_0211	RH	Unknown	
ERH_0230	ERH_0231	HR	Unknown	
ERH_0270	ERH_0269	RH	Unknown	
ERH_0310	ERH_0309	RH	Unknown	
ERH_0313	ERH_0312	RH	Unknown	
ERH_0805	ERH_0806	HR	Similar to AgrA family	35
ERH_0872	ERH_0871	RH	Unknown	
ERH_0920	ERH_0921	RH	Unknown	
ERH_0981	ERH_0982	RH	Similar to phosphatase assimilation	26, 27
ERH_1188	ERH_1189	RH	Unknown	
ERH_1193	ERH_1194	RH	Similar to LytTR family	35
	ERH_1319	Orphan[b]		
ERH_1430	ERH_1429	RH	Unknown	
ERH_1493	ERH_1494	RH	Unknown	

[a] Organization of each kinase-regulator pair on the *E. rhusiopathiae* chromosome (RH, 5′ response regulator-3′ histidine kinase; HR, 5′ histidine kinase-3′ response regulator).

[b] Orphan, a response regulator gene that is not directly associated with a histidine kinase gene.

TABLE 7. BLAST results for *E. rhusiopathiae* capsular polysaccharide biosynthetic homologs

Locus tag	Size (aa)[a]	GenBank accession no.	Gene product	Bacterial species	Hit length/length of best-hit homolog (% aa sequence identity)
ERH_0855	442	EEG30921	Sugar transferase	*Clostridium methylpentosum*	182/469 (38)
ERH_0856	590	ADE82409	O-antigen polymerase	*Prevotella ruminicola*	90/344 (26)
ERH_0857	401	EFI08982	Glycosyltransferase	*Bacteroides* sp. 3119	174/407 (42)
ERH_0858	337	EEI59831	UDP-glucose 4-epimerase (CapD)	*Enterococcus faecium*	238/335 (71)
ERH_0859	369	EEV61046	NAD-dependent epimerase/dehydratase	*Enterococcus faecium*	224/370 (60)
ERH_0860	374	EFF20431	UDP-*N*-acetylglucosamine 2-epimerase	*Enterococcus faecium*	299/374 (79)
ERH_0861	390	EEK97598	Glycosyltransferase	*Bacillus cereus*	172/360 (47)

[a] aa, amino acids.

dlt operon encoding the enzymes that catalyze the incorporation of D-alanine residues into WTA or LTA is always composed of *dltABCD* genes in Gram-positive bacteria (34), the gene order is not conserved, and the *dltD* gene is missing in *E. rhusiopathiae* (Fig. 3), suggesting that the organism contains atypical WTA and/or LTA. The *dltABCD* genes have been detected in all *Firmicutes* bacteria with small genomes sequenced so far (19, 34), with the exception of *Oenococcus oeni*, which is a lactic acid bacterium with the smallest genome of all *Firmicutes* (Table 3). In the future, it will be worthwhile to intensively analyze the structures and chemical compositions of WTA and LTA of *E. rhusiopathiae*.

(iii) Capsular polysaccharide synthesis. Immunological and microscopic approaches were used to demonstrate that *E. rhusiopathiae* produces a capsule that plays an important role in virulence (49, 51, 52); however, its chemical and biological properties are uncharacterized. We previously showed that in the representative acapsular mutant 33H6, which was generated by transposon mutagenesis with Tn*916* (52), the transposon was inserted into a gene (now corresponding to ERH_0855) (48). The *E. rhusiopathiae* genome analysis revealed that this gene is located in a cluster of genes encoding seven proteins (ERH_0855 to ERH_0861) that appear to be involved in capsular polysaccharide biosynthesis (Table 7). Reverse transcription-PCR analysis of the intergenic regions of the seven genes indicates that these genes are transcribed as a polycistronic mRNA forming an operon (data not shown).

(iv) Protein secretion systems and surface-associated or extracellular enzymes/proteins. Gram-positive bacteria produce a variety of extracellular or cell surface-associated proteins, many of which play important roles in virulence (13). In Gram-positive bacteria, these proteins could be translocated across the membrane via three pathways: the Sec (general secretory)-dependent pathway, the signal recognition particle (SRP)-dependent pathway, and the twin-arginine translocation (Tat) pathway (38).

E. rhusiopathiae encodes homologs for signal peptidase I, signal peptidase II (lipoprotein signal peptidase), SRP, and a full set of Sec proteins. In contrast, as seen in other members of *Firmicutes* with small genomes (*Streptococcus* species and lactic acid bacteria) and in *Mollicutes* (7), *E. rhusiopathiae* does not contain the Tat secretion system or any recognizable Tat substrates.

During the secretion process in Gram-positive bacteria, many secreted proteins are covalently attached to peptidoglycan through their carboxyl termini by transpeptidases, called sortases, to be exposed as surface proteins (33). *E. rhusiopathiae* contains a single sortase (ERH_0013) and 21 potential sortase substrates that contain a sortase recognition sequence (LPXTG) followed by a membrane-spanning hydrophobic domain and a positively charged tail (Table 8). They include various hydrolyzing enzymes, such as proteases and peptidases, and proteins that can potentially mediate host-bacterium interactions. Three of these proteins possess KXW repeat modules, as well, which are conserved in several adhesins of Gram-positive bacteria belonging to *Firmicutes* and have been shown to play important roles in biofilm formation (50).

Of particular interest are three hyaluronidases (ERH_0150, ERH_0765, and ERH_1210) and one neuraminidase (ERH_0299) that contain LPXTG motifs. Cell surface associations of hyaluronidases and neuraminidase via the LPXTG motif have been shown only in *S. pneumoniae* (13). It is most likely that these four enzymes of *E. rhusiopathiae* are also surface associated. Notably, the organism encodes an additional neuraminidase (ERH_0761) that lacks the LPXTG motif and thus is probably extracellularly secreted.

E. rhusiopathiae possesses another type of surface protein with glycine-tryptophan (GW) dipeptide repeat modules, which attach surface proteins to the cell wall through noncovalent interactions (59). In addition to the major surface protective antigen SpaA.1 protein (ERH_0094), *E. rhusiopathiae* strain Fujisawa contains two surface proteins (ERH_0407 and ERH_0768) with the GW dipeptide repeat modules. Thus, like many other Gram-positive pathogens (13), *E. rhusiopathiae* elaborates a number of cell surface components and extracellular products to interact with its target cells and initiate infection.

(v) Putative virulence factors required for intracellular survival. *E. rhusiopathiae* is vulnerable to oxidative stress, and the escape from reactive oxygen species (ROS) is a major strategy for the intracellular survival of the organism (51). Genome analysis revealed that *E. rhusiopathiae* possesses nine CDSs encoding enzymes that potentially confer ROS resistance. They include a superoxide dismutase, two thioredoxins, two thioredoxin reductases, a thiol peroxidase, and a glutaredoxin (Table 8). Although *E. rhusiopathiae* lacks catalase, the organism may have evolved these redundant defense mechanisms against oxidative stress within host cells. Furthermore, the two alkyl-hydroperoxide reductases may be important in protecting the organism against damage caused by nitric oxide (5).

In addition to these anti-ROS proteins, *E. rhusiopathiae* contains additional enzymes that potentially help the organism

TABLE 8. Possible virulence factors of *E. rhusiopathiae*

Locus tag	Gene	Predicted function and/or description	Motif/modules[a]
Surface proteins			
ERH_0075		Collagen-binding protein	LPXTG
ERH_0094	*spaA.1*	Unknown, protective antigen	GW
ERH_0150	*hylA*	Hyaluronidase	LPXTG
ERH_0161		Peptidase M14	LPXTG
ERH_0201		Pectin lyase fold-containing protein	LPXTG
ERH_0221		Glycoside hydrolase, family 16	LPXTG
ERH_0260		Proteinase	LPXTG
ERH_0278		Unknown	LPXTG
ERH_0299	*nanH.1*	Neuraminidase	LPXTG
ERH_0407	*cbpA*	Unknown, choline-binding protein	GW
ERH_0561		Glycosyl hydrolase, family 85	LPXTG
ERH_0668	*rspA*	Biofilm formation, protective antigen	LPXTG/KXW
ERH_0669	*rspB*	Biofilm formation	LPXTG/KXW
ERH_0728		Internalin-like	LPXTG
ERH_0765	*hylB*	Hyaluronidase	LPXTG
ERH_0768	*cbpB*	Unknown, choline-binding protein	GW
ERH_0777		Dipeptidase	LPXTG
ERH_1139	*ushA*	5′-Nucleotidase	LPXTG
ERH_1210	*hylC*	Hyaluronidase	LPXTG
ERH_1258		Unknown	LPXTG
ERH_1436		Collagen-binding protein	LPXTG
ERH_1454		Unknown	LPXTG
ERH_1472		Internalin-like	LPXTG
ERH_1687	*rspC*	Biofilm formation	LPXTG/KXW
Antioxidant proteins			
ERH_0162	*tpx*	Thiol peroxidase	
ERH_0175	*ahpC*	Alkyl-hydroperoxide reductase	
ERH_0356	*nrdH*	Glutaredoxin	
ERH_0375	*trxA.1*	Thioredoxin	
ERH_1065	*sodA*	Superoxide dismutase	
ERH_1311	*trxB.1*	Thioredoxin-disulfide reductase	
ERH_1345	*ahpD*	Alkylhydroperoxide reductase	
ERH_1500	*trxA.2*	Thioredoxin	
ERH_1541	*trxB.2*	Thioredoxin-disulfide reductase	
Phospholipase			
ERH_0072		Patatin-like phospholipase	
ERH_0083		Phospholipase/Carboxylesterase family	
ERH_0148	*pldB*	Lysophospholipase	
ERH_0333	*cls*	Cardiolipin synthetase	
ERH_0334		Patatin-like phospholipase	
ERH_0347		Phospholipase/carboxylesterase	
ERH_0388		Phospholipase D	
ERH_1214		Lysophospholipase	
ERH_1433		Lysophospholipase	
Hemolysins			
ERH_0467		Hemolysin-related protein	
ERH_0649		Hemolysin III	
Other extracellular proteins/enzymes			
ERH_0761	*nanH.2*	Neuraminidase	
ERH_1034		Fibronectin-binding protein	
ERH_1356		Adhesin	
ERH_1467		Biofilm formation	

[a] LPXTG, sortase motif (33); GW, choline-binding module (59); KXW, module identified in extracellular matrix-binding proteins of Gram-positive bacteria (50).

survive within phagocytic cells (Table 8). Phospholipases are considered virulence factors in many intracellular pathogens (43). The *E. rhusiopathiae* genome contains at least nine CDSs encoding proteins with sequence homology to phospholipase family proteins, including phospholipase D and patatin-like phospholipases. Patatin, a storage protein found in potatoes, also has a phospholipase activity (29). *E. rhusiopathiae* possesses an extremely high number of phospholipolytic enzymes compared to other intracellular bacteria (1). The abundance of these enzymes of *E. rhusiopathiae* may be a reflection of the lack of fatty acid biosynthesis pathways. Efficient acquisition of fatty acids from the host membrane may be achieved by their

combined actions. These phospholipases may allow the organism to escape from phagosomes into the cytoplasm by disrupting the phagosomal membrane (43). In fact, electron microscopic observation revealed that the organism multiplies predominantly within the cytoplasm of macrophages at the inoculation sites of mice (46), suggesting that the cytoplasm serves as a privileged *in vivo* niche for *E. rhusiopathiae*, which allows the organism to circumvent host immune responses. Antioxidant enzymes and phospholipases could also modulate immunological cell-signaling pathways (2, 44). Thus, the organism may regulate host cell functions by these enzymes for successful intracellular survival.

Genome comparison. By bidirectional best-hit analysis with the draft sequence of *E. rhusiopathiae* strain ATCC 19414, we found that 1,594 (93.5%) CDSs of Fujisawa, including the genes for capsular polysaccharide biosynthesis (ERH_0855 to ERH_0861), are conserved in ATCC 19414. The orthologs exhibited a high level of sequence similarity (99.3% amino acid sequence identity on average). The result of dot plot analysis also revealed a high level of genomic synteny of the two genomes (see Fig. S1 in the supplemental material). These data indicate that the genomic backbone is highly conserved between the two strains.

In ATCC 19414, 71 out of the 1,645 CDSs were strain specific (see Table S1 in the supplemental material). In Fujisawa, 110 CDSs were strain specific (see Table S2 in the supplemental material). These Fujisawa-specific CDSs include the 46 genes on PP_Erh_Fujisawa (ERH_0581 through ERH_0626) and 23 genes that were also clustered in the genome (ERH_1273 through ERH_1295) (Fig. 1; see Table S2 in the supplemental material). Because this gene cluster contains genes for an integrase and a replication protein, as well as several IS elements, it is most likely that it represents an integrative element, although direct-repeat sequences were not found at the boundaries. This integrative element, designated IE_Erh_Fujisawa, includes the genes for a restriction-modification system and a heavy-metal transport system. Moreover, Fujisawa appears to have a strain-specific pathway for polysaccharide biosynthesis (ERH_1439 through ERH_1444). The G+C contents of most of the strain-specific CDSs in the genomes of Fujisawa and ATCC 19414 differ significantly from those of other parts of the genome (see Tables S1 and S2 in the supplemental material), suggesting that they have been acquired by each of the strains through horizontal gene transfer.

We further analyzed conservation of the Fujisawa CDSs in other *Erysipelotrichia* strains. We excluded the *T. sanguinis* strain from this analysis because it is very likely that the species is not a true member of *Erysipelotrichia*, as mentioned above in the discussion of the phylogenetic position of *E. rhusiopathiae*. This analysis revealed that between 52 and 61% of the CDSs of Fujisawa are conserved in each of these strains (see Table S3 in the supplemental material) and that a total of 625 CDSs (37%) are shared by all *Erysipelotrichia* strains examined. Although this analysis is not complete because the genome sequence information on all these *Erysipelotrichia* strains consists of draft sequences with various levels of sequence quality, these 625 CDSs can be regarded as roughly representing the core gene set of the bacterial group. It might be worth mentioning that it appears that these *Erysipelotrichia* strains also lack the *dltABCD* operon.

Conclusions. This study describes the reductive genome revolution of *E. rhusiopathiae* and its unique strategy for intracellular parasitism. The phylogenetic study using the genome sequence information revealed that *Erysipelotrichia* strains, including *E. rhusiopathiae*, form a cluster distinct from other *Firmicutes* and are phylogenetically closest to *Mollicutes*. The genomic features of *E. rhusiopathiae* also represent evolutionary traits of both *Firmicutes* and *Mollicutes*. Like *Mollicutes*, during genome reduction, *E. rhusiopathiae* lost the genes necessary for fatty acid biosynthesis pathways and evolved redundant phospholipolytic enzymes, which may be utilized for fatty acid acquisition *in vivo*. Furthermore, the organism possesses many antioxidant factors, suggesting that *E. rhusiopathiae* specifically adapted to the intracellular environments of phagocytic cells and showing that its evolutionary adaptation is unique in the phylum *Firmicutes*. Comparative analysis with a draft genome sequence of another *E. rhusiopathiae* strain (ATCC 19414) revealed that these genomic features are highly conserved in the strain.

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