



Genomic Analysis of *Tepidimicrobium xylanilyticum*: Another Key to Lactate Metabolic Pathway in Thermophilic Anaerobic Digestion

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Table S1: The quality scores of the sequencing data of *T. xylanilyticum* EN5CB1.

Type of data	Length (bp)	#Reads	#Bases	Q20 (%)	Q30 (%)
Raw data	150	7407790	1.11E+09	97.18	92.08
After trimming	149.24	7398522	1.10E+09	97.28	92.19

Note: Length (bp)-Average read average length (bp), Reads-Read count, Bases-Base count, Q20 (%)-Percentage of bases that have less than 1% probability of error, Q30 (%)-Percentage of bases that have less than 0.1% probability of error.

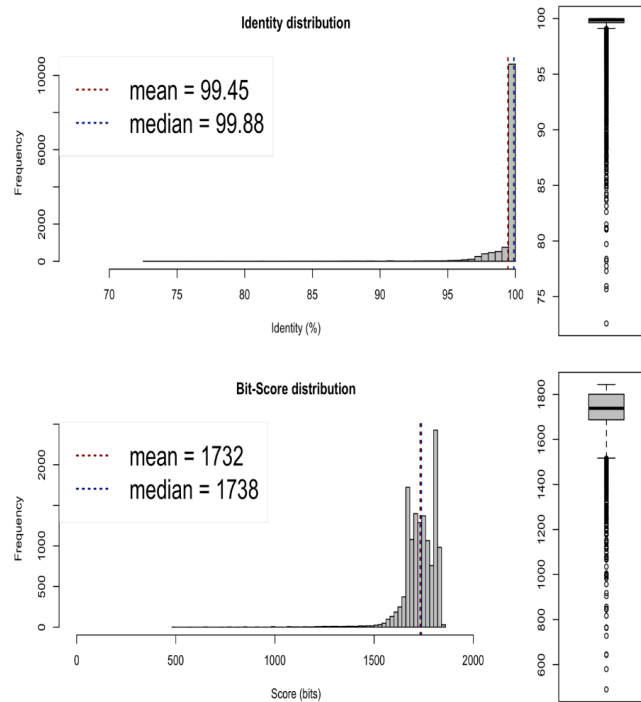


Figure S1: Average nucleotide identity and distribution between *T. xylanilyticum* EN5CB1 and DSM 23310

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Received: 06-Mar-2024, Manuscript No. JBP-24-25094; Editor assigned: 11-Mar-2024, Pre QC No. JBP-24-25094 (PQ); Reviewed: 25-Mar-2024, QC No. JBP-24-25094; Revised: 01-Apr-2024, Manuscript No. JBP-24-25094 (R); Published: 08-Apr-2024, DOI: 10.35248/2155-9597.24.15.505

Citation: Tseng HC, Matsutani M, Fujimoto N, Ohnishi A (2024) Genomic Analysis of *Tepidimicrobium xylanilyticum*: Another Key to Lactate Metabolic Pathway in Thermophilic Anaerobic Digestion. J Bacteriol Parasitol. 15:505.

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Table S2: Predicted gene products related to lactate metabolic pathway of *T. xylanilyticum*, *C. butyricum* and *M. elsdenii*

<i>Tepidimicrobium xylanilyticum</i>				<i>Clostridium butyricum</i> *			<i>Megasphaera elsdenii</i> *		
Group	Locus (EN5CB1)	Locus (DSM 23310)	Predicted gene products	Group	Locus	Predicted gene products	Group	Locus	Predicted gene products
T1	EN5CB1_01870		Lactate racemase	B1	NPD4_RS12485	Lactate racemase	M1	C6Y28_RS02170 C6Y28_RS02720	Lactate racemase
	EN5CB1_01710		D-lactate dehydrogenase						
	EN5CB1_01760		D-lactate dehydrogenase						
	EN5CB1_01860		D-lactate dehydrogenase						
T2	EN5CB1_14070	SAMN05660923_02883	L-lactate dehydrogenase	B2			M2		
	EN5CB1_23690	SAMN05660923_01484	D-lactate dehydrogenase		NPD4_RS08835	L-lactate dehydrogenase		C6Y28_RS01105	L-lactate dehydrogenase
	EN5CB1_23750	SAMN05660923_01490	D-lactate dehydrogenase		NPD4_RS18600	D-lactate dehydrogenase		C6Y28_RS00445	D-lactate dehydrogenase
T3	EN5CB1_16200	SAMN05660923_00950	Pyruvate: ferredoxin oxidoreductase	B3	NPD4_RS14035	Pyruvate-ferredoxin oxidoreductase	M3	C6Y28_RS09185 C6Y28_RS03555 C6Y28_RS07300	Pyruvate-ferredoxin oxidoreductase
T4	EN5CB1_21680	SAMN05660923_02693	Pyruvate dehydrogenase E1 alpha subunit						
	EN5CB1_21690	SAMN05660923_02692	Pyruvate dehydrogenase E1 beta subunit						
	EN5CB1_21700	SAMN05660923_02691	Dihydrolipoamide acetyltransferase						
	EN5CB1_21710	SAMN05660923_02690	Dihydrolipoyl dehydrogenase						
	EN5CB1_21720	SAMN05660923_02687	Dihydrolipoyl dehydrogenase						
	EN5CB1_27380	SAMN05660923_02610	Dihydrolipoyl dehydrogenase						
	EN5CB1_27390	SAMN05660923_02612	Dihydrolipoamide acetyltransferase						
	EN5CB1_27400	SAMN05660923_02613	Dihydrolipoyl dehydrogenase						
	EN5CB1_27410	SAMN05660923_02614	Dihydrolipoamide acetyltransferase						
	EN5CB1_27420	SAMN05660923_02615	Pyruvate dehydrogenase E1 beta subunit						
	EN5CB1_27430	SAMN05660923_02616	Pyruvate dehydrogenase E1 alpha subunit						
T5	EN5CB1_09570	SAMN05660923_00530	Phosphotransacetylase	B4	NPD4_RS11155	Phosphotransacetylase	M4	C6Y28_RS06795	Phosphotransacetylase

T6	EN5CB1_19990	SAMN05660923_01676	Acetate kinase	B5	NPD4_RS11160	Acetate kinase	M5	C6Y28_RS09165	Acetate kinase
	EN5CB1_20000	SAMN05660923_01677	Acetate kinase						
T7	EN5CB1_13180	SAMN05660923_01843	[FeFe] hydrogenase 4Fe-4S dicluster domain-containing protein	B6	NPD4_RS03860	[FeFe] hydrogenase related protein	M6	C6Y28_RS03325	[FeFe] hydrogenase related protein
	EN5CB1_18020	SAMN05660923_02196	[FeFe] hydrogenase H-cluster maturation GTPase HydF						
	EN5CB1_18030	SAMN05660923_02195	[FeFe] hydrogenase H-cluster radical SAM maturase HydG						
	EN5CB1_18040	SAMN05660923_02194	[FeFe] hydrogenase H-cluster radical SAM maturase HydE						
	EN5CB1_18050	SAMN05660923_02193	[FeFe] hydrogenase HydA						
	EN5CB1_06550	SAMN05660923_02851	[NiFe] hydrogenase expression/formation protein HypE						
T8	EN5CB1_07860	SAMN05660923_00696	[NiFe] hydrogenase nickel incorporation protein HypB	B7	NPD4_RS20385	Acetyl-CoA acetyltransferase			
	EN5CB1_07880	SAMN05660923_00694	[NiFe] hydrogenase nickel incorporation protein HypA						
	EN5CB1_24920	SAMN05660923_02142	[NiFe] hydrogenase accessory proteins HypC						
	EN5CB1_24930	SAMN05660923_02143	[NiFe] hydrogenase accessory proteins HypF						
	EN5CB1_24940	SAMN05660923_02144	[NiFe] hydrogenase accessory proteins HypD						
					NPD4_RS03875	3-hydroxybutyryl-CoA dehydrogenase			
					NPD4_RS03895	Crotonase (enoyl-CoA hydratase)			
					NPD4_RS12590	Butyryl-CoA dehydrogenase (acyl-CoA dehydrogenase)			

B8	NPD4_RS04355	Phosphate butyryltransferase
	NPD4_RS04350	Butyrate kinase
B9	WP_118472575	Butyryl-CoA: acetate CoA- transferase
M7	C6Y28_RS06180	Propionyl-CoA reductase
M8	C6Y28_RS06190	Lactyl-CoA dehydratase (acyl-CoA dehydratase)
M9	$\frac{C6Y28_RS06205}{C6Y28_RS08475}$	Acrylyl-CoA reductase

	EN5CB1_12810	SAMN05660923_01802	Na (+)-translocating NADH-quinone reductase subunit A
	EN5CB1_12820	SAMN05660923_01803	Na (+)-translocating NADH-quinone reductase subunit F
	EN5CB1_12830	SAMN05660923_01804	Na (+)-translocating NADH-quinone reductase subunit E
T9	EN5CB1_12840	SAMN05660923_01805	Na (+)-translocating NADH-quinone reductase subunit D
	EN5CB1_12850	SAMN05660923_01806	Na (+)-translocating NADH-quinone reductase subunit C
	EN5CB1_12860	SAMN05660923_01807	Na (+)-translocating NADH-quinone reductase subunit B
T10	EN5CB1_00220	SAMN05660923_01297	Na ⁺ /H ⁺ antiporter NhaC
	EN5CB1_01200	SAMN05660923_01206	<i>Sodium:proton antiporter</i>

	EN5CB1_09300	SAMN05660923_00556	<i>Sodium:proton antiporter</i>
	EN5CB1_18790	SAMN05660923_02824	<i>Sodium:proton antiporter</i>
	EN5CB1_03710	SAMN05660923_00260	AtpZ/AtpI family protein
	EN5CB1_03720	SAMN05660923_00259	ATP synthase subunit I
	EN5CB1_03730	SAMN05660923_00258	ATP synthase subunit a
	EN5CB1_03740	SAMN05660923_00257	ATP synthase subunit c
T11	EN5CB1_03750	SAMN05660923_00256	ATP synthase subunit b
	EN5CB1_03760	SAMN05660923_00255	ATP synthase subunit delta
	EN5CB1_03770	SAMN05660923_00254	ATP synthase subunit alpha
	EN5CB1_03780	SAMN05660923_00253	ATP synthase subunit gamma
	EN5CB1_03790	SAMN05660923_00252	ATP synthase subunit beta
	EN5CB1_03800	SAMN05660923_00251	ATP synthase subunit epsilon

Note: *The predicted genes products of *C. butyricum* and *M. elsdenii* were based on the genomic data of *C. butyricum* strain CDC_51208 (GenBank assembly acc. no.: GCA_001886875.1), and *M. elsdenii* strain NCIMB702410 (GenBank assembly acc. no.: GCA_003006415.1).

Table S3: Specific gene products related to carbohydrate metabolism of *T. xylanilyticum* EN5CB1

	Locus tag (EN5CB1)	Contig no.	Product
	EN5CB1_00960	Contig 1	Alpha-mannosidase
	EN5CB1_00980	Contig 1	Mannose-6-phosphate isomerase
	EN5CB1_01530	Contig 1	Transketolase
	EN5CB1_01540	Contig 1	Transketolase
	EN5CB1_01580	Contig 1	Aldolase
	EN5CB1_01600	Contig 1	Tagatose-6-phosphate kinase
	EN5CB1_01610	Contig 1	L-fucose-phosphate aldolase
	EN5CB1_01710	Contig 1	D-lactate dehydrogenase
	EN5CB1_01720	Contig 1	Ketohydroxyglutarate aldolase
	EN5CB1_01740	Contig 1	2,4-dihydroxyhept-2-ene-1,7-dioic acid aldolase

EN5CB1_01750	Contig 1	Ketohydroxyglutarate aldolase
EN5CB1_01760	Contig 1	D-lactate dehydrogenase
EN5CB1_01770	Contig 1	Transketolase
EN5CB1_01780	Contig 1	Transketolase
EN5CB1_01850	Contig 1	Fructose-bisphosphate aldolase
EN5CB1_01860	Contig 1	D-lactate dehydrogenase
EN5CB1_01880	Contig 1	Glyceraldehyde-3-phosphate dehydrogenase
EN5CB1_01890	Contig 1	Phosphoglycerate kinase
EN5CB1_02550	Contig 1	Alcohol dehydrogenase
EN5CB1_02610	Contig 1	Sorbitol-6-phosphate 2-dehydrogenase
EN5CB1_04220	Contig 1	UDP-2,4-diacetamido-2,4,6-trideoxy-beta-L-altropy ranose hydrolase
EN5CB1_07740	Contig 2	Glutaconyl-CoA decarboxylase subunit beta
EN5CB1_12880	Contig 3	Methylglyoxal synthase
EN5CB1_25970	Contig 10	Glucosamine-fructose-6-phosphate aminotransferase

Note: The area in light blue represented the gene products located in the same contig.

Table S4: Annotations and CD-HIT pattern of specific gene clusters (Cluster-LU1 and Cluster-LU2) of *T. xylanilyticum* EN5CB1

Locus (EN5CB1)	Locus tag	Closely related species	Predicted gene products	Identity	CD-HIT pattern (L70S90)*
EN5CB1_01510	SAMN05660923_01173	<i>Tepidimicrobium xylanilyticum</i>	Glycine betaine transporter	96.8	BLV68 EN5CB1 SAMN05660923
EN5CB1_01520	SAMN05660923_01172	<i>Tepidimicrobium xylanilyticum</i>	Membrane dipeptidase	98.8	BLV68 EN5CB1 SAMN05660923
EN5CB1_01530	CPJCM30710_03870	<i>Clostridium polyendosporum</i>	Transketolase	61.9	EN5CB1
EN5CB1_01540	Clopa_0951	<i>Clostridium pasteurianum</i>	Transketolase	66.2	EN5CB1
EN5CB1_01550	NSA23_RS16330	<i>Anaerolibacter massiliensis</i>	PTS sugar transporter subunit IIA	59	EN5CB1
EN5CB1_01560	BET03_04055	<i>Thermohalobacter berrensii</i>	PTS galactitol transporter subunit IIB	66.3	EN5CB1
EN5CB1_01570	BET03_04060	<i>Thermohalobacter berrensii</i>	PTS galactitol transporter subunit IIC	71.1	EN5CB1
EN5CB1_01580	BET03_04065	<i>Thermohalobacter berrensii</i>	Aldolase	55	EN5CB1
EN5CB1_01590	BET03_04070	<i>Thermohalobacter berrensii</i>	Hypothetical protein	50.9	EN5CB1
EN5CB1_01600	BET03_04075	<i>Thermohalobacter berrensii</i>	Hypothetical protein	57	EN5CB1
EN5CB1_01610	BET03_04080	<i>Thermohalobacter berrensii</i>	Hypothetical protein	53.3	EN5CB1
EN5CB1_01620	BET03_04085	<i>Thermohalobacter berrensii</i>	Hypothetical protein	52.9	EN5CB1
EN5CB1_01630	LQK79_02175	<i>Clostridium guangxiense</i>	Transcriptional regulator	45.7	EN5CB1
EN5CB1_01640		No significant similarity found	Hypothetical protein		EN5CB1
EN5CB1_01650	IW830_001170	<i>Enterococcus faecium</i>	Hypothetical protein	77.2	EN5CB1
EN5CB1_01660	SAMN05660923_01171	<i>Tepidimicrobium xylanilyticum</i>	Beta-aspartyl-dipeptidase (metallo-type)	94.4	BLV68 EN5CB1 SAMN05660923
EN5CB1_01670	SAMN05660923_01170	<i>Tepidimicrobium xylanilyticum</i>	Hypothetical protein	98	BLV68 EN5CB1 SAMN05660923
EN5CB1_01680	CULT_1160014	<i>Clostridium ultunense</i>	Transcriptional regulator	79.5	EN5CB1
EN5CB1_01690	H8707_13160	<i>Paratissierella segnis</i>	PTS sugar transporter subunit IIB	53.9	EN5CB1
EN5CB1_01700	EQM13_14145	<i>Acidilutibacter cellobiosedens</i>	PTS fructose transporter subunit IIA	38.1	EN5CB1
EN5CB1_01710	Theth_1754	<i>Pseudothermotoga thermarum</i>	D-Lactate dehydrogenase	53.2	EN5CB1
EN5CB1_01720	NCTC11087_01627	<i>Faecalicoccus pleomorphus</i>	Ketohydroxyglutarate aldolase	63	EN5CB1

EN5CB1_01730	SAMN04487929_10896	<i>Clostridium innocuum</i>	Demethylmenaquinone methyltransferase	71.6	EN5CB1
EN5CB1_01740	EQM13_03990	<i>Acidilutibacter cellobiosedens</i>	2,4-dihydroxyhept-2-ene-1,7-dioic acid aldolase	64.8	EN5CB1
EN5CB1_01750	EQM13_11950	<i>Acidilutibacter cellobiosedens</i>	Ketohydroxyglutarate aldolase	86	EN5CB1
EN5CB1_01760	CINTURNW_3284	<i>Clostridium intestinale</i>	D-Lactate dehydrogenase	52.3	EN5CB1
EN5CB1_01770	Gferi_18190	<i>Geosporobacter ferrireducens</i>	Transketolase	66.9	EN5CB1
EN5CB1_01780	Gferi_18195	<i>Geosporobacter ferrireducens</i>	Transketolase	78.9	EN5CB1
EN5CB1_01790	EQM13_11945	<i>Acidilutibacter cellobiosedens</i>	PTS sugar transporter subunit IIB	75.6	EN5CB1
EN5CB1_01800	EQM13_11940	<i>Acidilutibacter cellobiosedens</i>	PTS sugar transporter subunit IIC	85.5	EN5CB1
EN5CB1_01810	EQM13_11935	<i>Acidilutibacter cellobiosedens</i>	PTS sugar transporter subunit IID	83	EN5CB1
EN5CB1_01820	MKD05_13045	<i>Clostridium innocuum</i>	PTS sugar transporter subunit IIA	54.7	EN5CB1
EN5CB1_01830	EQM13_11930	<i>Acidilutibacter cellobiosedens</i>	Hypothetical protein	64.6	EN5CB1
EN5CB1_01840	MKC73_17455	<i>Clostridium innocuum</i>	Zinc-binding dehydrogenase	58.4	EN5CB1
EN5CB1_01850	EQM13_09920	<i>Acidilutibacter cellobiosedens</i>	Fructose-bisphosphate aldolase	63.4	EN5CB1
EN5CB1_01860	CLJU_c11620	<i>Clostridium ljungdahlii</i>	D-Lactate dehydrogenase	46.2	EN5CB1
EN5CB1_01870	LKJ23_04000	<i>Clostridium luticellarii</i>	Lactate racemase	65.6	EN5CB1
EN5CB1_01880	I6U51_13895	<i>Clostridium aciditolerans</i>	Glyceraldehyde-3-phosphate dehydrogenase	77.8	EN5CB1
EN5CB1_01890	NBE98_20940	<i>Clostridium swellfunianum</i>	Phosphoglycerate kinase	64.6	EN5CB1
EN5CB1_01900	SAMN05660923_00432	<i>Tepidimicrobium xylanilyticum</i>	Polysaccharide deacetylase	100	BLV68 EN5CB1 SAMN05660923
Identical					
EN5CB1_01910	SAMN05660923_00431	<i>Tepidimicrobium xylanilyticum</i>	Transcriptional regulator	100	BLV68 EN5CB1 SAMN05660923

Note: *CD-HIT patterns were compared between genomic sequencing data of *T. xylanilyticum* EN5CB1 (EN5CB1), GenBank (SAMN05660923) and RefSeq database (BLV68) of strain DSM 23310 with a sequence overlap threshold of 70% and sequence identity of 90%.

Table S5: The specific gene products related to lactate utilization (Group T1) and proton transportation (Group T11) detected in *T. xylanilyticum* EN5CB1

Group	Locus (EN5CB1)	Locus (DSM 23310)	Predicted gene products	CD-HIT pattern (L70S90)	Number of cluster (L70S90)	Length (L70S90)	Identity(%) (L70S90)
T1	EN5CB1_01870		Lactate racemase	EN5CB1	1	420	*
	EN5CB1_01710		D-lactate dehydrogenase	EN5CB1	1	333	*
	EN5CB1_01760		D-lactate dehydrogenase	EN5CB1	1	315	*
	EN5CB1_01860		D-lactate dehydrogenase	EN5CB1	1	306	*
	EN5CB1_14070	SAMN05660923_02883	L-lactate dehydrogenase	BLV68 EN5CB1 SAMN05660923	3	324	100
	EN5CB1_23690	SAMN05660923_01484	D-lactate dehydrogenase	BLV68 EN5CB1 SAMN05660923	3	329	99.39
	EN5CB1_23750	SAMN05660923_01490	D-lactate dehydrogenase	BLV68 EN5CB1 SAMN05660923	3	320	100

T7	EN5CB1_13180	SAMN05660923_01843	[FeFe] hydrogenase 4Fe-4S dicluster domain-containing protein	BLV68 EN5CB1 SAMN05660923	3	500	100
	EN5CB1_18020	SAMN05660923_02196	[FeFe] hydrogenase H-cluster maturation GTPase HydF	BLV68 EN5CB1 SAMN05660923	3	396	100
	EN5CB1_18030	SAMN05660923_02195	[FeFe] hydrogenase H-cluster radical SAM maturase HydG	BLV68 EN5CB1 SAMN05660923	3	456	100
	EN5CB1_18040	SAMN05660923_02194	[FeFe] hydrogenase H-cluster radical SAM maturase HydE	BLV68 EN5CB1 SAMN05660923	3	351	100
	EN5CB1_18050	SAMN05660923_02193	[FeFe] hydrogenase HydA	BLV68 EN5CB1 SAMN05660923	3	567	99.65
T8	EN5CB1_06550	SAMN05660923_02851	[NiFe] hydrogenase expression/formation protein HypE	BLV68 EN5CB1 SAMN05660923	3	331	99.4
	EN5CB1_07860	SAMN05660923_00696	[NiFe] hydrogenase nickel incorporation protein HypB	BLV68 EN5CB1 SAMN05660923	3	222	100
	EN5CB1_07880	SAMN05660923_00694	[NiFe] hydrogenase nickel incorporation protein HypA	BLV68 EN5CB1 SAMN05660923	3	113	98.23
	EN5CB1_24920	SAMN05660923_02142	[NiFe] hydrogenase accessory proteins HypC	BLV68 EN5CB1 SAMN05660923	3	161	100
	EN5CB1_24930	SAMN05660923_02143	[NiFe] hydrogenase accessory proteins HypF	BLV68 EN5CB1 SAMN05660923	3	629	100
	EN5CB1_24940	SAMN05660923_02144	[NiFe] hydrogenase accessory proteins HypD	BLV68 EN5CB1 SAMN05660923	3	312	100
T11	EN5CB1_03710	SAMN05660923_00260	AtpZ/AtpI family protein	EN5CB1	1	75	*
	EN5CB1_03720	SAMN05660923_00259	ATP synthase subunit I	EN5CB1	1	122	*
	EN5CB1_03730	SAMN05660923_00258	ATP synthase subunit a	EN5CB1	1	222	*
	EN5CB1_03740	SAMN05660923_00257	ATP synthase subunit c	EN5CB1	1	89	*
	EN5CB1_03750	SAMN05660923_00256	ATP synthase subunit b	EN5CB1	1	170	*
	EN5CB1_03760	SAMN05660923_00255	ATP synthase subunit delta	BLV68 EN5CB1 SAMN05660923	3	180	100
	EN5CB1_03770	SAMN05660923_00254	ATP synthase subunit alpha	BLV68 EN5CB1 SAMN05660923	3	500	100
	EN5CB1_03780	SAMN05660923_00253	ATP synthase subunit gamma	BLV68 EN5CB1 SAMN05660923	3	285	100
	EN5CB1_03790	SAMN05660923_00252	ATP synthase subunit beta	BLV68 EN5CB1 SAMN05660923	3	465	100
	EN5CB1_03800	SAMN05660923_00251	ATP synthase subunit epsilon	BLV68 EN5CB1 SAMN05660923	3	140	100

Note: *CD-HIT patterns were compared between genomic sequencing data of *T. xylanilyticum* strain EN5CB1 (EN5CB1), GenBank (SAMN05660923) and RefSeq database (BLV68) of strain DSM 23310 with a sequence overlap threshold of 70% and sequence identity of 90%, *N represented value was below the overlap threshold of 70% and sequence identity of 90%.



Figure S2: Phylogenetic tree of ATPase operon (EN5CB1_03710-03800) based on amino acid sequence. (A)-EN5CB1_03710, (B)-EN5CB1_03720, (C)-EN5CB1_03740, (D)-EN5CB1_03760, (E)-EN5CB1_03770, (F)-EN5CB1_03790 and (G)-EN5CB1_03800. **Note:** The closely related species and gene product was marked by red circle. Scale bar, 0.1 substitutions per amino acid sites. Numerals indicate the statistical reliability of the branching order determined by bootstrap analysis (1000 duplications).