NAD Biosynthesis Evolution in Bacteria: Lateral Gene Transfer of Kynurenine Pathway in Xanthomonadales and Flavobacteriales

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The biosynthesis of quinolinate, the de novo precursor of nicotinamide adenine dinucleotide (NAD), may be performed by two distinct pathways, namely, the bacterial aspartate (aspartate-to-quinolinate) and the eukaryotic kynurenine (tryptophan-to-quinolinate). Even though the separation into eukaryotic and bacterial routes is long established, recent genomic surveys have challenged this view, because certain bacterial species also carry the genes for the kynurenine pathway. In this work, both quinolinate biosynthetic pathways were investigated in the Bacteria clade and with special attention to Xanthomonadales and Bacteroidetes, from an evolutionary viewpoint. Genomic screening has revealed that a small number of bacterial species possess some of the genes for the kynurenine pathway, which is complete in the genus *Xanthomonas* and in the order Flavobacteriales, where the aspartate pathway is absent. The opposite pattern (presence of the aspartate pathway and absence of the kynurenine pathway) in close relatives (*Xylella* ssp. and the order Bacteroidales, respectively) points to the idea of a recent acquisition of the kynurenine pathway through lateral gene transfer in these bacterial groups. In fact, sequence similarity comparison and phylogenetic reconstruction both suggest that at least part of the genes of the kynurenine pathway in *Xanthomonas* and Flavobacteriales is shared by eukaryotes. These results reinforce the idea of the role that lateral gene transfer plays in the configuration of bacterial genomes, thereby providing alternative metabolic pathways, even with the replacement of primary and essential cell functions, as exemplified by NAD biosynthesis.

Background

For several decades, it has been known that nicotinamide adenine dinucleotide (NAD) plays a major role as a coenzyme in numerous oxidation–reduction reactions. According to a widely accepted concept, a combination of de novo and salvage pathways contributes to the biosynthesis of NAD (Rongvaux et al. 2003). Depending on the organism, three different building blocks can be used for the biosynthesis of NAD, namely, quinolinic acid (or quinolinate) as the precursor in the so-called de novo pathway, nicotinic acid, and nicotinamide in the salvage pathway (Mattevi 2006).

Two different biosynthetic pathways are responsible for de novo quinolinate production (Begley et al. 2001) (fig. 1). In prokaryotes, quinolinate is usually formed from aspartate and dihydroxyacetone phosphate, in a two-step pathway involving the enzymes NadB (L-aspartate oxidase, EC 1.4.3.16, encoded by *nadB*, which oxidizes the amino group of L-aspartate to the imino group, forming α-iminoaspartate) and NadA (quinolinate synthase, encoded by *nadA*, responsible for the production of quinolinic acid from α-iminoaspartate and dihydroxyacetone phosphate) (Katoh and Hashimoto 2004).

In eukaryotes, NAD biosynthesis is coupled to tryptophan catabolism through the so-called kynurenine pathway (or tryptophan-to-quinolinate pathway), whereas the common enzymatic substrate resides in α-amino-β-carboxymuconic acid ϵ-semialdehyde. This compound may either cyclize nonenzymatically to yield quinolinate or it can be enzymatically decarboxylated by a-amino-b-carboxy muconic acid e-semialdehyde decarboxylase (Fukuoka et al. 2002). In the first step of the kynurenine pathway, tryptophan is cleaved by tryptophan-2,3-dioxygenase (TDO, EC 1.13.11.11, encoded by *kynA*) to yield *N*-formylkynurenine. Alternatively, in some organisms (as in Fungi), this step may be performed by indoleamine 2,3-dioxygenase (EC 1.13.11.52). Removal of the formyl group by *N*-formylkynurenine formamidase (or arylformamidase, KFA, EC 3.5.1.9, encoded by *kynB*) results in L-kynurenine, which is then hydroxylated by kynurenine-3-monooxygenase (KMO, EC 1.14.13.9, encoded by *kmo*), a FAD-dependent monooxygenase that incorporates molecular oxygen into 3-hydroxykynurenine. Cleavage of the amino acid side chain by the pyridoxal-5′-phosphate-dependent enzyme kynureninase (KYN, EC 3.7.1.3, encoded by *kynU*) generates 3-hydroxyanthranilic acid, which is then converted by 3-hydroxyanthranilate-3,4-dioxygenase (HAD, EC 1.13.11.6, encoded by *haaO*) to an unstable aliphatic compound, α-amino-β-carboxymuconic acid ϵ-semialdehyde, which spontaneously cyclizes to quinolinic acid (Kurnasov, Goral et al. 2003).

Pioneer studies dating from four to five decades ago have demonstrated that certain bacteria, such as *Streptomyces antibioticus*, *Pseudomonas fluorescens*, and *Xanthomonas pruni*, might utilize this pathway to synthesize quinolinic acid (Davis et al. 1951; Wilson and Henderson 1963; Tremblay et al. 1967) or at least present related enzymatic activities, as in *Pseudomonas aureofaciens* and *Streptomyces parvulus* (Salcher and Lingens 1980; Brown et al. 1986). In a keynote work undertaken by Brown and Wagner (1970), enzymatic assays of the five steps in the kynurenine pathway were performed in *X. pruni*, whereby all the activities were found to be present.

However, until recently, very little was known regarding identification of the primary sequence or structural prediction of such bacterial enzymes (Kurnasov, Jablonski, et al. 2003), and no glimpse was given to clarify the evolutionary scenario of the quinolinate biosynthetic pathway. Recent extensive bacterial genome sequencing allowed us to address this evolutionary question. Analyses of these pathways help to understand how both de novo pathways are distributed and how they have evolved within bacterial clades, when taking into special consideration two groups, Xanthomonadales and Bacteroidetes.

Key words: lateral gene transfer, *Xanthomonas*, Flavobacteriales, NAD metabolism, kynurenine pathway, tryptophan catabolism.

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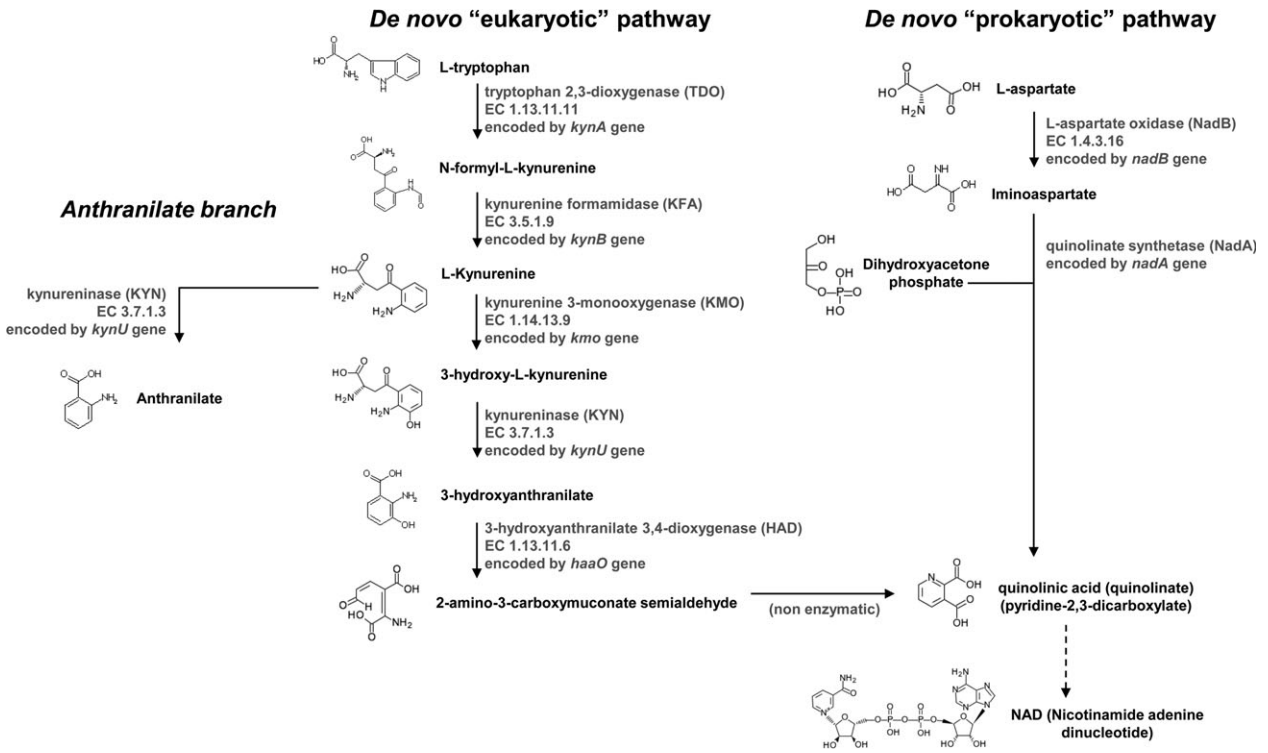

FIG. 1.—Schematic representation of the two biosynthetic pathways to quinolinic acid: the five-step "eukaryotic" route (from L-tryptophan) and the two-step "prokaryotic" route (from L-aspartate). On the left, the alternative anthranilate branch. The enzyme name and synonyms and the EC number are shown in each text box.

Methods

Identification of the Quinolinate Biosynthetic Pathway

Sequence similarity analyses were performed (using BlastP and TBlastN programs) using a current data set of more than 600 complete or almost complete genomes (locally downloaded on February 7, 2008), from NCBI (Pruitt et al. 2005), Expasy Proteomics Server (Gasteiger et al. 2003), or CMR-TIGR (Peterson et al. 2001) databases. Metabolic pathways were analyzed through the KEGG web service (Kanehisa et al. 2008), BRENDA enzyme database (Schomburg et al. 2004), Genome Properties server (Selengut et al. 2007), and SEED Subsystems (Overbeek et al. 2005), up to April 2008. Genes coding for enzymes involved in the quinolinate biosynthesis were identified both by similarity (using as seed sequences homologs with a functional assignment [see supplementary table S1, Supplementary Material online]; threshold inclusion values were e-value $<10^{-05}$ and similarity percent higher than 40%) or keyword searches and, wherever possible, by genome context analysis (for a review, see Osterman and Overbeek 2003). GenBank accession numbers for the most relevant genomes used in this study are presented in supplementary table S2, Supplementary Material online. Data concerning the other genomes here analyzed are available upon request to the authors. Protein sequences to all open reading frames (ORFs) referred in table 1 are provided as Supplementary Material online.

Phylogenetic Analyses

Protein sequences were aligned by using ClustalX 2.0 program (Larkin et al. 2007), whereby regions of the alignments that were ambiguous, hypervariable, or containing gaps were excluded from subsequent analysis (GENEDOC program, Nicholas et al. 1997). Pairwise genetic distances were computed by using a PROTDIST program from the PHYLIP 3.67 package (Felsenstein 2005) or MEGA 4.0 (Tamura et al. 2007), with the Jones–Taylor–Thornton (JTT) model of amino acid replacement, gamma-distributed rate (four categories) and inferred α parameter. Distance-based phylogenetic trees were generated through the Neighbor-Joining (NJ) algorithm (NJ program). Bootstrap assessment of tree topology (1,000 replicates) was done with the SEQBOOT program. Maximum likelihood (ML) trees were set up with RAxML 7.0.4 (Stamatakis 2006), with the JTT model, and parameters for invariable sites and gamma-distributed rate heterogeneity (four categories). One-thousand bootstrap replicates were executed and bootstrap values drawn up on the best-scoring ML tree. Trees were visualized using MEGA 4.0 and TREEVIEW (Page 1996) programs and were arbitrarily rooted at midpoint (although trees should be fundamentally viewed as unrooted).

Results and Discussion

Identification of the Quinolinate Biosynthetic Pathway in Bacteria

In a review, Kurnasov, Goral, et al. (2003) described the presence of the kynurenine pathway in certain bacterial species (including *Cytophaga hutchinsonii*, *Gemmata* sp., *Polaribacter filamentus*, *Ralstonia metallidurans*, and *Xanthomonas axonopodis*), thus providing evidence that this pathway is found not only in Eukaryotes as formerly

Table 1
Genes Related to Quinolinate Biosynthesis in Xanthomonadales and Bacteroidetes Genomes

Order	Organism	ORF Number	*kynA*	*kynB*	*kmo*	*kynU*	*haaO*	*nadA*	*nadB*
Xanthomonadales	*Xanthomonas axonopodis* pv. *citri* 306	XAC	0448	2393[a]	1600	1601	1603	—	—
	Xanthomonas campestris pv. *campestris*		0432[b]	2285[a]	1552	1553	1555	—	—
	ATCC33913	XCC	1210						
			0446[b]	1830[a]	2682	2681	2679	—	—
	Xanthomonas campestris pv. *campestris* 8004	XC_	3032						
	Xanthomonas campestris pv. vesicatoria str. 85-10	XCV	0478	2590[a]	1641	1642	1645	—	—
	Xanthomonas oryzae KACC10331	XOO	4075	2719[a]	2429	2428	2424	—	—
	Xanthomonas oryzae MAFF 311018	XOO	3850	2564[a]	2306	2305	2302	—	—
	Stenotrophomonas maltophilia R551-3[d]	SmalDRAFT_	3710	—	2540	2539	2538	—	—
	Stenotrophomonas maltophilia K279a	Smlt	4336	—	3161	3160	3159	—	—
	Xylella fastidiosa 9a5c	XF	—	—	—	—	—	1923	1924
	Xylella fastidiosa Temecula1	PD	—	—	—	—	—	0869	0868
Flavobacteriales	*Croceibacter atlanticus* HTCC2559[d]	CA2559_	02610	04895[c]	07400	07761	02445	—	—
				11953[a]					
	Dokdonia donghaensis MED134[d]	MED134_	07811	10550[c]	12926	12936	09816	—	—
	Flavobacteria bacterium BAL38	FBBAL38_	04720	06760[c]	03445	03480	04995	—	—
	Flavobacteria bacterium BBFL7[d]	BBFL7_	01803	01193[c]	01115	01114	01456	—	—
			05105	03570[c]	16541	16566	06825	—	—
	Flavobacteriales bacterium HTCC2170[d]	FB2170_		01482[b]					
	Flavobacterium johnsoniae UW101	Fjoh_	0731	1374[c]	0495	0506	1221	4055	4056
	Flavobacterium psychrophilum JIP02/86	FP	0228	0207[c]	2212	2215	2235	—	—
			00575	02150[c]	14850	14845	00180	—	—
	Flavobacterium sp. MED217[d]	MED217_		10327[a]					
			1393[b]	0068[c]	2711	2720	3400	—	—
	*Gramella forsetii*KT0803	GFO_	3168	2628[b]					
	Polaribacter irgensii 23-P[d]	PI23P_	04822	01552[c]	07155	07150	07175	—	—
	Psychroflexus torquis ATCC 700755[d]	P700755_	02427	—	14750	14755	10058	—	—
			—	04070[c]	02455	02490	07695	—	—
	Robiginitalea biformata HTCC2501[d]	RB2501_	00881[a]						
	Tenacibaculum sp. MED152[d]	MED152_	09370	12039[c]	12759	12754	12779	—	—
Bacteroidales	*Bacteroides fragilis* NCTC9343	BF	—	—	—	—	—	4375	0022
	Bacteroides thetaiotaomicron VPI-5482	BT	—	—	—	—	—	3164	3184
	Porphyromonas gingivalis W83	PG	—	—	—	—	—	1578	1576

NOTE.—ORF number refers to the name given to each gene during the sequencing project.

[a] *kynB*—esterase/lipase domain.

[b] *G. forsetii* carry two close copies of *kynA* (possibly result of a recent duplication), whereas the two strains of *X. campestris pv. campestris* carry two divergent copies: The copy shared by all Xanthomonadales (XCC0432 and XC_0446) has similarity to *Firmicutes* species, whereas the second copy (XCC1210 and XC_3032) is more similar to eukaryotic orthologs.

[c] kynB-Cyclase/hydrolase domain.

[d] Organisms with genome sequencing still in progress.

thought. However, since then hundreds of new genomes have been sequenced and published. Consequently, an up-to-date analysis is required to afford a broader evolutionary view of NAD biosynthesis in bacteria.

Attempts have been made to identify all the genes belonging to both the aspartate and kynurenine pathways for the more than 600 genomes publicly available, figure 2 representing a summary of all the collected data. According to current assumptions, a large majority of genomes bear only those genes responsible for the aspartate pathway (*nadA* and *nadB*). However, the presence of kynurenine pathway–coding genes in bacterial groups is not restricted as previously thought. In fact, six groups of Bacteria carry at least some of the genes for this pathway, although patchily distributed within each group: Actinobacteria (from the order Actinomycetales), Firmicutes (only species from the order Bacillales), Bacteroidetes (only Flavobacteriales), Alpha-proteobacteria (Rhizobiales and Rhodobacterales), Beta-proteobacteria (Burkholderiales), and Gamma-proteobacteria (Xanthomonadales and some Pseudomonadales). Most of these groups bear only *kynA*, *kynB*, and *kynU* genes (fig. 2), *kmo* and *haaO* having a very restricted phyletic distribution and being present only in the suborder Micromonosporineae and the orders Flavobacteriales and Xanthomonadales.

The presence of *kynA*, *kynB*, and *kynU* in few bacterial species has been linked to tryptophan catabolism by way of the anthranilate pathway (fig. 1) (Farrow and Pesci 2007). In *Escherichia coli* and many other bacteria, tryptophan degradation proceeds through pyridoxal phosphate–dependent tryptophanase TnaA (encoded by *tnaA*) (Vederas et al. 1978). In eukaryotes, catabolism of tryptophan is coupled to NAD biosynthesis through the kynurenine pathway, using as common intermediate 2-amino-3-carboxymuconate semialdehyde (Fukuoka et al. 2002). However, in some species of Pseudomonadaceae and Bacillaceae, the oxidative degradation of L-tryptophan occurs through the anthranilate pathway by using TDO, KFA, and KYN enzymes (encoded by *kynA, kynB*, and *kynU* genes, respectively), whereby tryptophan is converted to formylkynurenine and then to kynurenine by TDO and KFA, with the final conversion of kynurenine to anthranilic acid catalyzed by the kynureninase KYN (fig. 1) (D'Argenio et al. 2002; Kurnasov et al. 2003; Farrow and Pesci 2007). Interestingly,

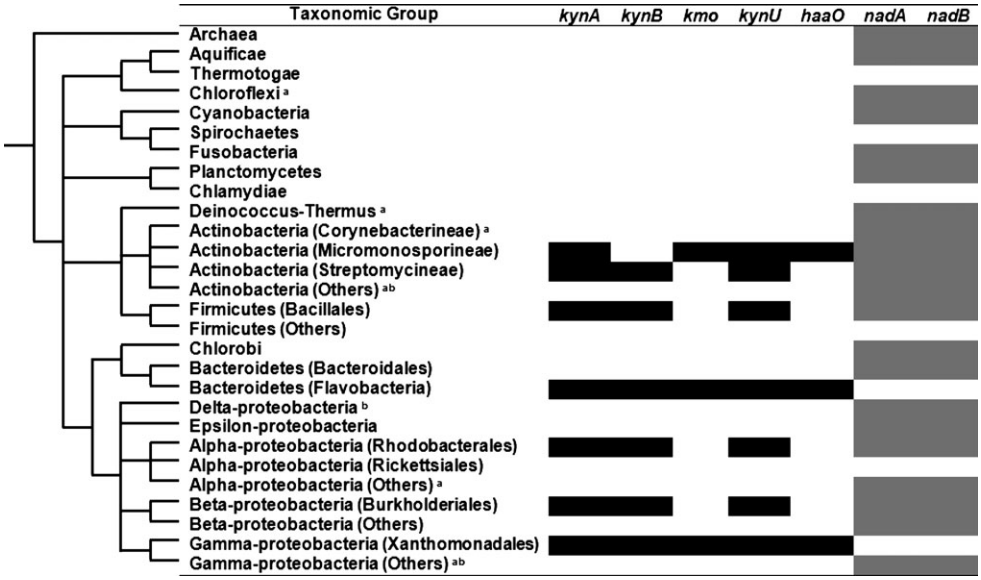

FIG. 2.—Schematic profile of the presence and absence of seven genes of the kynurenine (*kynA*, *kynB*, *kmo*, *kynU*, and *haaO*) and aspartate pathway (*nadA* and *nadB*). A consensus phylogenetic tree for the major bacterial groups is presented. Exceptions within each group are as follows: (*a*) species bearing *kynA*, *kynB*, and *kynU*: *Herpetosiphon aurantiacus* (Chlorofexi); *Deinococcus* ssp. (Deinococcus-Thermus); *Kineococcus radiotolerans* and *Rhodococcus* sp. RHA1 (Actinobacteria); *Bradyrhizobium* ssp., *Mesorhizobium loti*, *Sphingopyxis alaskensis*, and *Erythrobacter litoralis* (alpha-proteobacteria); *Pseudomonas aeruginosa* PAO1; *Pseudomonas fluorescens* Pf-5 and *Photorhabdus luminescens* TTO1 (Gamma-proteobacteria); (*b*) species bearing *kynA* or *kynB* and *kynU*: *Renibacterium salmoninarum*, *Arthrobacter* ssp., *Thermobifida fusca*, and *Saccharopolyspora erythraea* (Actinobacteria); *Anaeromyxobacter* and *Bdellovibrio* (Delta-proteobacteria); *Acinetobacter baumannii*, *Idiomarina loihiensis*, *Nitrococcus mobilis*, and *Marinomonas* sp. (Gamma-proteobacteria).

the anthranilate branch and the aspartate-to-quinolinate pathway always co-occur in the given species, playing different roles within the cell, such as quinolinate production and tryptophan degradation.

Nevertheless, only the order Flavobacteriales and *Xanthomonas* present the entire set of genes responsible for the complete kynurenine pathway (*kynA*, *kynB*, *kmo*, *kynU*, and *haaO*) and not those for the classical prokaryotic pathway (comprising *nadA* and *nadB* genes). The order Xanthomonadales is mainly composed of three genera (*Xanthomonas*, *Stenotrophomonas*, and *Xylella*), although only *Xanthomonas* and *Stenotrophomonas* bear genes for the kynurenine pathway, as *Xylella* shows the opposite profile by carrying only *nadA* and *nadB* (table 1). The same occurs for Bacteroidetes: Although species from the order Flavobacteriales reveal the same pattern seen in *Xanthomonas*, those from the order Bacteroidales bear only genes for the aspartate-to-quinolinate pathway (table 1).

Genomic Organization of the Kynurenine Pathway in Flavobacteriales and *Xanthomonas*

Genes responsible for the kynurenine pathway are normally found clustered along the genome. In *Xanthomonas*, the genetic arrangement is similar for all completely sequenced genomes (supplementary fig. S1, Supplementary Material online): Three genes are clustered together in a putative operon—*kmo*, *kynU*, and *haaO*—interrupted by one or two hypothetical genes, depending on the organism. The genes *kynA* and *kynB* do not belong to the same cluster and may be separated from the main operon by more than 1 Mb. This configuration gives support to the idea that these three genes were transferred en bloc, given their common evolutionary history (see below). Moreover, this pathway has been previously identified as part of a genomic island potentially originating from lateral gene transfer (LGT) in *Xanthomonas* (Lima et al. 2005, 2008).

Very few genomes display genes spread throughout the entire chromosome, this being the case of Flavobacteriales. Those related to the kynurenine pathway are not directly connected physically, *kmo* and *kynU* being the only ones closer together in some cases (even though separated by at least 5,000 bp). Moreover, there is no collinearity: each gene appearing in a different position, depending on the genome considered (supplementary fig. S1, Supplementary Material online, shows only the scheme for completely sequenced Flavobacteriales genomes). This fact may reflect gene dispersion throughout the genome by recombination, although this is difficult to prove, and other operons are easily found in the genome of these bacteria (data not shown).

Identification of the *kynB* gene has been a matter of debate, because it occurs in at least two nonorthologous forms, one of which presenting a cyclase or metal-dependent hydrolase domain, functionally characterized in some bacteria (as in *Pseudomonas aeruginosa*, *R. metallidurans*, and *Bacillus cereus*) (Kurnasov, Goral, et al. 2003; Kurnasov, Jablonski, et al. 2003) and the other possessing a conserved esterase/lipase domain (from the alpha/beta-hydrolase family), characterized as the kynurenine formamidase in eukaryotes (Pabarcus and Casida 2002, 2005). The distribution of these two different proteins differs significantly according to the bacteria analyzed, as shown in table 1. *Xanthomonas* homologs were identified based on their similarity to the

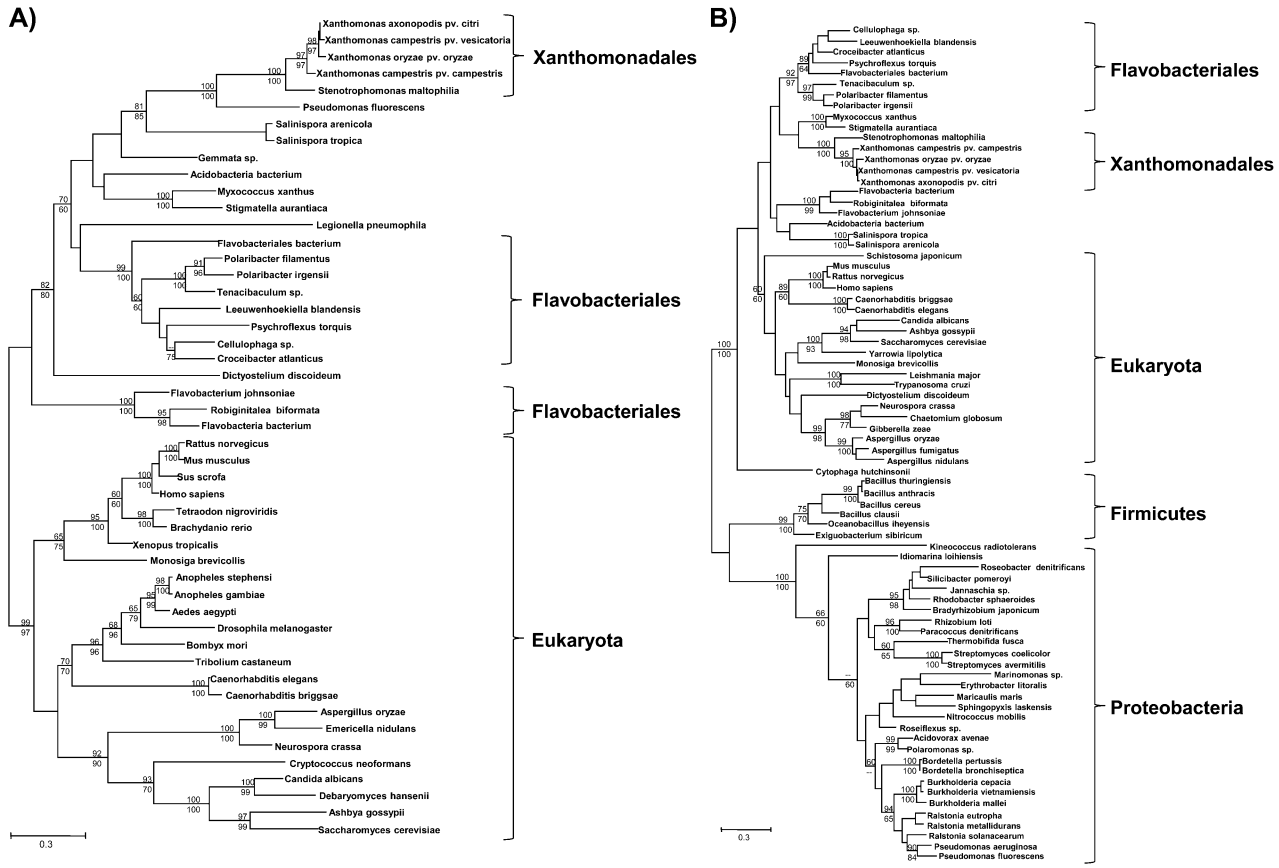

FIG. 3.—ML tree of *kmo* (*A*) and *kynU* (*B*) genes. Branch lengths are proportional to the number of amino acid substitutions per site. Numbers at the nodes represent the percentage of bootstrap support (upper values for the ML tree and lower values for the NJ tree. Only values >60% are shown).

eukaryotic kynB (reciprocal BlastP searches confirm these genes as being similar to KFA-coding genes in mice and humans). On the other hand, Flavobacteriales homologs were identified based on their similarity to the cyclase form (as identified by Kurnasov, Goral, et al. 2003; Kurnasov, Jablonski, et al. 2003). However, some species of this order also carry the eukaryotic esterase form (table 1). All genes identified as putative KFA possess the conserved catalytic residues characteristic of both esterase and cyclase-like genes (supplementary fig. S2, Supplementary Material online). Therefore, the functional characterization of these two homolog forms is still necessary to understand which protein is fulfilling the role of kynurenine formamidase in these organisms.

Evolution of the Kynurenine Pathway in Bacteria

The discovery of a typical eukaryotic metabolic pathway in bacterial species is uncommon and evokes an evolutionary explanation that accounts for such a scenario. In order to trace the evolution of each gene belonging to the kynurenine pathway, phylogenetic reconstructions were conducted (fig. 3 and supplementary fig. S3, Supplementary Material online). The general pattern observed reinforces the relationship between the eukaryotic homologs and the genes present in *Xanthomonas* and Flavobacteriales. In fact, the branching of both groups with Eukaryotes and the high level of sequence similarity to eukaryotic genes suggest a potential foreign origin for such genes.

As reflected by the profile of presence and absence (fig. 2), *kmo* (fig. 3*A*) and *haaO* (supplementary fig. S3, Supplementary Material online) genes are those that present the most restricted phyletic distribution. Both genes are present in eukaryotic species, mainly those belonging to Unikonts (comprising Metazoa and Fungi; for a review, see Cavalier-Smith 2002), as well as in *Xanthomonas* and Flavobacteriales. Few bacterial species (mostly proteobacterial) possess the *kmo* gene and a few more, including species belonging to Firmicutes and Proteobacteria, a copy of the *haa*O. However, these bacterial *haaO* homologs form a monophyletic group, independent of those found in *Xanthomonas*, Flavobacteriales, and Eukaryotes.

The *kynU* gene is present in a wider range of bacterial species of the Firmicutes and Proteobacteria (fig. 3*B*). However, the homologs of *Xanthomonas* and Flavobacteriales branch with Eukaryotes and only a few other bacteria species (those bearing *kmo* and *haaO* genes, such as *Myxococcus xanthus*, *Salinispora* ssp., and *Acidobacteria bacterium*). The separation of these homologs from the main group of Bacteria is supported by high bootstrap values, both for ML and NJ trees.

Phylogenetic reconstructions conducted for *kynA* gene (data not shown) place Flavobacteriales with Eukarya (although with low bootstrap support) and Xanthomonadales branches with gram-positive species (Actinobacteria and

Firmicutes); Proteobacteria form a distinct and monophyletic group. Interestingly, the second copy of *kynA* gene from *X. campestris* clusters with Eukarya and Flavobacteriales, thus indicating an independent origin for this, probably recently acquired by an ancestor of both *X. campestris* pv. *campestris* strains (table 1).

As stated before, degradation of tryptophan in bacteria may be performed by the tryptophanase TnaA or by way of the anthranilate-degrading pathway, although production of quinolinic acid is mostly carried out by the aspartate pathway (*nadA* and *nadB* genes). Both *nadA* and *nadB* genes are absent in eukaryotes, with a few exceptions in the Viridiplantae kingdom. On the other hand, the production of quinolinate is fulfilled by the kynurenine pathway, similar to what occurs in *Xanthomonas* and Flavobacteriales. The question is, how did this profile arise during the evolution of these bacteria?

The simplest and most parsimonious explanation for the widespread presence of the aspartate route and the scattered distribution of the anthranilate pathway would be an ancient bacterial origin for genes belonging to both pathways. Genes related to the anthranilate pathway would have been lost in several bacterial groups, probably due to functional redundancy in other systems for amino acid degradation. However, another scenario should be evoked to explain the presence and distribution of an entire kynurenine pathway in *Xanthomonas* and Flavobacteriales. The odd phyletic distribution of *kyn* genes (especially *kynU*, *kmo*, and *haaO*) in Xanthomonadales and Bacteroidetes, along with the results of phylogenetic reconstructions and similarity searches, points to the lateral acquisition of kynurenine pathway genes in these groups. The phylogenetic placement of *Xanthomonas*, Flavobacteriales, and Eukaryotes, branching together even when homologs are present in other Bacteria (as in the case of *kynU* and *haaO* genes), supports the differential origin of such genes in *Xanthomonas* and Flavobacteriales. The polarity of LGT is difficult to assess because of uncertain rooting, but data indicate that these genes, necessary for the complete kynurenine pathway, would have been acquired later by gene transfer to an ancestor of both. They possibly originated from Eukaryotes, where homologs are widely distributed in Unikonts and then recruited to NAD biosynthesis.

Moreover, the apparent phylogenetic proximity of the homologs between *Xanthomonas* and Flavobacteriales indicates that transfer might have occurred to one of these bacteria, then spreading in one of the groups, before being transferred to the other. In fact, further exchange of genes between Bacteroidetes and Xanthomonadales may have also occurred. In a recent study, certain genes of the arginine biosynthetic pathway (*argF*, *argG*, and *argH*) were described as having been laterally transferred among Xanthomonadales, Bacteroidetes, and Eukaryotes (Lima and Menck 2008).

An alternative explanation, a bacterial origin for these genes followed by a gene transfer to an ancestor of the Unikonts, cannot be excluded. However, this is unlikely, as it would presuppose a massive gene loss in most of the bacterial groups. The loss would be particularly recent in *Xylellas* and Bacteroidales, thus arguing against the bacterial origin of such genes.

The loss of *nadA* and *nadB* genes in *Xanthomonas* and Flavobacteriales due to redundancy in the kynurenine pathway may be an example of xenologous pathway replacement. In fact, the success and maintenance of one quinolinate biosynthetic pathway over another in a given organism is probably governed by complex factors, and it is interesting to discuss the ecological reasons for this. In any scenario (of gene transfer from a bacterial or eukaryotic donor), the introgression of kynurenine genes may have coexisted with the aspartate pathway, which was then lost, due to duplicated function.

In both groups, there are free living and pathogenic species. *Xylellas* are obligatory plant parasites, living in an extremely dilute nutritional environment and present an intermediate insect vector, where it lives in the gut (Hopkins 1989; Monteiro-Vitorello et al. 2005; Moreira et al. 2005). Similarly, Bacteroidales are obligatory anaerobic parasites or symbionts of the mammalian intestinal tract (Xu et al. 2003, 2007). The presence of sufficient aspartate and/or tryptophan in the milieu of ancestral bacteria, during a specific period in evolution, may have dictated the fate of those pathways that were retained in these bacteria. However, these bacterial groups also present genes that potentially encode key proteins for the synthesis of aspartate from the citrate cycle (aspartate aminotransferase) or tryptophan (tryptophan synthase). Another explanation would be the aerobic or anaerobic status of each organism, as the kynurenine pathway requires three molecules of oxygen (in the reactions catalyzed by TDO, KMO, and HAD), whereas no oxygen is required for the aspartate pathway. Bacteroidales species are strict anaerobics and *Xylella* ssp., although growing in aerobic conditions in in vitro cultures, possess an unusual and least energy-efficient aerobic respiratory system, indicating that this is not the prevalent type of energy metabolism in these organisms (Bhattacharyya et al. 2002).

In any case, it is difficult to account for the benefit of such a change in the metabolic network. The aspartate pathway seems to be the most primitive. In an interesting discussion, Cleaves and Miller (2001) stated that NadA and NadB functions were even dispensable for the interconversion of aspartate and dihydroxyacetone phosphate into quinolinate in a hypothetically primitive world. Therefore, this pathway might have arisen very early during evolution, independent of any enzymatic chemical reaction. On the other hand, the kynurenine pathway is a multistep process with a rate-limiting substrate (as tryptophan is one of the rarest amino acids). The successful pathway replacement in both bacterial groups reveals the degree of complexity during evolution.

Conclusion

Lateral gene transfer is well accepted as changing bacterial gene content in prokaryotes by the increment of genes and/or pathways for secondary metabolism, thus likely increasing fitness (such as antibiotic resistance, pathogenesis, etc.). The transfer of genes involved in primary metabolism is considered to be a less frequent process (Nakamura et al. 2004; Pal et al. 2005; Merkl 2006; Homma et al. 2007), although these events have been reported (Boucher et al.

2003; Omelchenko et al. 2003). The results described in this work provide evidence that transfer may have occurred for pathways involved in the biosynthesis of quinolinate, an essential precursor of NAD, together with de novo acquisition of genes involved in the kynurenine pathway. In this case, the transfer of such a primary metabolic pathway could have triggered the replacement of a previously well-established pathway, which further supports the high relevance of these lateral gene transfer processes during the evolution of bacterial genomes.

Supplementary Material

Supplementary table S1 (List of seed sequences used to similarity search analysis) and table S2 (Genome's GenBank accession numbers); supplementary figure S1 (Chromosomal arrangement of the kynurenine pathway genes), figure S2 (Alignment showing the conserved residues in *kynB* orthologs), and figure S3 (phylogenetic tree of *haaO* gene); and protein sequences (in fasta format) for all ORFs referred in table 1 are available at *Molecular Biology and Evolution*online (http://www.mbe.oxfordjournals.org/).

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