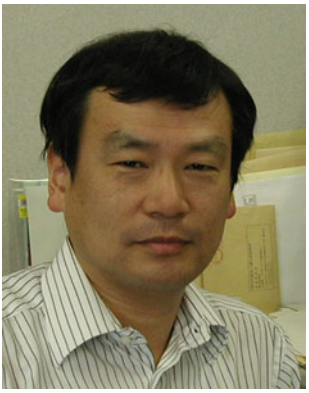
Biodata of **Yukata Kawarabayasi**, author of "***Acido- and Thermophilic Microorganisms: Their Features, and the Identification of Novel Enzymes or Pathways***."

Professor Yukata Kawarabayasi is currently the Dean of the Faculty of Agriculture in Kyushu University, Japan. He obtained his Ph.D. from the Kyoto University in 1987 and he then joined the staff of Tohoku University, Japan. Then, he worked at the Kazusa DNA research institute and the National Institute of Advanced Industrial Science and Technology. Professor Kawarabayasi's scientific interests are in the areas of genomics of thermophilic archaea, identification of useful enzymes from genomic data, and isolation of novel genes by environmental genomics.

E-mail: **kyutaka@agr.kyushu-u.ac.jp**, **kawarabayasi.yutaka@nifty.com**



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ACIDO- AND THERMOPHILIC MICROORGANISMS: THEIR FEATURES, AND THE IDENTIFICATION OF NOVEL ENZYMES OR PATHWAYS

YUTAKA KAWARABAYASI
Laboratory on Functional Genomics of Extremophiles, Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan

1. Introduction to Acido- and Thermophilic Microorganisms

Numerous species of acidophilic and acidotolerant bacteria have been identified. For example, many lactic acid bacteria have been identified from traditional yogurt, and some species of *Helicobacter* are tolerant of acidic conditions. Most bacterial species with acidophilic features grow at normal temperatures, around 25–37 °C. Only few microorganisms from the domain *Bacteria* have been found to exhibit both acidophilic and thermophilic features. As shown in Table 1, only two genera in *Bacteria* have been identified as acido- and thermophilic microorganisms. The optimum growth temperature of these microorganisms is around 45–65 °C, which is slightly lower than optimum for similar microorganisms from the domain *Archaea*.

By contrast, a large number of genera in *Archaea* have been identified as acido- and thermophilic microorganisms, including members of the phyla *Creanarchaeota* and *Euryarchaeota* (Table 1). The optimum growth temperature of the acido- and thermophilic microorganisms within *Crenarchaeota* is around 65–85 °C. Moreover, some of these microorganisms are capable of growing under aerobic condition, as many of the microorganisms in *Crenarchaeota* were isolated from geothermal environments, like hot springs. In addition to being hot, geothermal environments typically contain high concentrations of sulfur or hydrogen sulfide, which make them acidic, so that microorganisms growing in these environments should have acido- and thermophilic features. It is thought that these aerobic organisms and the gene products derived from them or produced recombinantly in *E. coli* cells will be convenient for industrial application.

The typical features specific to the acido- and thermophilic microorganisms are extracted from the genus *Sulfolobus* as model microorganism and summarized in the following section.

2. Characteristics of Microorganisms in the Genus *Sulfolobus*

Among the acido- and thermophilic microorganisms in *Archaea*, those in the genus *Sulfolobus* are the most extensively characterized. In this section, therefore, the features of three *Sulfolobus* species will be summarized as typical of acido- and thermophilic microorganisms.

Table 1. List of acidothermophilic microorganisms.

Domain	Genus	Optimum growth temperature (°C)	Optimum growth pH	Phyla[a]
Archaea				
	Acidianus	70–85	0.8–2.5	C
	Acidilobus	80	3.5–3.8	C
	Caldisphaera	75	3.5	C
	Caldivirga	85	3.5	C
	Metallosphaera	65–75	2.5–4.0	C
	Sulfolobus	65–80	2.0–3.5	C
	Sulfurisphaera	75–80	3.5	C
	Picrophilus	55	1.0	E
	Thermoplasma	60	2.0	E
Bacteria				
	Alicyclobacillus	45–60	3.0–4.0	
	Hydrogenobaculum	65	3.0	

[a]*C* Crenarchaeota; *E* Euryarchaeota.

Table 2. Characteristics of typical species in genus *Sulfolobus*.

	S. acidocaldarius	***S. solfataricus***	***S. tokodaii***
Origin	Thermal soils from Hot springs	Hot springs in Agnano, Napoli, Italy	Hot spring in Beppu, Japan
Morphology	Coccus	Coccus	Coccus
Temperature optimum	70–75	87	75–80
pH optimum	2–3	3.5	2–3
Growth conditions	Aerobic	Aerobic	Aerobic
Electron acceptor	S^0	S^0	S^0
Glucose metabolism	Modified ED pathway	Modified ED pathway	Modified ED pathway
Genome type	Circular DNA	Circular DNA	Circular DNA
Genome size	2,225,959	2,992,245	2,694,756

The characteristics of *S. acidocaldarius* (Brock et al., 1972), *S. solfataricus* (Millonig et al., 1975), and *S. tokodaii* (Suzuki et al., 2002) are summarized in Table 2. These species were isolated from geothermal environments (hot springs and hot soils) and, as is typical of such organisms, they exhibited aerobic features. Their optimum growth temperatures are around 70–80 °C, and their optimum growth pHs are between 1 and 3. These microorganisms are thus tolerant to both high temperatures and low pHs.

Electron microscopic observation revealed that all *Sulfolobus* species are cocci; i.e., they are round in shape (Millonig et al., 1975; Suzuki et al., 2002).

For energy production, glycolysis (degradation of glucose) is one of the most important pathways in any organism, and glucose is usually phosphorylated

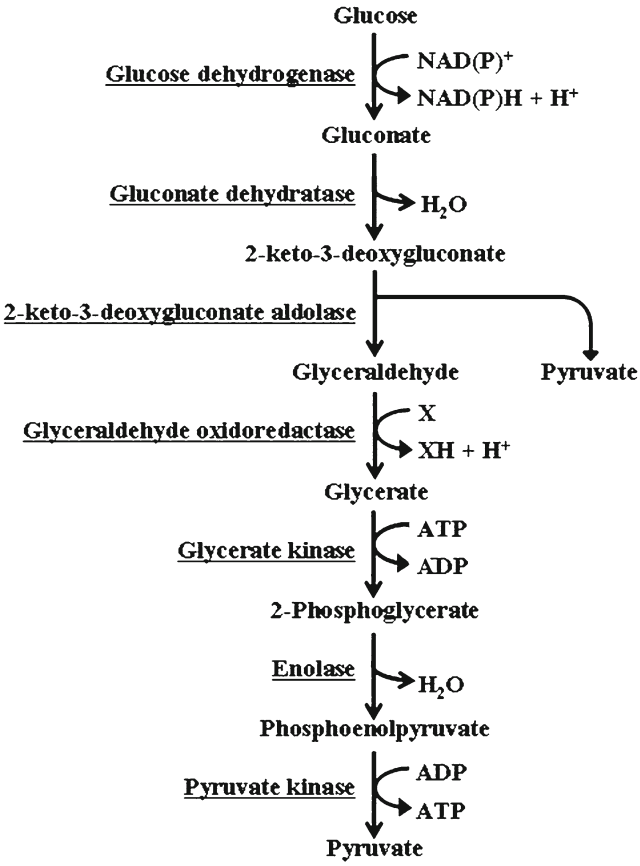

Figure 1. Nonphosphorylated Entner-Doudoroff pathway for metabolism of glucose identified in genus *Sulfolobus*. The enzymes catalyzing each reaction are *underlined*.

at the first step of the glycolytic pathway. However, the glycolysis pathway identified in *Sulfolobus* species is absolutely different from that identified in other organisms. These organisms in genus *Sulfolobus* utilized the nonphosphorylated Entner-Doudoroff pathway as the main glycolysis pathway (De Rosa et al., 1984). The enzymes catalyzing the reactions of this pathway and compounds converting through this pathway are shown in Fig. 1. In this pathway, glucose is converted into gluconate by glucose dehydrogenase (Lamble et al., 2003; Milburn et al., 2006) without phosphorylation of glucose. The gluconate is then converted to 2-keto-3-deoxygluconate by gluconate dehydratase (Lamble et al., 2004), which is in turn converted to glyceraldehyde by 2-keto-3-deoxygluconate aldolase (Theodossis et al., 2004) and then to glycerate by glyceraldehyde oxidoreductase. Glycerate is phosphorylated to 2-phosphoglycerate by glycerate kinase. In this

Table 3. List of *Sulfolobus* enzymes involved in the nonphosphorylated ED pathway.

Enzyme name	EC number	*S. acidocaldarius*	*S. solfataricus*	*S. tokodaii*
Glucose dehydrogenase	1.1.1.47	Saci_1079 (364 aa)	SSO3204(36 0 aa)	ST1704 (360 aa)
Gluconate dehydratase	4.2.1.39	Saci_0885 (395 aa)	SSO3198 (395 aa)	ST2366 (396 aa)
2-Keto-3-deoxygluconate aldolase	4.1.2.-	Saci_0225 (288 aa)	SSO3197 (308 aa)	ST2479 (290 aa)
Glyceraldehyde oxidoreductase	1.2.1.3	Saci_1700 (481 aa)	SSO3117 (478 aa)	ST1116 (490 aa)
Glycerate kinase	2.7.1.31	Saci_0113 (393 aa)	SSO0666 (400 aa)	ST2037 (399 aa)
Enolase	4.2.1.11	Saci_1377 (416 aa)	SSO0913 (419 aa)	ST1212 (416 aa)
Pyruvate kinase	2.7.1.40	Saci_1648 (441 aa)	SSO0981 (452 aa)	ST1617 (418 aa)

Numerals within parentheses indicate the length of amino acid residues in the corresponding enzyme.

pathway, two molecules of pyruvate and two protons are produced. The pyruvate, the final product of this pathway, is then utilized in the TCA cycle. All genes encoding each enzyme involved in the nonphosphorylated Entner-Doudoroff pathway were detected within the genomic data from three *Sulfolobus* species and are summarized in Table 3.

It is noteworthy that although microorganisms in genus *Sulfolobus* grow in an environment at an around pH 2.0, their cytoplasmic pH appears to be nearly neutral (Wakagi and Oshima, 1985). This means that protons entering the cells must be pumped out through the cytoplasmic membrane to maintain the neutral intracellular pH. As such, the cytoplasmic membrane from *Sulfolobus* species exhibits proton-pumping activity driven by respiration (Anemüller et al., 1985). Moreover, a novel ATPase comprised of three subunits, an α subunit with a molecular weight of 69,000, a β subunit with a molecular weight of 54,000, and a γ subunit with a molecular weight of 28,000, has been isolated from the membrane of *S. tokodaii* (Konishi et al., 1987). The characteristics of the ATPase include stability at high temperature, a pH optimum around 5, stimulation by bisulfite and inactivation by 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole, which is similar to other archaeal ATPases, though the presence of the γ subunit is exceptional (Konishi et al., 1987). The genes encoding the three subunits of the *S. tokodaii* ATPase have been isolated and their nucleotide sequences were determined (α subunit, Denda et al., 1988a; β subunit, Denda et al., 1988b; γ subunit, Denda et al., 1989). Analyses of the nucleotide sequences of the genes encoding each subunit confirmed that this ATPase belongs to a different family than the F_1-ATPase.

The sequencing of the *S. solfataricus*, *S. tokodaii*, and *S. acidocaldarius* genomes was completed in 2001, 2001, and 2005, respectively (She et al., 2001; Kawarabayasi et al., 2001; Chen et al., 2005). The size of each genome is shown in Table 2. The genomic features and information extracted from the nucleotide sequences will be described in the next section.

3. Features Detected from the Genomic Information of Microorganisms in *Sulfolobus*

During the last decade, progress in genome sequencing has provided biological scientists with a huge amount of information that would be nearly unimaginable to traditional biologists. The entire sequencing of the *S. solfataricus*, *S. tokodaii*, and *S. acidocaldarius* genomes has brought to light previously unavailable information, which I have summarized in the following section.

One of the most important finding derived from the genomic sequences of *Sulfolobus* species is that *S. tokodaii* makes use of a eukaryotic-type tRNA system. Generally, the sequence CCA is present at the 3 end of tRNA molecule and is required for aminoacylation of tRNA molecules. In bacteria and archaea, the CCA sequence is usually present within tRNA genes and is contained within the tRNA transcripts themselves. By contrast, eukaryotic transcripts from tRNA genes do not contain the CCA sequence. Instead, the sequence is added after transcription in a reaction catalyzed by the tRNA nucleotidyltransferase. It was detected that most tRNA genes predicted on the genomic sequences of *S. tokodaii* do not contain a CCA sequence at their 3 ends (Kawarabayasi et al., 2001). Moreover, a gene encoding the tRNA nucleotidyltransferase was detected within the genomic information from this microorganism (Kawarabayasi et al., 2001). Because the tRNA system employed by *S. tokodaii* is very similar to the eukaryotic tRNA system, it has been proposed that this archaeal system is likely the origin of the eukaryotic tRNA system.

Among total 46 tRNA genes predicted inform the *S. tokodaii* genomic sequence, 24 were detected as the interrupted genes in which intron was present within tRNA coding region (Kawarabayasi et al., 2001). This means that more than 50 % of tRNA genes predicted from the archaeal genomic sequence contain an intron within their tRNA coding regions. It was therefore of interest to know whether these introns found within tRNA genes predicted from the *S. tokodaii* genome are actually removed during their maturation.

Splicing sites in the tRNA genes from *Aeropyrum pernix* were easily determined by comparing immature and mature forms of cDNA molecules (Yamazaki et al., 2005). However, when cDNAs were constructed from mature and immature tRNA molecules and compared, this approach did not yield conclusive results (Yamazaki et al., 2011). To determine the correct digestion site for introns in *S. tokodaii* tRNA molecules, immature tRNA molecules were digested in vitro using recombinant stEndA, a tRNA intron endonuclease from *S. tokodaii* (Yoshinari et al., 2005). The results indicated that all predicted tRNA molecules were expressed in *S. tokodaii* and that all of the predicted introns were correctly spliced. Furthermore, analysis of the structures surrounding the splicing sites indicated that the common archaeal bulge-helix-bulge structure was detected at all intron-surrounding sites in *S. tokodaii* tRNA genes (Yamazaki et al., 2011). This means that although the unusually sized and positioned introns were detected within *S. tokodaii* tRNA genes, the microorganism makes use of a common splicing mechanism to remove those introns.

Table 4. List of genes related to sulfur metabolism.

ORF ID	Length (aa)	Predicated product	Reaction predicted
ST0615	384	Sulfide dehydrogenase	$H_2S \rightarrow S + H_2$
ST0971	390	Sulfide dehydrogenase	$H_2S \rightarrow S + H_2$
ST1010	208	Sulfite oxidase	$SO_3^{2-} + O_2 + H_2O \rightarrow SO_4^{2-} + H_2O_2$
ST1839	270	Thiosulfate reductase electron transport protein	$H_2S \rightarrow S_2O_3^{2-}$
ST2564	293	Thiosulfate sulfurtransferase	$S_2O_3^{2-} + \text{cyanide} \rightarrow SO_3^{-} + \text{thiocyanide}$
ST2566	628	Sulfite reductase	$H_2S + 3\text{Fe-oxy} + 3H_2O \rightarrow SO_3^{2-} + 3\text{Fe-Red}$
ST2567	239	Phosphoadenosine phosphosulfate reductase	$SO_4^{2-} + H_2 \rightarrow SO_3^{2-} + H_2O$
ST2568	412	Sulfate adenylyltransferase	$ATP + SO_4^{2-} \rightarrow PPi + AMP\text{-}SO_4^{2-}$

Table 5. Repetitive sequences detected in the *S. tokodaii* genome.

Type of repetitive sequence	Length (nt)	Number of repetitions
Tn-like element	1,779	7
Truncated Tn-like element	345	9
Dispersed repetitive sequence		
Type I	1,459	2
Type II	1,322	4
Type III	844	5
Type IV	518	2
LR-SR-type repetitive sequence		
Type LS-I	SR unit: 24 bp	3
Type LS-II	SR unit: 25 bp	2

S. tokodaii strain 7 is known to produce energy by oxidizing hydrogen sulfide to sulfate. A search for genes related to this reaction yielded a total of eight open reading frames (ORFs) involved in sulfide metabolism (Kawarabayasi et al., 2001). The predicted enzymes and the reactions they catalyzed are summarized in Table 4. These enzymes appear to be sufficient to oxidize hydrogen sulfide to sulfate. But to confirm their activities, heterologous expression in *E. coli* and functional analysis of the enzymes will be required.

Three types of repetitive sequences were detected in the *S. tokodaii* genome (Kawarabayasi et al., 2001). As shown in Table 5, seven full-size transposon (Tn)-like repetitive sequences and nine truncated Tn-like repetitive sequences were detected. This result indicates that Tn-like mobile elements did and do move within the *S. tokodaii* genomic DNA and that nine truncated forms of Tn-like elements were constructed through recombination after transfer of the

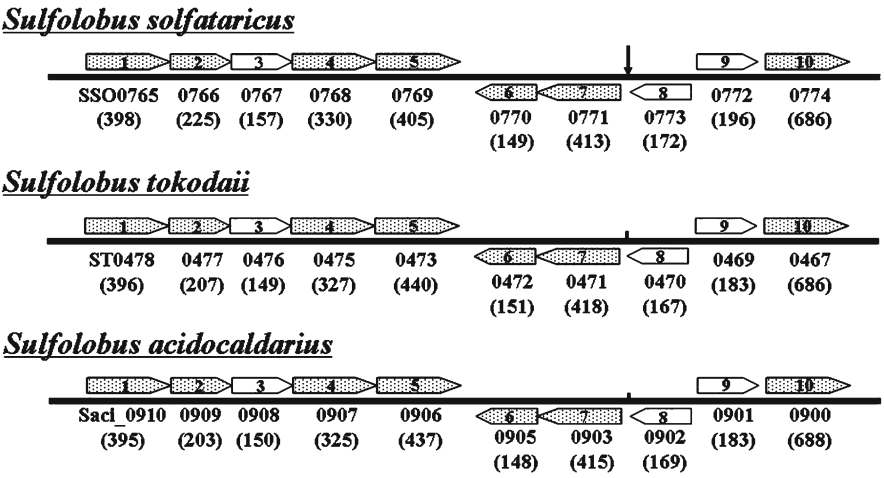

Figure 2. Structures surrounding the predicted replication origin of three species in the genus *Sulfolobus. Numbers* indicate the corresponding ORF ID in each species. In *parentheses* are the numbers of amino acid residues encoded in each ORF. *Numbers* within *arrows* are defined in Table 6 and indicate the predicted function of each ORF. *Vertical arrow* indicates the assigned replication origin.

elements. The second type of repetitive sequence was the dispersed repetitive sequence without Tn-like features, which consisted of four subtypes (types I–IV) with different sequences. The longest one repeated two times, the second longest repeated four times, the third longest five times, and the shortest two times. The third type of typical repetitive sequence was the LR-SR-type repetitive sequence. Two structurally similar but sequentially different LR-SR-type repetitive sequences were detected. One was an LS-I type and contained from 47 to 117 repeats of a 24-bp-long short repetitive segment following a 223–310-bp-long repetitive unit. The other was an LS-II type, which contained 73–113 repeats of a 25-bp-long short repetitive segment following a 228-bp-long repetitive unit.

The origin for genomic replication was well analyzed in *S. solfataricus*, and it was found that multiple regions throughout the genome are utilized as the origin for genomic replication (Robinson et al., 2004). In one instance, the region surrounding the replication origin contained ten genes, including MCM and Cdc6. The genes from gatA to MCM and the ordering of those genes within the region surrounding the replication origin are conserved among the three *Sulfolobus* species studied, as shown in Fig. 2 and Table 6. This structural feature appears to be unique to microorganisms in the genus *Sulfolobus*. Even other archaea from different genera do not conserve this structure of the replication origin. Apparently, the structure of the replication origin conserved among *Sulfolobus* species is not necessary for other archaeal species, though it is thought that this structure plays a key role during DNA replication in *Sulfolobus* species.

Table 6. List of *Sulfolobus* genes involved in the predicted replication origin.

	Predicted products	Gene name	*S. acidocaldarius*	*S. solfataricus*	*S. tokodaii*
1	Glutamyl-tRNA(Gln) amidotransferase	gatA	Saci_0910	SSO0765	ST0478
2.	Proteasome β subunit		Saci_0909	SSO0766	ST0477
3.	Hypothetical protein		Saci_0908	SSO0767	ST0476
4.	Replication factor C small subunit	rfcS	Saci_0907	SSO0768	ST0475
5.	Replication factor C large subunit	rfcL	Saci_0906	SSO0769	ST0473
6.	Molybdenum cofactor biosynthesis protein	moaC	Saci_0905	SSO0770	ST0472
7.	Cell division control protein 6	Cdc6	Saci_0903	SSO0771	ST0471
8.	Hypothetical protein		Saci_0902	SSO0773	ST0470
9.	Hypothetical protein		Saci_0901	SSO0772	ST0469
10.	Mini-chromosome maintenance protein	MCM	Saci_0900	SSO0774	ST0467

4. Resources Identified from *Sulfolobus* Species

In the following section, the identification of two novel enzymatic activities from the acido- and thermophilic archaeon *S. tokodaii* is described. In one example, a novel enzyme that was not predicted from the genomic data was isolated using traditional purification techniques, after which its gene was isolated. In the second example, unexpected novel activity not predicted from the genomic information was detected through activity analysis of proteins heterologously expressed in *E. coli*.

4.1. HEXOSE KINASE

As mentioned, *Sulfolobus* species mainly utilize the nonphosphorylated Entner-Doudoroff pathway to derive energy from glucose (Fig. 1). However, ATP-dependent glucose-phosphorylating activity was detected in the cell extracts of *S. solfataricus* (De Rosa et al., 1984), though the enzyme responsible for the activity was not isolated until much later. An enzyme catalyzing the ATP-dependent glucose phosphorylation was purified from extracts of *S. tokodaii*, after which the N-terminal amino acid sequence was used to determine the gene encoding this enzyme to be ST2354, the original annotation of which was just as a hypothetical protein (Nishimasu et al., 2006). The isolated ATP-dependent hexokinase accepted a broad range of sugar substrates, including glucose, mannose, 2-deoxyglucose, glucosamine, and *N*-acetylglucosamine (Nishimasu et al., 2006). Furthermore, the four resolved crystal structures of this hexokinase showed that large conformational changes are induced by the binding of sugars

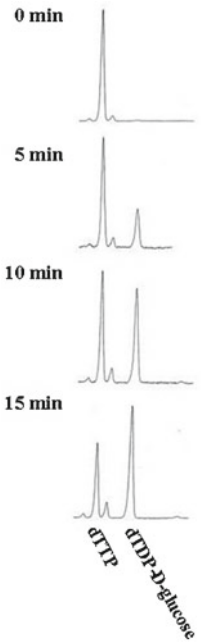

Figure 3. HPLC elution profile of the products of the glucose-1-phosphate nucleotidyltransferase activity. Substrates were incubated with the ST0452 protein for the indicated times at 80 °C. The scale was automatically adjusted depending on the amount of material detected.

to the enzyme (Nishimasu et al., 2007), which may provide an explanation for the enzyme's substrate specificity.

4.2. ST0452 PROTEIN AS A SUGAR-1-PHOSPHATE NUCLEOTIDYLYL-TRANSFERASE AND AN AMINO-SUGAR-1-PHOSPHATE ACETYLTRANSFERASE

The ST0452 gene was annotated as a sugar-1-phosphate nucleotidyltransferase gene based on its similarity to the bacterial enzyme glucose-1-phosphate thymidylyltransferase. However, the ST0452 protein contains a long extra region that is not present in the other similar genes, which encodes a segment at the C-terminus of the ST0452 protein. To characterize the activity of this protein and determine the function of the extra C-terminal region, the ST0452 gene was successfully expressed using a pET vector system in *E. coli* BL21codonplus RIL cells. This system yielded a soluble form of the ST0452 protein, which was stable for 30 min at 80 °C.

Initially, the ability of the ST0452 protein to catalyze the synthesis of nucleotide-sugar molecules from thymidine triphosphate (TTP) and glucose-1-phosphate was examined. As shown in Fig. 3, incubation of TTP and glucose-1-phosphate with the ST0452 protein yielded a nucleotide-sugar product, TDP-glucose, in a

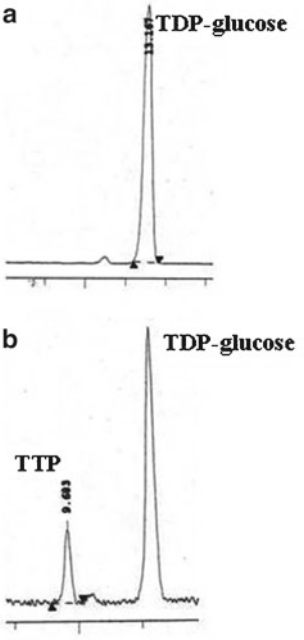

Figure 4. HPLC elution profile of the products of the reverse direction of glucose-1-phosphate nucleotidyltransferase activity of the ST0452 protein. The HPLC elution profiles show the products before (**a**) and after (**b**) 20 min of incubation at 80 °C with the ST0452 protein. The TDP-glucose and PPi were added as substrates for the primary reaction.

time-dependent manner. This confirms that the ST0452 protein possesses glucose-1-phosphate thymidylyltransferase activity. The reverse reaction was also analyzed. As shown in Fig. 4, the ST0452 protein catalyzed the production of TTP using TDP-glucose and pyrophosphate as substrates. Thus the ST0452 protein appears capable of catalyzing both directions of the sugar-1-phosphate nucleotidyltransferase reaction (Zhang et al., 2005).

The substrate specificity of the ST0452 protein is summarized in Table 7. The ST0452 protein was capable of combining all dNTPs with glucose-1-phosphate. Interestingly, the ST0452 protein also catalyzed the synthesis of UDP- or TDP-*N*-acetyl-D-glucosamine from UDP or TTP and *N*-acetyl-D-glucosamine-1-phosphate. Moreover, this activity was three or five times greater than the glucose-1-phosphate thymidylyltransferase activity. This observation suggests the ST0452 protein may catalyze the last reaction in the UDP-*N*-acetyl-D-glucosamine biosynthetic pathway. Therefore, the C-terminal region was carefully searched again, and a motif that repeated 24 times and was originally identified in acyl- and acetyltransferases was detected (Zhang et al., 2010). Because it was expected that the ST0452 protein would also be capable of catalyzing the acetyltransferase reaction for synthesis of *N*-acetyl-D-glucosamine-1-phosphate from glucosamine-1-phosphate, this

Table 7. Substrate specificity of the sugar-1-phosphate nucleotidyltransferase activity of the ST0452 protein.

Substrate A (0.1 mM)	Substrate B (10 mM)	Relative activity
dTTP	α-D-Glucose-1-phosphate	100
dATP		35
dCTP		7
dGTP		1
UTP		130
ATP/CTP/GTP		ND[a]
dTTP	*N*-Acetyl-D-glucosamine-1-phosphate	320
UTP		540

[a]*ND* not detected.

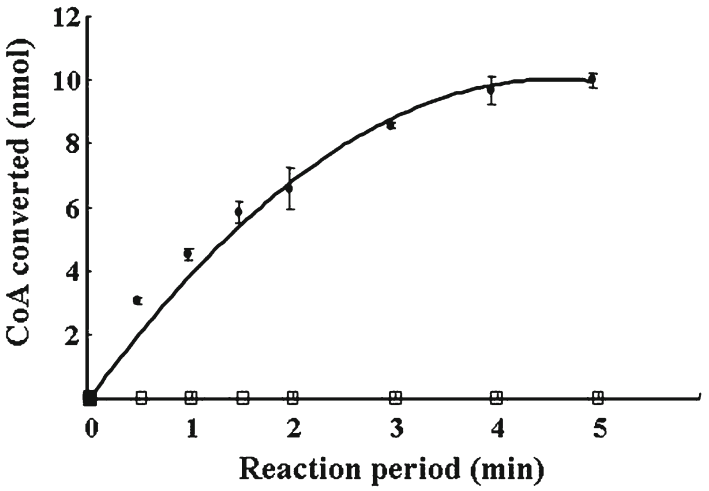

Figure 5 Time course of CoA production catalyzed by the ST0452 protein. Ellman's reaction was used to monitor CoA production. The amount of CoA was calculated from the absorbance measured at 412 nm. The reaction was run at 80 °C in the presence (*closed circles*) or absence (*open squares*) of the ST0452 protein.

acetyltransferase activity was examined. In that reaction, acetyl-CoA usually served as the acetyl group donor, yielding free CoA. Since CoA exhibits absorbance at 412 nm by using Ellman's reaction (Riddles et al., 1983) but acetyl-CoA does not, acetyltransferase activity of the ST0452 protein can be estimated based on the absorbance at 412 nm (Zhang et al., 2010).

As expected, within a reaction mixture containing glucosamine-1-phosphate, acetyl-CoA, and the ST0452 protein, there was a time-dependent increase in the absorbance at 412 nm (Fig. 5), indicating just the release of the acetyl group from acetyl-CoA. However, this observation does not indicate production of the

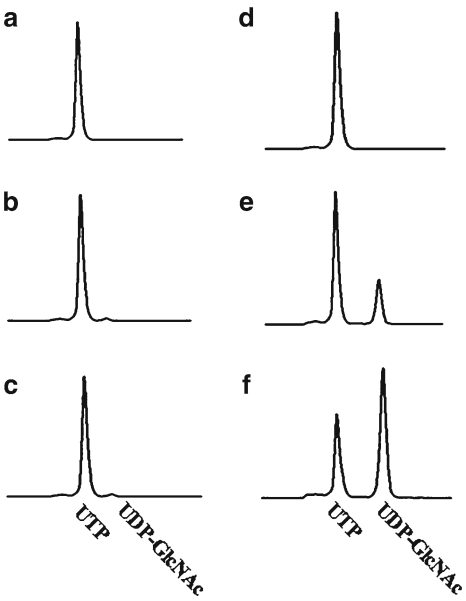

Figure 6. HPLC elution profile of the product of the coupling reaction catalyzed by the ST0452 protein. The products were eluted on a Wakosil 5C18-200 column. The reaction was run at 80 °C with 0.1 mM UTP and 2 mM glucosamine-1-phosphate (**a**–**c**) or with 0.1 mM UTP, 2 mM acetyl-CoA, and 2 mM glucosamine-1-phosphate (**d**–**f**). Shown are the elution patterns for compounds in the reaction mixture before starting the reaction (**a**, **d**) and after running the reaction for 5 min (**b**, **e**) or 10 min (**c**, **f**). The elution positions of standard UTP and UDP-*N*-acetylglucosamine are indicated by "UTP" or "UDP-GlcNAc" under panels **c** and **f**. The scale was automatically adjusted depending on the amount of material detected.

expected product. Thus, the coupling reaction, in which the sugar-1-phosphate nucleotidyltransferase activity of the ST0452 protein was used, was designed to detect the product of the acetyltransferase activity, because the facility of direct detection of modified sugar-1-phosphate was not available in my laboratory. The sugar-1-phosphate nucleotidyltransferase activity of the ST0452 protein was supported by *N*-acetyl-D-glucosamine-1-phosphate, the expected product of the acetyltransferase reaction, but not by glucosamine-1-phosphate, the substrate added into the acetyltransferase reaction mixture. Therefore, the acetyltransferase reaction was run in the presence of UTP. As shown in Fig. 6a–c, when acetyl-CoA was not added to the reaction mixture, only UTP was detected after incubation. However, the final product, UDP-*N*-acetylglucosamine, was detected when the reaction was run with glucosamine-1-phosphate; UTP and acetyl-CoA were added to the reaction mixture (Fig. 6d–f). It thus appears that the ST0452 protein can catalyze transfer of the acetyl group from acetyl-CoA to glucosamine-1-phosphate to produce *N*-acetylglucosamine-1-phosphate (Zhang et al., 2010).

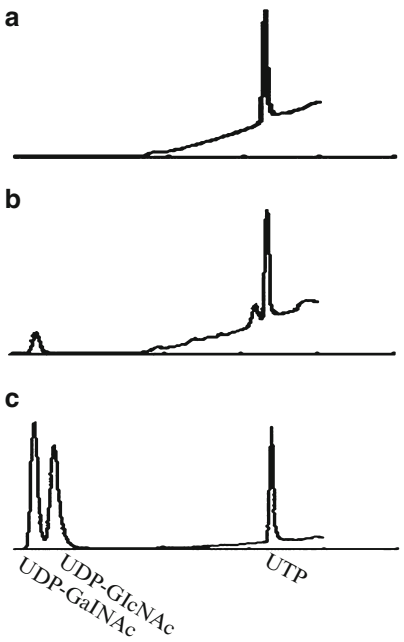

Figure 7. HPLC elution profile of the standard molecules and the product by the activities of the ST0452 protein. Standard UTP, UDP-acetyl-aminosugar, and the products were eluted on a CarboPac PA1 column. The reaction was run at 80 °C for 10 min with 0.1 mM UTP and 2 mM galactosamine-1-P (**a**) or with 0.1 mM UTP, 2 mM acetyl-CoA, and 2 mM galactosamine-1-P (**b**). Elution profile of a mixture of the standard UTP, UDP-*N*-acetylglucosamine, and UDP-*N*-acetylgalactosamine (**c**). Each elution position is indicated by UTP, UDP-GlcNAc, and UDP-GalNAc, respectively.

When the substrate specificity of the acetyltransferase activity of the ST0452 protein was analyzed, increases in absorbance at 412 nm indicating release of the acetyl group were observed upon addition of galactosamine-1-phosphate and acetyl-CoA to the reaction mixture as acetyl acceptor and donor, respectively. The product of this reaction was then analyzed using the same coupling reaction used to detect glucosamine-1-phosphate activity. Only when acetyl-CoA and UTP were added to the reaction mixture, UDP-*N*-acetylgalactosamine, the final product of this reaction was detected (Fig. 7). This confirms that the ST0452 protein is capable of acetylating galactosamine-1-phosphate to *N*-acetyl-D-galactosamine-1-phosphate as well as glucosamine-1-phosphate to *N*-acetyl-D-glucosamine-1-phosphate and of catalyzing the synthesis of UDP-*N*-acetylgalactosamine from *N*-acetyl-D-galactosamine-1-phosphate and UTP. Notably, the detection of the ST0452 protein's galactosamine-1-phosphate acetyltransferase activity was the first discovery of this activity in any organism.

These findings indicate that although enzymatic activity may be predicted from the genomic information based on similarity to known genes, unexpected activity is often hidden behind the predicted activity. It can therefore be said that the genomic data often contains a large number of hidden gems waiting to be discovered by biologists.

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6. References

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