Table S1. Primer sequences for site-directed mutagenesis.

Mutant	Primer Sequences [a]	
K162A	K162A-F	cgcaggagtagttcaatgtGCGgatgcattttacgggcaacatg
	K162A-R	catgttgcccgtaaaatgcatcCGCacattgaactactcctgcg
	K162A-sF	caggagtagttcaatgtGCG
H169A	H169A-F	gatgcattttacgggcaaGCGgagccagaacgtatgccag
	H169A-R	ctggcatacgttctggctcCGCttgcccgtaaaatgcatc
	H169A-sF	atgcattttacgggcaaGCG
H169D	H169D-F	gcattttacgggcaaGACgagccagaacgtatgccag
	H169D-R	ctggcatacgttctggctcGTCttgcccgtaaaatgcatc
	H169D-sF	atgcattttacgggcaaGAC
H169N	H169N-F	gatgcattttacgggcaaAACgagccagaacgtatgccag
	H169N-R	ctggcatacgttctggctcGTTttgcccgtaaaatgcatc
	H169N-sF	atgcattttacgggcaaAAC
V220D	V220D-F	ggatcagatttccttgtGgACggtaatcaagaacgcaacg
	V220D-R	cgttgcgttcttgattaccGTcCacaaggaaatctgatcc
	V220D-sR	tgcgttcttgattaccGTcC
V220E	V220E-F	ggatcagatttccttgtGgAaggtaatcaagaacgcaacg
	V220E-R	cgttgcgttcttgattacctTcCacaaggaaatctgatcc

	V220E-sR	tgcgttcttgattaccctTcC

Thermococcus_barophilus
Thermococcus_onnurineus
Thermococcus_gammatolerans
Bacteroides_capillosus
Eubacterium_ventriosum
Sebaldella_termitidis
Treponema_phagedenis
butyrate-producing_bacterium/
Eubacterium_cellulosolvens
Clostridium
Blautia_hydrogenotrophica
Erysipelotrichaceae_bacterium
Parvimonas_micra
Anaerococcus_lactolyticus
Finegoldia_magna
Fusobacterium_sp._D12
Fusobacterium_mortiferum
Fusobacterium_varium
Fusobacterium_ulcerans
Granulicatella_adiacens
Granulicatella_elegans
Clostridium_asparagiforme
Clostridium_sp._M62/1
Roseburia_inulinivorans
Streptococcus_pyogenes ✳
Clostridium_hiranonis
Clostridiales_bacterium
Clostridium_bolteae
Coprococcus_catus
Dorea_longicatena
Ruminococcus_sp._5_1_39B_FAA
Ruminococcus_torques
Ruminococcus_obeum_A2-162
Ruminococcus_sp._SR1/5
Ruminococcus_obeum_ATCC_29174
Ruminococcus_torques
Clostridium_nexile
Dorea_formicigenerans
Clostridium_scindens
Clostridium_saccharolyticum
Clostridium_hathewayi
Abiotrophia_defectiva
Clostridium_perfringens
Clostridium_butyricum
Clostridium_botulinum
Clostridium_cellulovorans
Clostridium_beijerinckii
Eubacterium_saburreum
Anaerofustis_stercorihominis
Holdemania_filiformis
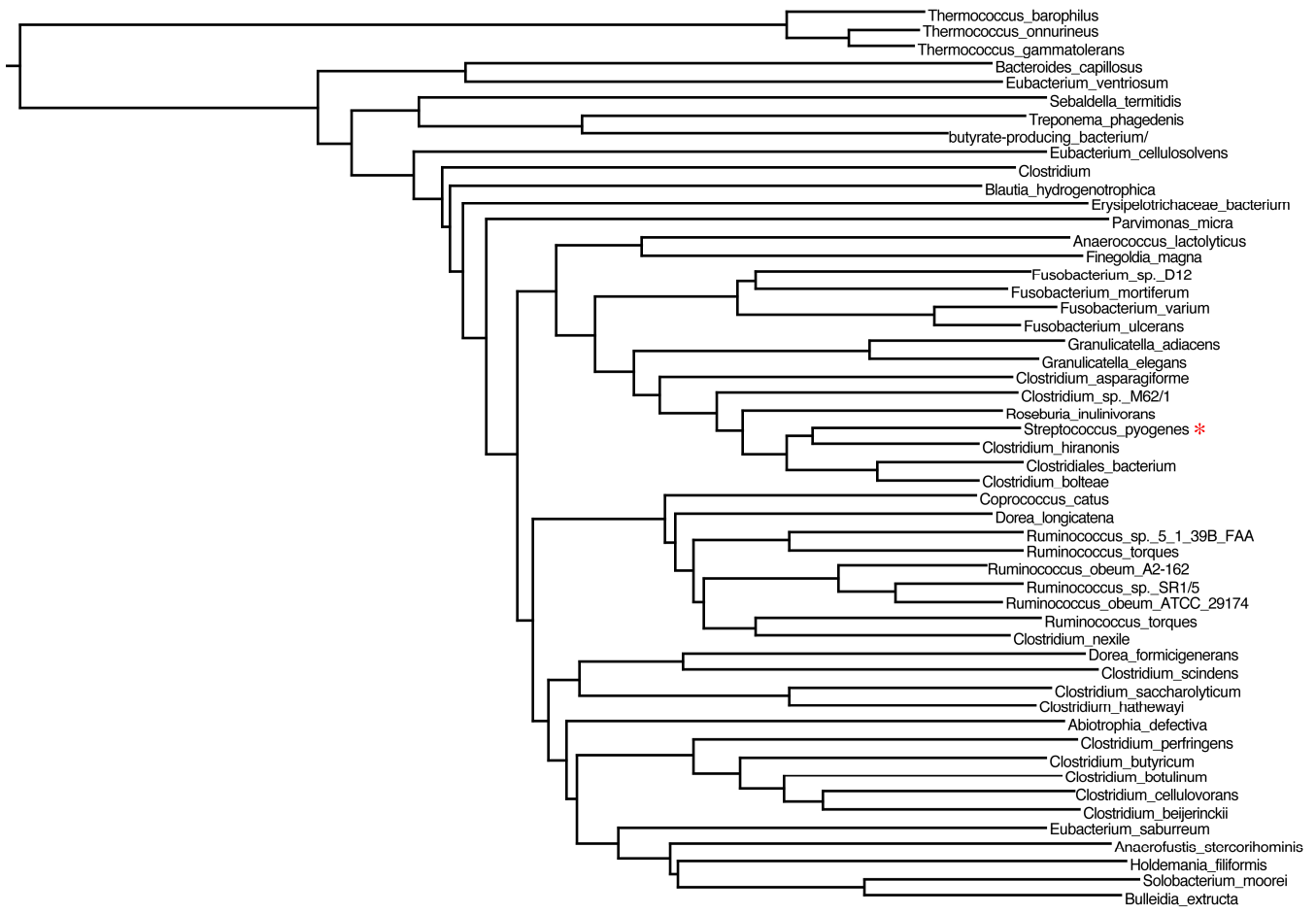Solobacterium_moorei
Bulleidia_extructa

Figure S1. The branch of the phylogenetic tree of uridine phosphorylases depicting the subfamily that contains *Streptococcus pyogenes* UP (SpUp). The tree was generated using ClustalW. A red asterisk highlights the position of SpUP.
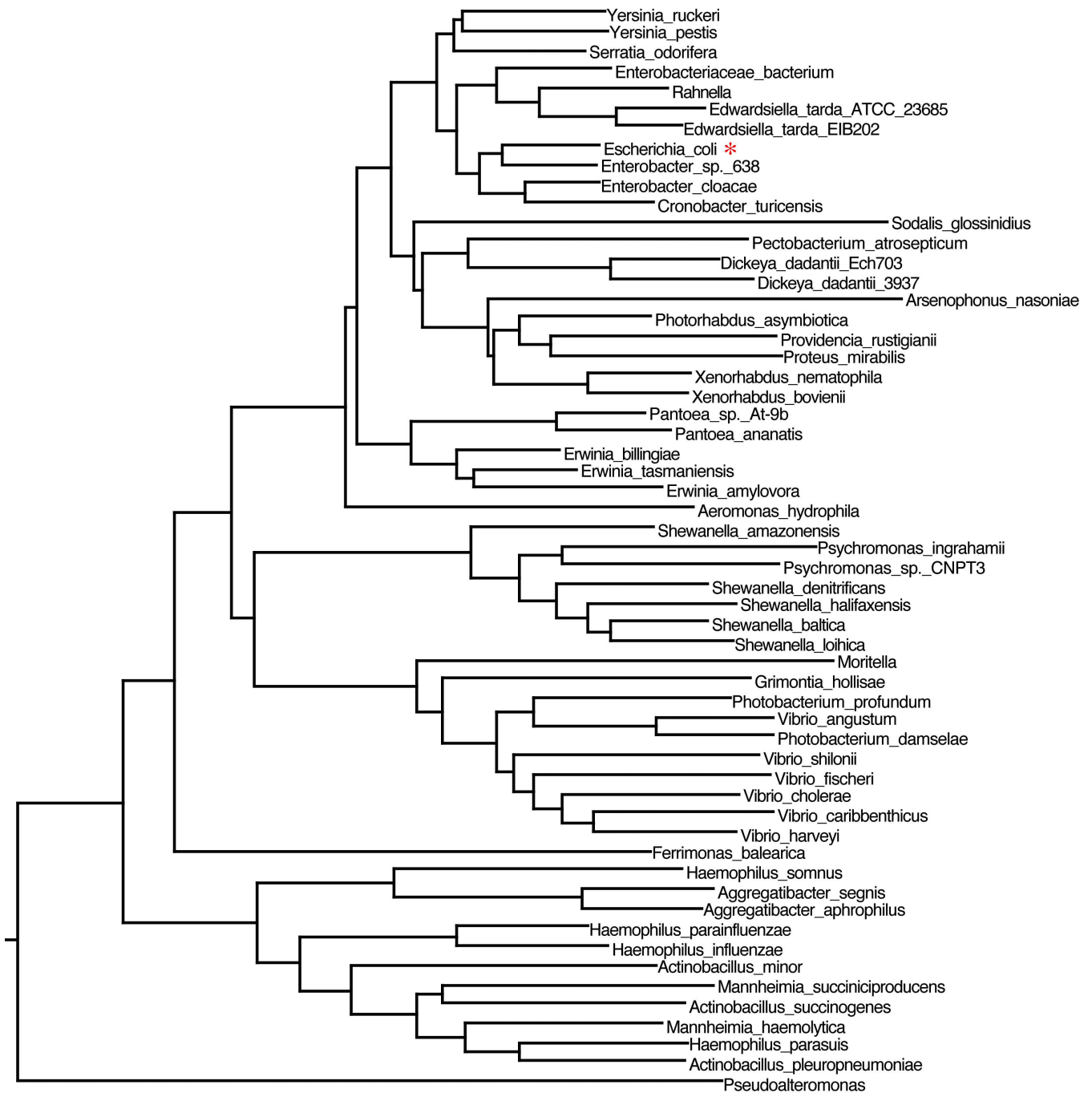
Figure S2. The branch of the phylogenetic tree of uridine phosphorylases depicting the subfamily that contains *Excherichia coli* UP (EcUP). The tree was generated using ClustalW. A red asterisk highlights the position of EcUP.