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Seryl-tRNA Synthetases from Methanogenic Archaea: Suppression of Bacterial Amber Mutation and Heterologous Toxicity

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Summary

Methanogenic archaea possess unusual seryl-tRNA synthetases (SerRS), evolutionarily distinct from the SerRSs found in other archaea, eucaryotes and bacteria. Our recent X-ray structural analysis of *Methanosarcina barkeri* SerRS revealed an idiosyncratic N-terminal domain and catalytic zinc ion in the active site. To shed further light on substrate discrimination by methanogenic-type SerRS, we set up to explore *in vivo* the interaction of methanogenic-type SerRSs with their cognate tRNAs in *Escherichia coli* or *Saccharomyces cerevisiae*. The expression of various methanogenic-type SerRSs was toxic for *E. coli*, resulting in the synthesis of erroneous proteins, as revealed by β-galactosidase stability assay. Although SerRSs from methanogenic archaea recognize $tRNAs^{Ser}$ from all three domains of life i*n vitro*, the toxicity presumably precluded the complementation of endogenous SerRS function in both, *E. coli* and *S. cerevisiae*. However, despite the observed toxicity, coexpression of methanogenic-type SerRS with its cognate tRNA suppressed bacterial amber mutation.

Key words: seryl-tRNA synthetase, heterologous toxicity, amber suppression, methanogenic archaea

Introduction

Aminoacyl-tRNA synthetases (AARSs) are essential for the faithful translation of the genetic code. These enzymes ensure the fidelity of protein synthesis by correctly acylating tRNA with its cognate amino acid (*1*). The ability of a heterologous tRNA synthetase to functionally replace an endogenous synthetase requires specificity of aminoacylation and the level of activity necessary to sustain cell growth. Failure to crossaminoacylate cognate tRNAs or, on the other hand, misacylation of noncognate tRNA species may limit the ability of an aminoacyl-tRNA synthetase to function heterologously. In most cases of heterologous toxicity related to aminoacyl-tRNA synthetases (*2*–*5*), the cause of host cell growth inhibition was misaminoacylation of noncognate tRNAs by the heterologously expressed synthetase. Tyrosyl-tRNA synthetase (TyrRS) from *Bacillus stearothermophilus* is toxic for *Escherichia coli*, due to misaminoacylation of noncognate tRNAs, but so is the *E. coli* TyrRS when expressed from the same plasmid (*2*). *Rhizobium meliloti* GluRS exhibits the *in vivo* toxic effect following the expression of *R. meliloti gltX* gene in *E. coli.* Surprisingly, *R. meliloti* GluRS is unable to ligate glutamate to *E. coli* $tRNA^{Glu}$, but is able to ligate it to unfractionated *E. coli* tRNA suggesting that one or several *E. coli* tRNAs are mischarged with glutamate. Since this misacylating activity probably leads to errors in protein biosynthesis, it explains the *in vivo* toxic effect (*3*). Reduced *E. coli* growth, which is observed when *Bacillus subtilis* GluRS is overexpressed in *E. coli*, was due to the mischarging of $tRNA^{Gln}$ with glutamate (*4*). Owning to its ability to misacylate $tRNA^{Asn}$, heterologous overexpression of *Helicobacter pylori* ND-AspRS is toxic to *E. coli* (*5*).

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tRNA recognition by seryl-tRNA synthetases (SerRSs) is of particular interest in methanogenic archaea (*Methanococcales*), due to the existence of highly diverged serine-charging enzymes. N-terminal tRNA-binding domain of methanogenic-type SerRS shares very low sequence homology and structural resemblance with the tRNA-binding domain of the bacterial-type SerRS (*6*). Our biochemical data (*7*,*8*) revealed that both bacterial and eukaryotic $tRNA^{Ser}$ are recognized by the methanogenic-type SerRSs *in vitro*, indicating that both tRNA-binding domains have the same function and similar specificity. This suggests that at least some aspects of tRNA recognition are conserved between the two SerRS types. Both bacterial- (bMbSerRS) and methanogenic-type (mMbSerRS) seryl-tRNA synthetases co-exist in a methanogenic archaeon *Methanosarcina barkeri*. We have recently shown that expression of *M. barkeri* bacterial-type SerRS gene in *E. coli* complements the function of thermolabile SerRS at nonpermissive temperature, while mMbSerRS does not (*9*), which implies that tRNA recognition pattern by two *M. barkeri* SerRSs may differ *in vivo*. However, co-expression of *M. barkeri* seryl-tRNA synthetase gene, encoding either bacterial- or methanogenic-type SerRS, with the gene for cognate archaeal suppressor tRNA leads to suppression of bacterial amber mutations, implying that *E. coli* translation machinery can use serylated tRNA from methanogenic archaea as a substrate in protein synthesis (*9*).

To shed further light on the nature of serylation in methanogenic archaea, we set out to explore the interaction of other methanogenic-type SerRSs with their cognate tRNAs *in vivo*, by attempting to complement the function of *E. coli* or *Saccharomyces cerevisiae* SerRS, and by testing the suppression of bacterial amber mutations by coexpressing methanogenic-type SerRSs with their homologous suppressor tRNAs.

Materials and Methods

Materials

Oligonucleotides were synthesized and DNAs were sequenced by the Keck Foundation Biotechnology Resource Laboratory at Yale University. [^{14}C]serine (160 μCi/mmol) was from Perkin Elmer Life Sciences Inc., Waltham, MA, USA. Restriction enzymes were from New England Biolabs, Ipswich, MA, USA. *E. coli* tRNAs were from Subriden, Washington, DC, USA.

Growth media

Plates for *E. coli* were supplemented with ampicillin (100 μg/mL), chloramphenicol (30 μg/mL), thymine (200 μg/mL), arginine (40 μg/mL) tryptophan (40 μg/mL), IPTG (0.2 g/mL), X-gal (66 μg/mL) and/or arabinose (0.001–0.2 %) as required.

Selection for yeast auxotrophic markers was done in a medium containing 0.67 % nitrogen base and 2 % glucose lacking amino acids, supplemented as needed with adenine (20 mg/mL), uracil (20 mg/mL) and amino acids (20–30 mg/mL).

Strains and plasmids

Strains and plasmids used in this work are listed in Table 1 (*10–16*). Genes for methanogenic-type SerRSs were obtained as described (*7*). *E. coli serS* gene was PCR--amplified and cloned into pBAD24 plasmid. The design

Table 1. Genotype and description of the strains and plasmids used in this study

Strain of *E. coli* used	Description	Reference
KL229	F$^-$ *serS15*(ts) *thyA6 rpsL120*	*10*
JR104	F′ *trpA*(*UAG*)*211*/*glyV55* Δ(*tonB-trpAB*) *argE*(*UAG*) *rpoB*	*11*
XACΔ14	F′ *ara argE*(*UAG*) *rpoB gyrA* Δ*lac pro*/F′ *lacI-Z pro AB*$^+$	*12*
XAC-A24	F′ *ara argE*(*UAG*) *rpoB gyrA* Δ*lac pro*/F′ *lacI*(*UAG*)-*Z proAB*	*12*
Plasmid used in *E. coli*		
pBAD24	contains inducible BAD promoter	*13*
pBADEcSerRS	plasmid pBAD24 containing the gene for *E. coli* SerRS cloned behind BAD promoter	this work
pBADMmSerRS	plasmid pBAD24 containing the gene for *M. maripaludis* SerRS cloned behind BAD promoter	this work
pBADMmC315A	plasmid pBAD24 containing the gene for *M. maripaludis* SerRS C315A cloned behind BAD promoter	this work
pBADMjSerRS	plasmid pBAD24 containing the gene for *M. jannaschii* SerRS cloned behind BAD promoter	this work
pTech	contains constitutive lpp promoter	*14*
pTechsupD	plasmid pTech containing the gene for *E. coli* suppressor $tRNA^{Ser}$ cloned behind lpp promoter	this work
pTechsupSMm	plasmid pTech containing the gene for *M. maripaludis* suppressor $tRNA^{Ser}$ cloned behind lpp promoter	this work
pTechsupSMj	plasmid pTech containing the gene for *M. jannaschii* suppressor $tRNA^{Ser}$ gene cloned behind lpp promoter	this work
Strain of *S. cerevisiae* used		
BR2727ΔSES1	*MAT***a** *ade2–1 arg4–9 his4 leu2–3, 112 lys2 trp1 ura3–1 SES1::LYS2*	*15*
Plasmid used in *S. cerevisiae*		
pVT100-L	multicopy, *ADH* promoter, *LEU-2d* selectable marker	*16*
pVTMmSerRS	plasmid pVT100-L containing the gene for *M. maripaludis* SerRS cloned behind ADH promoter	this work
pVTMjSerRS	plasmid pVT100-L containing the gene for *M. jannaschii* SerRS cloned behind ADH promoter	this work
pVTSES1	plasmid pVT100-L containing the gene for *S. cerevisae* SerRS cloned behind ADH promoter	*15*

of an inactive mutant of *Methanococcus maripaludis* SerRS was carried out by altering Cys315, which according to homology with the *M. barkeri* enzyme, coordinates the active site zinc ion to alanine. Site-directed mutagenesis was carried out by using a QuikChange® mutagenesis kit (Stratagene, La Jolla, CA, USA) as described previously (*17*).

Complementation assay in E. coli

E. coli strain KL229 was transformed with the control plasmid (empty), plasmids carrying genes for *E. coli*, *M. maripaludis* (wild type (wt) or mutant C315A) or *Methanocaldococcus jannaschii* SerRS. To test for complementation, transformants were plated on lysogeny broth (LB) or M9 minimal plates supplemented as indicated and grown overnight at 30, 37 or 42 °C. To test for toxicity, aliquots of overnight cultures of transformants grown in LB supplemented with required antibiotics at 30 °C were serially diluted, and 0.1-mL aliquots of the appropriate dilutions of bacterial culture were plated on LB plates supplemented with 0.2 % arabinose and ampicillin. Surviving cells formed visible colonies during overnight incubation at 30 or 37 °C, and the colonies were counted the next day. Number of colonies was plotted on a logarithmic scale. The values are the means of at least three independent experiments.

Aminoacylation assay

Aminoacylation was done at 30 °C as previously described (*7*) in reaction mixtures containing 50 mM Tris/HCl, pH=7.5, 15 mM $MgCl_2$, 4 mM dithiotreitol, 5 mM ATP and 1 mM ^{14}C-labelled serine. All values represent the average of three independent determinations, which varied by less than 10 %.

Complementation assay in S. cerevisiae

Complementation assay in *S. cerevisiae* was performed by plasmid shuffling as described previously (*15*). The haploid *S. cerevisiae* strain BR2727ΔSES1 (where viability of the cell is ensured by a wild type *SES1* gene on a *URA3*-containing plasmid pUN70*SES1*) was transformed with pVT100-L constructs (which carry the *LEU2*-selectable marker and a SerRS gene behind the *ADH* promoter). pUN70*SES1* was cured by growing double transformants in the presence of uracil, followed by plating the cells on selective plates. Leu^+Lys^+ colonies were replica-plated to 5-fluoroorotic acid-containing medium, which counterselects against colonies containing *URA3* plasmids.

Suppression assay

Genes encoding archaeal suppressor tRNAs were constructed from two overlapping synthetic oligonucleotides and inserted between *Bam*HI and *Pst*I sites of pTech plasmid vector, placing the transcription under control of the *lpp* promoter and the *rrnC* terminator. Their sequences (*18*) were converted to amber suppressor tRNAs by exchanging the sequence of the anticodon (GCT) with the sequence complementary to amber STOP codon (CUA).

Suppression of amber mutations in *E. coli* strains JR104 and XAC-A24 was tested by plating *E. coli* transformed with plasmids carrying genes for archaeal amber suppressor tRNAs (derived from pTech), and where indicated with genes for synthetases on a compatible plasmid (derived from pBAD24), on selective plates. Suppression of *argE* or *trpA* amber mutations in strains JR104 and XAC-A24 was tested by plating *E. coli* cells on selective M9 minimal glucose plates, while LB plates with IPTG and X-gal were used for testing *lacI*(*UAG*)*-lacZ* suppression. The efficiency of suppression was determined in the *E. coli* strain XAC-A24 by measuring the β-galactosidase activity produced from *lacI-lacZ* fusion harbouring a nonsense mutation in *lacI* portion (*19*). The truncated protein resulting from premature termination of protein synthesis at the *lacI* in-frame stop codon is unable to degrade chromogenic 2-nitrophenyl-β-D-galactopyranoside (ONPG), while the suppression of the nonsense mutation results in the synthesis of functional β-galactosidase in *E. coli* (*9*). Suppression is defined as 100 % from the β-galactosidase activity of *E. coli* strain XACΔ14, which contains a *lacI-Z* fusion with no amber mutation. Values are the average of triplicate measurements. Suppression of *argE*(*UAG*) and *lacI*(*UAG*)*-lacZ* is not dependent on the nature of the inserted amino acid (*12*), while the amber mutation at the position 211 in *trpA* can be suppressed with only a limited set of amino acids, including serine (*20*).

Thermal stability test of β-galactosidase

For assaying the thermal stability of β-galactosidase, plasmids pBAD24, pBADEcSerRS, pBADMmSerRS and pBADMmC315A were transformed into *E. coli* strain XACΔ14. Crude protein extracts were prepared by sonication, and proteins were separated from debris by centrifugation. Aliquots were incubated at 56 °C, and tested for β-galactosidase activity as described earlier (*9*,*21*). Values are the means of at least three independent experiments.

Results and Discussion

Heterologous recognition in vivo

In order to test the *in vivo* activity of *M. maripaludis* and *M. jannaschii* SerRS in *E. coli*, we inquired whether they could rescue the *E. coli* strain KL229, which carries a mutated *serS* gene responsible for the temperature-sensitive phenotype (*10*).

As shown in Table 2, we tested transformants for complementation at various temperatures and in different growth media. Furthermore, we varied the level of expression of SerRSs by varying the concentration of arabinose (which induces expression from pBAD plasmid). Complementation by methanogenic-type SerRSs could not be observed under any of those conditions, even though the protein extract from the induced *E. coli* KL229 transformants showed 60-fold higher serylation activity than the extract from noninduced cells (not shown).

In parallel, we tried to complement a null allele strain of *S. cerevisiae via* plasmid shuffling. *S. cerevisiae* strain BR2727ΔSES1 carries a deletion of the gene for cytosolic SerRS, which is rescued by supplying a functional SES1 gene on a centromeric plasmid pUN70. *S. cerevisiae* strain BR2727ΔSES1 was cotransformed with plasmids which

Table 2. Growth of *E. coli* KL229 transformants on various media

Plates/Amp (100 μg/mL)	*E. coli* KL229 cells transformed with											
	Control			MmSerRS			MjSerRS			EcSerRS		
Temperature/°C	30	37	42	30	37	42	30	37	42	30	37	42
LB+0.2 % arabinose	+++	++	–	++	–	–	++	–	–	+++	+++	+++
M9 glucose	+++	–	–	++	–	–	++	–	–	+++	–	–
M9 glucose+0.2 % arabinose	+++	–	–	–	–	–	–	–	–	+++	++	++
M9 glycerol	++	–	–	++	–	–	++	–	–	++	–	–
M9 glycerol+0.001 % arabinose	++	–	–	+	–	–	+	–	–	++	+	+
M9 glycerol+0.1 % arabinose	++	–	–	–	–	–	–	–	–	++	++	++
M9+0.2 % arabinose	++	–	–	–	–	–	–	–	–	++	++	++

Cells were tested for growth at permissive (30 °C), semipermissive (37 °C) and nonpermisive temperature (42 °C) on LB or M9 minimal plates supplemented as indicated. Cell growth is indicated as very good (+++), good (++), weak (+), or no growth (–)

carry the *LEU2-d* selectable marker and SerRS genes from *M. maripaludis*, *M. jannaschii* or *S. cerevisiae* behind the *ADH* promoter. Double transformants were grown in the presence of uracil and colonies were then replica plated to medium containing 5-fluoroorotic acid. This excludes the presence of *URA3*-containing plasmid with the wild type *S. cerevisiae SES1* gene. We could not select viable haploids carrying archaeal genes as the only source of seryl-tRNA synthetase activity, in contrast to haploids carrying the *SES1* gene on the same plasmid, which served as a positive control (not shown). Thus, seryl-tRNA synthetases from methanogenic archaea did not complement the function of *SES1* gene.

It may be that the lack of complementation is caused by toxic effects (*2,3*) or by the inability of methanogenic-type SerRSs to serylate all $tRNA^{Ser}$ isoacceptors in *E. coli* or in yeast.

Expression of methanogenic-type SerRSs is toxic for E. coli cells

In order to test the toxic hypothesis we performed serial dilutions of overnight cultures of *E. coli* KL229 transformants, which were incubated on LB plates overnight at 30 or 37 °C (Fig. 1). To confirm that active mMbSerRS is responsible for the toxic effect, an inactive synthetase mutant, bearing an altered Cys315, was constructed and assayed *in vivo*. Cys315, according to the homology with the *M. barkeri* enzyme, coordinates the active site zinc ion, required for binding the serine substrate. Impaired growth was observed for *E. coli* KL229 cells carrying a plasmid encoding methanogenic-type SerRS, but not for cells carrying the plasmid with the inactive mutant or the *E. coli* SerRS (Fig. 1). This toxic effect was not observed in the absence of arabinose and was more pronounced at elevated temperatures (Table 2, Fig. 1).

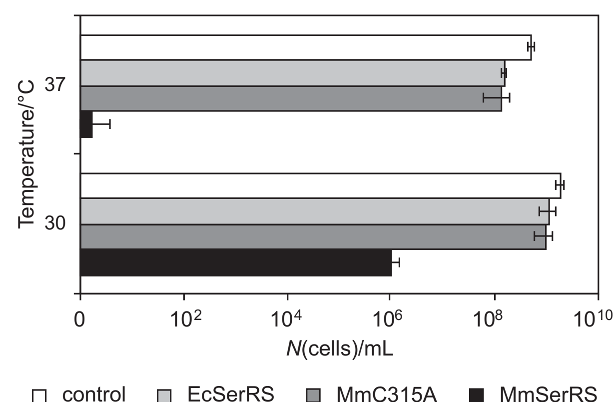

Fig. 1. Toxicity of methanogenic-type SerRS in *E. coli* strain KL229. Number of colonies after transformation and induction with arabinose was plotted on a logarithmic scale. The values are the means of at least three independent experiments

To further examine the observed toxicity, we measured the growth rate of different *E. coli* strains transformed with the plasmids mentioned above. The expression from the plasmid carrying the gene for SerRS from *M. maripaludis* (pBADMmSerRS) impairs the growth of *E. coli* strains XAC-A24, XACΔ14 and KL229, while the expression from the plasmid carrying the gene for SerRS from *E. coli* (pBADEcSerRS), plasmid carrying the gene for the inactive mutant of *M. maripaludis* SerRS (pBADC-315A) or empty plasmid (pBAD24) have no such effect (not shown). The observed toxicity (Table 2, Fig. 1) of heterologously expressed methanogenic-type enzymes might obscure the expected interaction of methanogenic-type SerRSs with bacterial or eukaryotic $tRNAs^{Ser}$, and thus preclude the detection of complementation.

The cause of host cell growth inhibition may be misaminoacylation of noncognate tRNAs by the heterologously expressed synthetase. In this case, the most likely targets for misaminoacylation are *E. coli* $tRNA^{Tyr}$ and $tRNA^{Leu}$, which contain a long extra arm (one of the major identity elements of $tRNA^{Ser}$). Our preliminary results show that the misaminoacylation of *E. coli* $tRNA^{Tyr}$ with serine is higher by the methanogenic-type SerRS than by the *E. coli* synthetase (Fig. 2). If such misaminoacylation were to happen in the *E. coli* cell, the incorporation of serine in place of tyrosine by the misacylated $tRNA^{Tyr}$ would result in the synthesis of erroneous proteins, and thus in cell growth inhibition.

Decrease in thermal stability of β-galactosidase

To determine whether the expression of methanogenic-type SerRSs affects translation and therefore alters structural properties of cellular proteins, we assayed the thermal stability of a test protein (β-galactosidase) as described (*2*). This assay is a good indicator of errors in translation. Strain XACΔ14 was transformed with pBAD24,

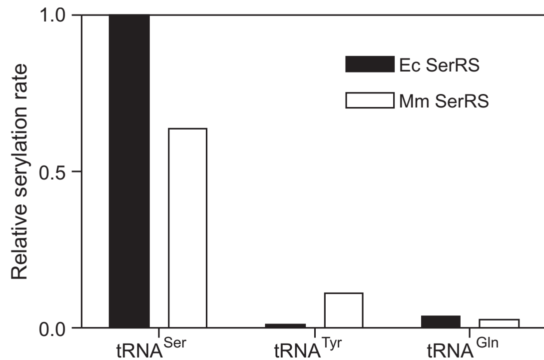

Fig. 2. Relative serylation rate of cognate and non-cognate tRNAs by SerRSs from *E. coli* (filled bars) and *M. maripaludis* (empty bars)

and pBAD24 plasmids carrying genes for *E. coli* SerRS, *M. maripaludis* SerRS and the inactive mutant of Mm-SerRS. Aliquots of crude protein extracts were incubated at 56 °C, and tested for β-galactosidase activity (Fig. 3). The decrease in thermal stability of β-galactosidase was more pronounced in the strain expressing *M. maripaludis* SerRS, than in the control strains (strain expressing *E. coli* SerRS, inactive mutant of *M. maripaludis* SerRS and the strain with the empty plasmid) (Fig. 3).

Thermal stability of a protein is compromised when there are errors in translation – incorrectly incorporated amino acids challenge the proper folding of a protein. Such errors may be the result of amino acid starvation, mischarging in the aminoacylation reaction (*2,3,5*), or depletion of certain tRNA isoacceptor species (*2,5*). In our system there is no decrease in thermal stability when *E. coli* SerRS is overexpressed, so we can rule out amino acid starvation. We are left with two possible explanations of toxicity and decreased thermal stability of β-galactosidase: misincorporation of amino acids into proteins mediated by erroneously aminoacylated tRNAs, or depletion of certain tRNA isoacceptor species caused by the unproductive interactions with methanogenic-type SerRS. Toxicity caused by overexpression of a heterologous protein is ruled out by the observation that there is no

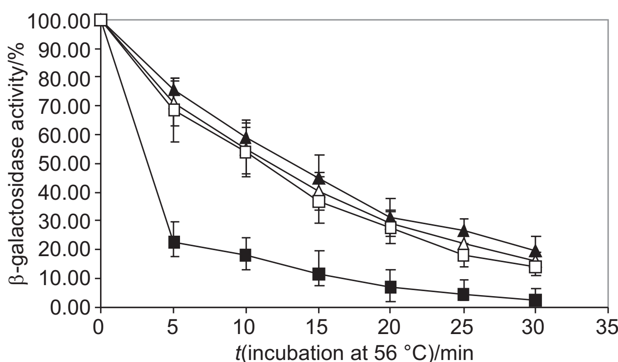

Fig. 3. Decrease in the thermal stability of β-galactosidase from strain XACΔ14 transformed with the empty plasmid pBAD24 (△), and pBAD24 plasmids carrying SerRSs genes from *E. coli* (▲), *M. maripaludis* (■) and the inactive mutant of *M. maripaludis* SerRS (□). Samples were incubated at 56 °C, and assayed for residual β-galactosidase activity in time intervals of 5 min. The values are the means of at least three independent experiments

decrease in the thermal stability of β-galactosidase when the inactive mutant of *M. maripaludis* SerRS is expressed from the same plasmid.

Suppression of E. coli amber mutations by archaeal tRNAs^{Ser}

Suppression of bacterial amber mutations is a well documented system for studying the ability of some aminoacyl-tRNA synthetases to recognize their cognate tRNAs *in vivo* (*9,22–24*). Bacterial strains JR104 and XAC-A24 (Table 1) carry several amber mutations, suppression of which may reflect recognition and aminoacylation levels of suppressor tRNA by selected aminoacyl-tRNA synthetases. Both strains have *argE*(*UAG*) mutation suppressible by any amino acid. Amber mutation in strain JR104, at the position 211 in *trpA* gene, can be suppressed with only a limited set of amino acids, including serine (*20*). The other suppressible marker in strain XAC-A24, in which an UAG in-frame codon has been inserted in the *lacI* part of a *lacI-lacZ* fusion gene, was used for quantification of suppression efficiency (*24–27*). Serine-specific tRNAs are especially suitable to be used in such assays, since in contrast to many other tRNAs, the anticodon is not a recognition element for interaction with the cognate synthetase, and consequently its alteration does not change the tRNA identity (*28*). Genes encoding suppressor tRNAs based on serine-specific tRNA genes from *M. maripaludis* and *M. jannaschii* were introduced into two *E. coli* strains bearing amber mutations (JR104 and XAC-A24). Suppression was checked by streaking the transformants on selective plates [M9 glucose for *argE*(*UAG*) or *trpE*(*UAG*) and LB supplemented with IPTG and X-gal for *lacI*(*UAG*)-*-lacZ*] supplemented with required antibiotics. No suppression of any amber mutation was observed by expressing solely archaeal tRNAs, showing that they are not efficiently recognized by the *E. coli* seryl-tRNA synthetase *in vivo*, which is in accordance with our *in vitro* results (*7*) (Fig. 4). In the second experiment the tested strains were cotransformed with a pair of compatible plasmids, the first of which carried the gene for a methanogenic--type synthetase, while the second carried the suppressor tRNA gene. Surprisingly, no suppression of *argE*(*UAG*) and *trpE*(*UAG*) amber mutations was detected (not shown). Suppression of *lacI*(*UAG*)-*lacZ* mutation, by the archaeal tRNA/synthetase pair, was observed on LB plates supplemented with X-gal and IPTG at 30 °C (Fig. 4a), but could not be reproduced on minimal plates (not shown), presumably due to more stringent growth conditions. No suppression was obtained when the MmSerRS variant, bearing an altered zinc ligand Cys315, was coexpressed with archaeal suppressor tRNAs (Fig. 4a), confirming that active archaeal seryl-tRNA synthetases are needed for efficient tRNA aminoacylation and suppression.

Suppression levels were quantitated by assaying β--galactosidase activity produced in strain XAC-A24 from the suppressed *lacI*(*UAG*)-*lacZ* gene by the archaeal Ser--tRNA^{Ser} species. The suppressor efficiencies of archaeal tRNAs charged with the methanogenic-type SerRSs in *E. coli* were poor but consistent (Fig. 4b). Importantly, Figs. 4a and b show that the interaction between archaeal SerRS and tRNA^{Ser} can be detected in *E. coli*, albeit with

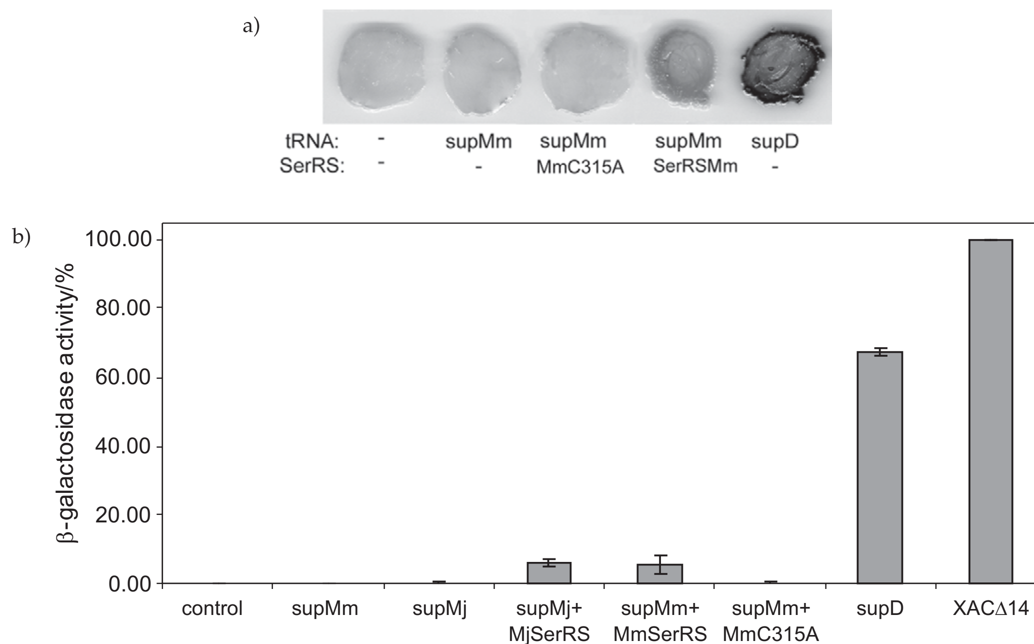

Fig. 4. Suppression of *E. coli* amber mutations by archaeal tRNAs^{Ser} in *E. coli* strain XAC-A24: a) suppression of *lacI*(*UAG*)-*lacZ* was checked by streaking the transformants on LB supplemented with arabinose, X-gal, IPTG and required antibiotics and incubated at 30 °C for 20 h. Activity of β-galactosidase (intensity of blue colour) indicates suppression of amber mutation; (b) suppression efficiency measured by assaying β-galactosidase activity. Values are given as percentages of that for the wild type *lacI-lacZ* fusion strain (XACΔ14), which gives 309 U of β-galactosidase under the same conditions. The values are the means of at least three independent experiments

some restrictions. Coexpression of homologous tRNAs with methanogenic-type SerRSs may overcome the toxicity by decreasing the extent of misaminoacylation. When the archaeal tRNA^{Ser} is expressed in the *E. coli* cell, it competes with the *E. coli* tRNA^{Tyr} for binding to the methanogenic SerRS, thus decreasing the amount of faulty Ser-tRNA^{Tyr}. Suppression was observed only under the conditions of weaker toxicity, *i.e.* in rich media and at lower temperatures.

Besides toxicity, low levels of suppression could be due to the inefficient recognition of archaeal suppressor tRNAs by bacterial elongation factor Tu or the ribosome.

Conclusion

All tested archaeal methanogenic-type SerRSs aminoacylated *E. coli* and *S. cerevisiae* tRNA *in vitro*, but could not complement the function of the respective SerRS genes *in vivo* (this work and *9*). *Methanococcus maripaludis* and *Methanocaldococcus jannaschii* methanogenic-type SerRSs investigated in this work exhibited barely detectible suppression efficiencies (<6 %), as opposed to *Methanosarcina barkeri* methanogenic-type SerRS (32.18 %). The two *M. barkeri* SerRSs (methanogenic- and bacterial-type) that coexist in the cell have the similar tRNA recognition pattern *in vitro* (*29*), but not *in vivo* (*9*). These two SerRSs may have been forced to a tRNA-driven alteration of the identity requirements to recognize the archaeal tRNA substrate in a highly specific manner (*29*). It may be assumed that *M. barkeri* mMbSerRS may have adopted a tRNA recognition pattern that differs from its methanogenic-type counterparts, thus mollifying the toxicity and enabling it to express its full serylation potential in the bacterial cell.

E. coli can tolerate mischarged tRNAs to some extent, but it tends to undergo abnormal protein synthesis when an excessive amount of mischarged tRNA is recognized by elongation factors (*14*). This abnormal protein synthesis is uniformly fatal for the host cell. We have shown that archaeal methanogenic-type seryl-tRNA synthetases are toxic when expressed in *E. coli*. There are two ways that methanogenic-type SerRS can produce mischarged tRNAs: by charging tRNA^{Ser} with a noncognate amino acid, or by recognizing noncognate tRNAs and charging them with serine. The methanogenic-type enzyme misacylates *E. coli* tRNA^{Tyr} with serine (this work), and also misactivates threonine to some extent *in vitro* (*6*). The promiscuity of the methanogenic-type enzyme towards both the tRNA and the amino acid may act synergistically, thus causing translational errors upon heterologous expression of these unusual synthetases. On the other hand, methanogenic-type SerRSs might harbour some other function which could be deleterious for the bacterial cell. Further work on understanding the causes of this toxic effect will provide us with more information on these unusual enzymes from methanogenic archaea.

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