# Two forms of Tyrosyl-tRNA Synthetase from *Pseudomonas aeruginosa*: Characterization and Discovery of Inhibitory Compounds

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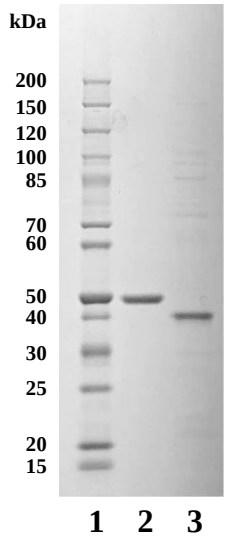
## Introduction

The genes encoding both forms of TyrRS (TyrRS-Z and TyrRS-S) from *P. aeruginosa* were cloned, the resulting over-expressed proteins were purified, and the kinetic parameters ($K_M$, $V_{max}$ and $k_{cat}$) governing the interaction with ATP, Tyr, and $tRNA^{Tyr}$ were determined for both enzymes. A high-throughput screening platform was then developed and optimized to screen for potential inhibitors of both forms of TyrRS activity using scintillation proximity assay (SPA) technology. Out of ~2000 chemical compounds, four compounds were identified that inhibited the activity of *P. aeruginosa* TyrRS (BCD37H06, BCD38C11, BCD49D09, and BCD54B04). All four of the compounds inhibited the activity of TyrRS-S, but only one inhibited the activity of TyrRS-Z. All four compounds were characterized for their effect on enzymatic activity, bacterial growth, and human cell cultures as well as assessing their effect on ATP and Tyr binding.

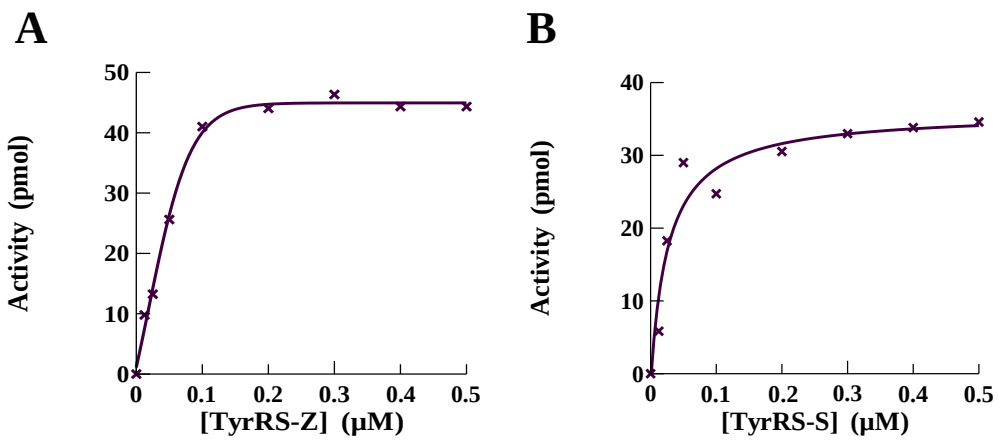
**Results**

The gene (*tyr*Z and *tyr*S) encoding the two forms of tyrosyl-tRNA synthetase from *P. aeruginosa* were cloned, expressed and purified as described in "Material and Methods". The purified proteins was greater than 95 % homogeneous as visualized by SDS-PAGE (Suppl. Fig. S1).




**Supplemental Figure S1. Purification of *P. aeruginosa* TyrRS-Z and TyrRS-S.** Both forms of purified *P. aeruginosa* TyrRS was analyzed on a 4-20% SDS-PAGE gel and the protein bands were visualized by staining with Coomassie blue. Lane #1, contains 10-200 kDa protein standard; lane #2 contains purified *P. aeruginosa* TyrRS-Z; and lane #3 contains purified *P. aeruginosa* TyrRS-S.

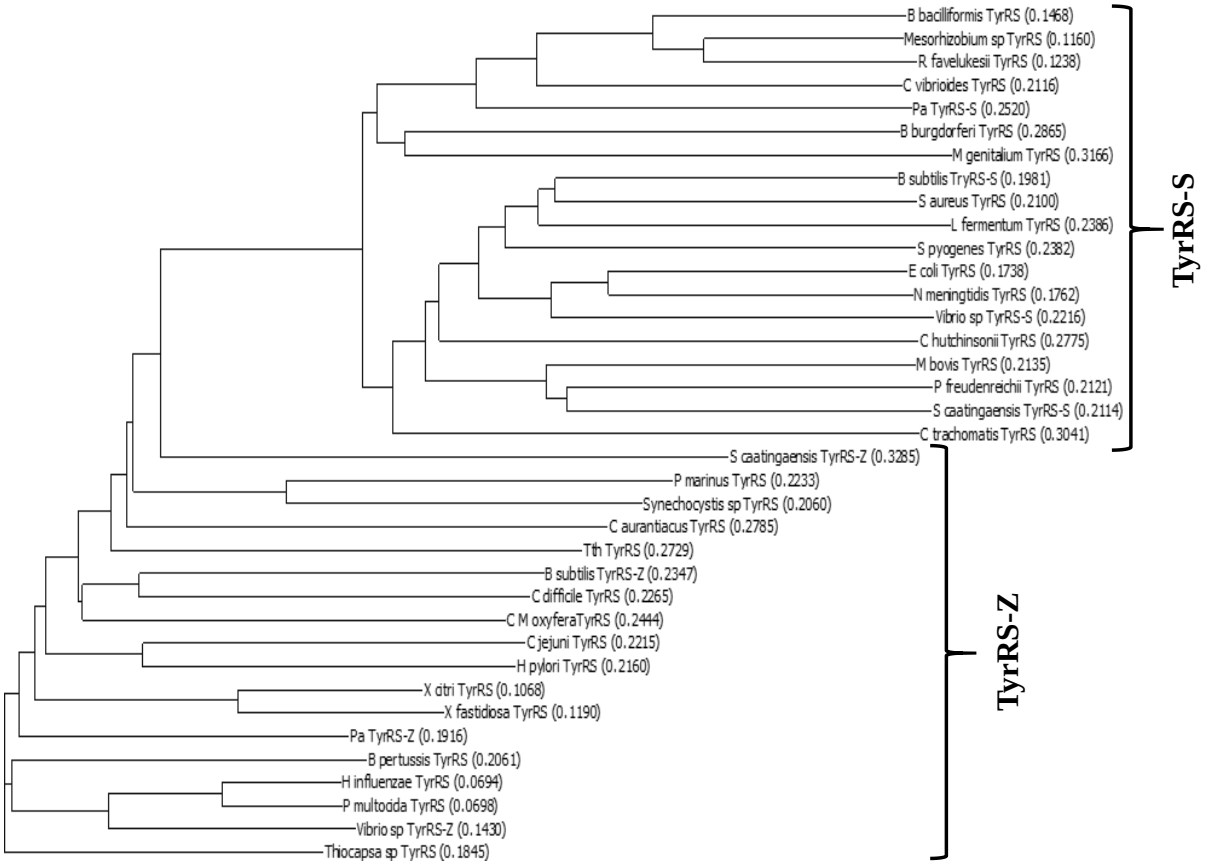
To determine the ability of the two forms of TyrRS to aminoacylate cognate tRNA, each of the purified enzymes was titrated into aminoacylation assays at varying concentrations (Fig. S2).



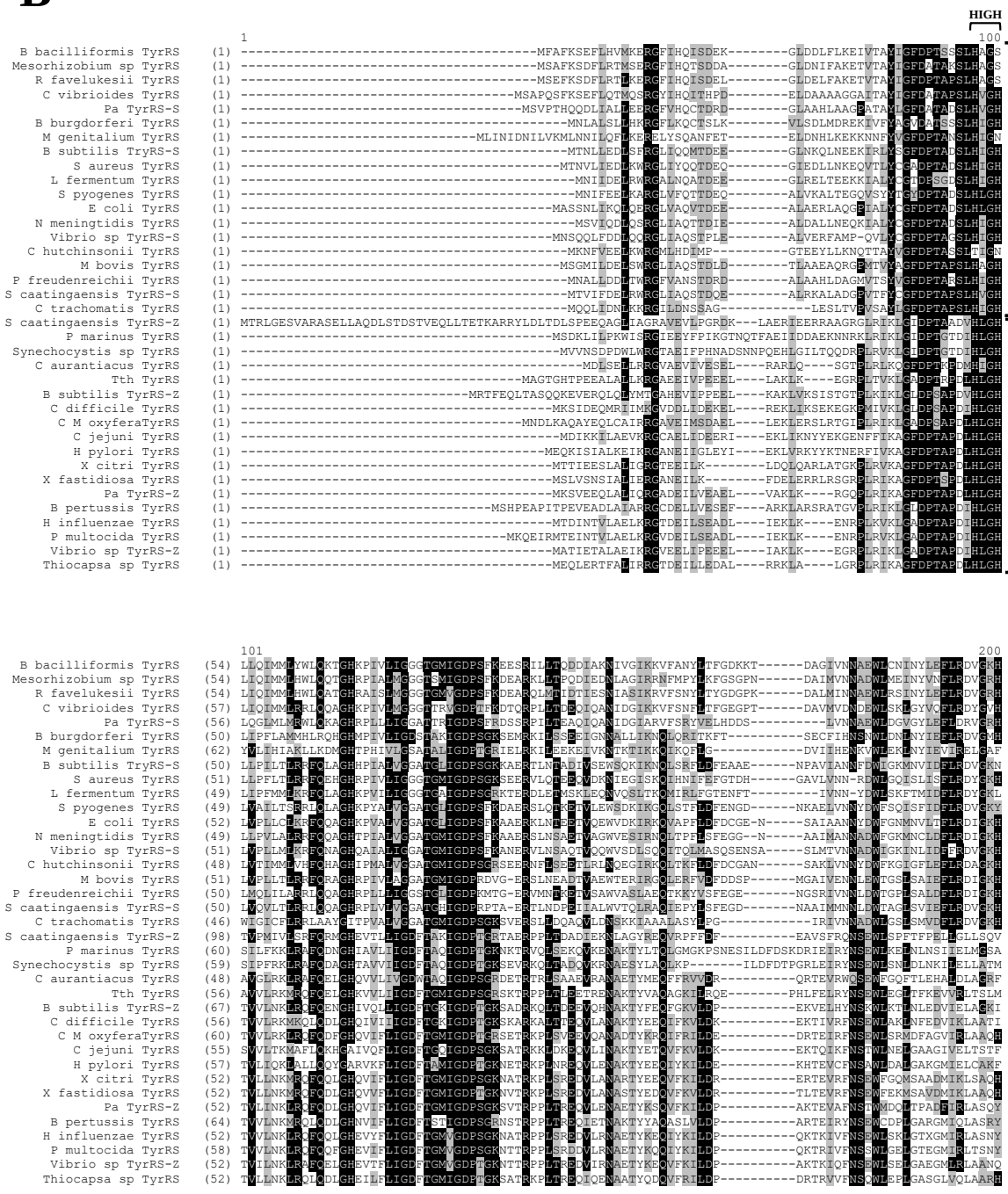

**Supplemental Figure S2. Determination of the activity of *P. aeruginosa* TyrRS-Z and TyrRS-S.** *P. aeruginosa* (**A**) TyrRS-Z and (**B**) TyrRS-S was titrated into the aminoacylation assay as described in the Methods and Materials in amounts varying from 0.0125 to 0.5 µM enzyme. Background activity was minimal and was subtracted from values at all concentrations of TyrRS.

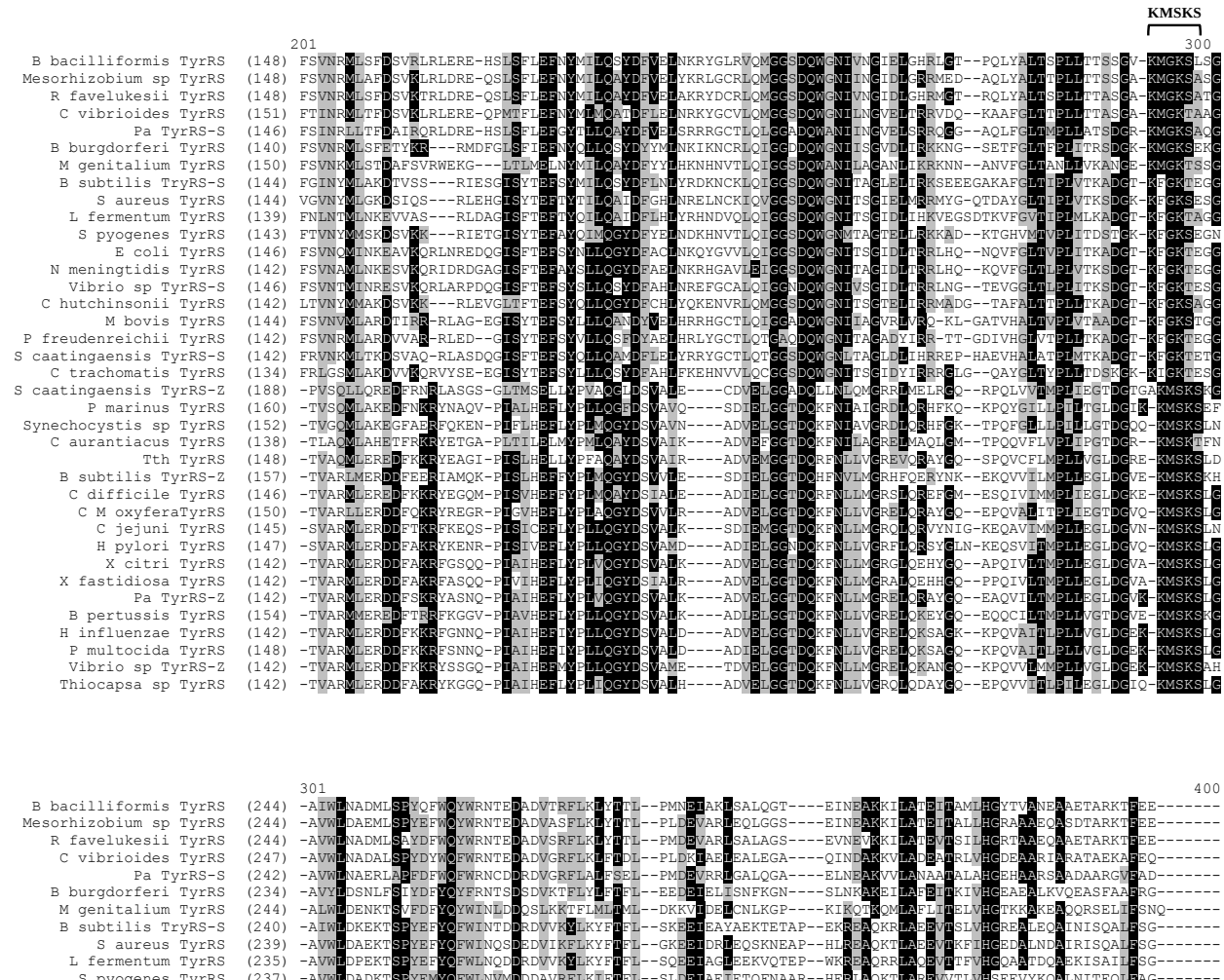
The bacteria containing one or the other TyrRS are not split along taxonomic lines and members of various taxa can contain either of the proteins. An alignment of thirty-seven proteins from various eubacteria resulted in two distinct grouping in the phylogenic tree (Fig. S3A). The gross alignment of these proteins show that although there is an overall conservation of certain amino acids, there is also subtle variations between the TyrRS-Z and the TyrRS-S proteins (Fig. S3B).

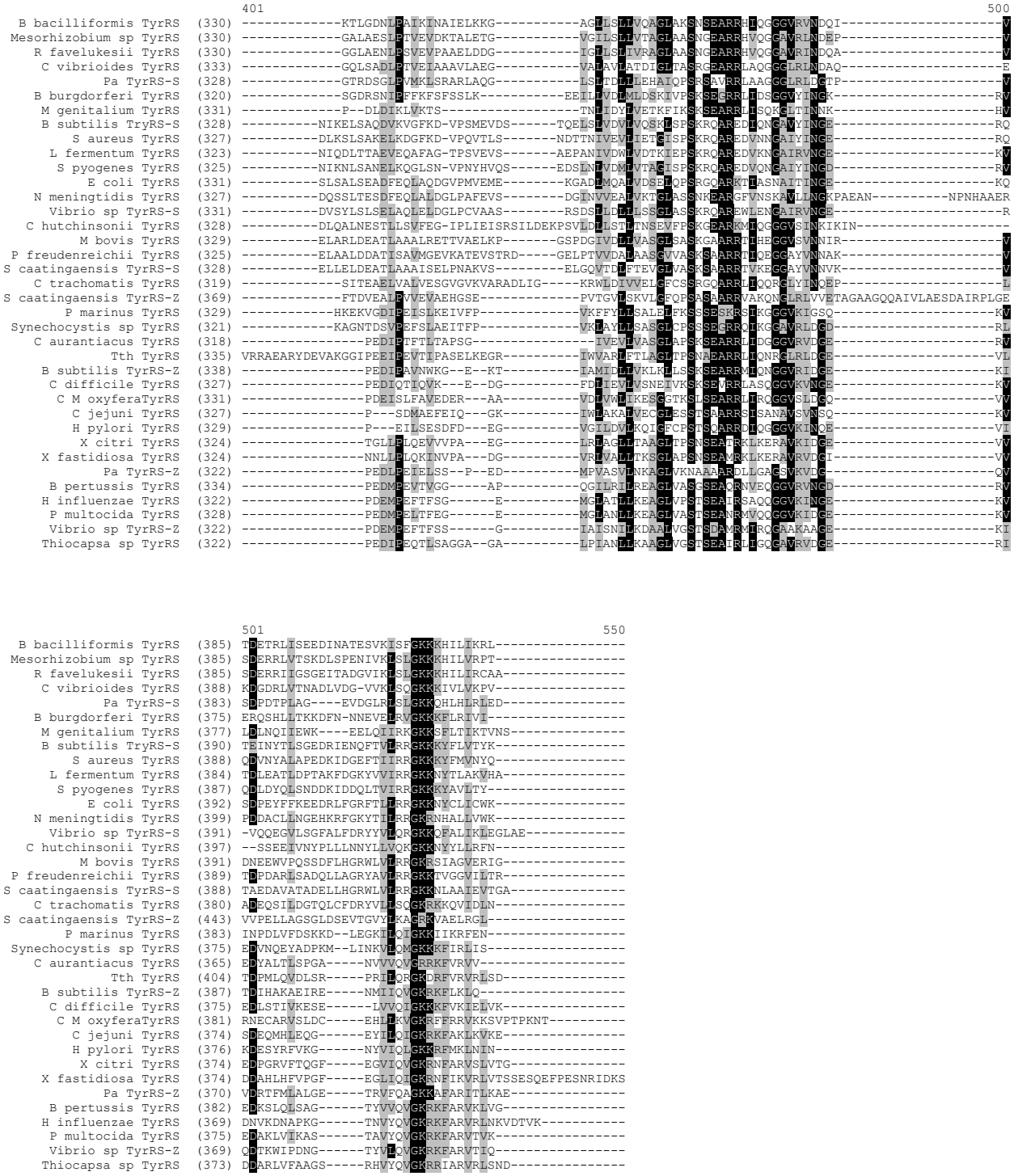
**A**


B bacilliformis TyrRS (0.1468)
Mesorhizobium sp TyrRS (0.1160)
R favelukesii TyrRS (0.1238)
C vibrioides TyrRS (0.2116)
Pa TyrRS-S (0.2520)
B burgdorferi TyrRS (0.2865)
M genitalium TyrRS (0.3166)
B subtilis TryRS-S (0.1981)
S aureus TyrRS (0.2100)
L fermentum TyrRS (0.2386)
S pyogenes TyrRS (0.2382)
E coli TyrRS (0.1738)
N meningtidis TyrRS (0.1762)
Vibrio sp TyrRS-S (0.2216)
C hutchinsonii TyrRS (0.2775)
M bovis TyrRS (0.2135)
P freudenreichii TyrRS (0.2121)
S caatingaensis TyrRS-S (0.2114)
C trachomatis TyrRS (0.3041)
TyrRS-S
S caatingaensis TyrRS-Z (0.3285)
P marinus TyrRS (0.2233)
Synechocystis sp TyrRS (0.2060)
C aurantiacus TyrRS (0.2785)
Tth TyrRS (0.2729)
B subtilis TyrRS-Z (0.2347)
C difficile TyrRS (0.2265)
C M oxyferaTyrRS (0.2444)
C jejuni TyrRS (0.2215)
H pylori TyrRS (0.2160)
X citri TyrRS (0.1068)
X fastidiosa TyrRS (0.1190)
Pa TyrRS-Z (0.1916)
B pertussis TyrRS (0.2061)
H influenzae TyrRS (0.0694)
P multocida TyrRS (0.0698)
Vibrio sp TyrRS-Z (0.1430)
Thiocapsa sp TyrRS (0.1845)
TyrRS-Z

# B


HIGH
1
100
101
200
TyrRS-S
TyrRS-Z
B bacilliformis TyrRS
Mesorhizobium sp TyrRS
R favelukesii TyrRS
C vibrioides TyrRS
Pa TyrRS-S
B burgdorferi TyrRS
M genitalium TyrRS
B subtilis TryRS-S
S aureus TyrRS
L fermentum TyrRS
S pyogenes TyrRS
E coli TyrRS
N meningtidis TyrRS
Vibrio sp TyrRS-S
C hutchinsonii TyrRS
M bovis TyrRS
P freudenreichii TyrRS
S caatingaensis TyrRS-S
C trachomatis TyrRS
S caatingaensis TyrRS-Z
P marinus TyrRS
Synechocystis sp TyrRS
C aurantiacus TyrRS
Tth TyrRS
B subtilis TyrRS-Z
C difficile TyrRS
C M oxyferaTyrRS
C jejuni TyrRS
H pylori TyrRS
X citri TyrRS
X fastidiosa TyrRS
Pa TyrRS-Z
B pertussis TyrRS
H influenzae TyrRS
P multocida TyrRS
Vibrio sp TyrRS-Z
Thiocapsa sp TyrRS

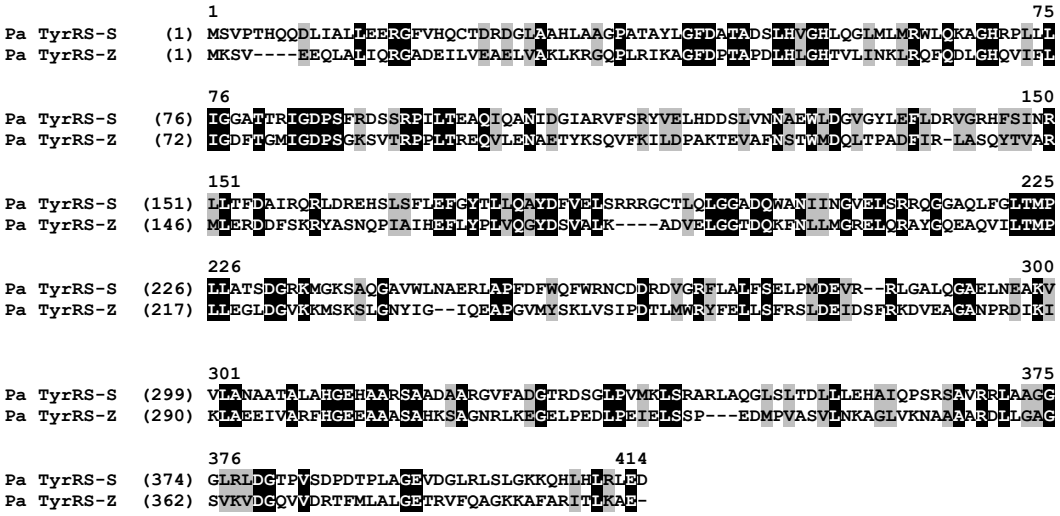

KMSKS
201
300
B bacilliformis TyrRS (148) FSVNRMLSFDSVRLRLERE-HSLSFLEFNYMILQSYDFVELNKRYGLRVQMGGSDQWGNIVNGIELGHRLGT--PQLYALTSPLLTTSSGV-KMGKSLSG
Mesorhizobium sp TyrRS (148) FSVNRMLAFDSVKLRLDRE-QSLSFLEFNYMILQAYDFVELYKRLGCRLQMGGSDQWGNIINGIDLIRRMED--AQLYALTTPLLTTSSGA-KMGKSASG
R favelukesii TyrRS (148) FSVNRMLSFDSVKTRLDRE-QSLSFLEFNYMILQAYDFVELAKRYDCRLQMGGSDQWGNIVNGIDLGHRMGT--RQLYALTSPLLTTASGA-KMGKSATG
C vibrioides TyrRS (151) FTINRMLTFDSVKLRLERE-QPMTFLEFNYMLMQAIDFLELNRKYGCVLQMGGSDQWGNILNGVELTRRVDQ--KAAFGLTTPLLTTASGA-KMGKTAAG
Pa TyrRS-S (146) FSINRLLTFDAIRQRLDRE-HSLSFLEFGVTLLQAYDFVELSRRRGCTLQLGGADQWANIINGVELSRRQGG--AQLFGLTVPLLATSDGR-KMGKSAQG
B burgdorferi TyrRS (140) FSVNRMLSFETYKR---RMDFGLSFIEFNYQLLQSYDYYMLNKIKNCRLQIGGDDQWGNIISGVDLIRKKNG--SETFGLTFPLLTRSDGK-KMGKSEKG
M genitalium TyrRS (150) FSVNKMLSTDAFSVRWEKG---LTLMELNYMILQAYDFYYLHKNHNVTLQIGGSDQWANILAGANLIKRKNN--ANVFGLTANLLVKANGE-KMGKTSSG
B subtilis TryRS-S (144) FGINYMLAKDTVSS---RIESGISYTEFSYMILQSYDFLNLYRDKNCKLQIGGSDQWGNITAGLELIRKSEEEGAKAFGLTIPLVTKADGT-KFGKTEGG
S aureus TyrRS (144) VGVNYMLGKDSIQS---RLEHGISYTEFTYTILQAIDFGHLNRELNCKIQVGGSDQWGNITSGIELMRRMYG-QTDAYGLTIPLVTKSDGK-KFGKSESG
L fermentum TyrRS (139) FNLNTMLNKEVVAS---RLDAGISFTEFTYQILQAIDFLHLYRHNDVQLQIGGSDQWGNITSGIDLIHKVEGSDTKVFGVTIPLMLKADGT-KFGKTAGG
S pyogenes TyrRS (143) FTVNYMMSKDSVKK---RIETGISYTEFAYQIMQGYDFYELNDKHNVTLQIGGSDQWGNMTAGTELIRKKAD--KTGHVMTVPLITDSTGK-KFGKSEGN
E coli TyrRS (146) FSVNQMINKEAVKQRLNREDQGISFTEFSYNLLQGYDFACLNKQYGVVLQIGGSDQWGNITSGIDLTRRLHQ--NQVFGLTVPLITKADGT-KFGKTEGG
N meningtidis TyrRS (142) FSVNAMLNKESVKQRIDRDGAGISFTEFAYSLLQGYDFAELNKRHGAVLQIGGSDQWGNITAGIDLIRRLHQ--KQVFGLTLPLVTKSDGT-KFGKTEGG
Vibrio sp TyrRS-S (146) FSVNTMINRESVKQRLARPDQGISFTEFSYSLLQSYDFAHLNREFGCALQIGGNDQWGNIVSGIDLIRRLNG--TEVGLTLPLITKSDGT-KFGKTESG
C hutchinsonii TyrRS (142) LTVNYMMAKDSVKK---RLEVGLTFTEFSYQLLQGYDFCHLYQKENVRLQMGGSDQWGNITSGTELIRRMADG--TAFALTTPLITKADGT-KFGKSAGG
M bovis TyrRS (144) FSVNVMLARDTIRR-RLAG-EGISYTEFSYLLLQANDYVELHRRHGCTLQIGGADQWGNITAGVRLVRQ-KL-GATVHALTVPLVTAADGT-KFGKSTGG
P freudenreichii TyrRS (142) FSVNRMLARDVVAR-RLED--GISYTEFSYVLLQSDYAELHRLYGCTLQTGAQDQWGNITAGADYIRR-TT-GDIVHGLVTPLITKADGT-KFGKTEGG
S caatingaensis TyrRS-S (142) FRVNKMLTKDSVAQ-RLASDQGISFTEFSYQLLQAMDFLELYRRYGCTLQTGGSDQWGNLTAGLDLIHRREP-HAEVHALATPLMTKADGT-KFGKTETG
C trachomatis TyrRS (134) FRLGSMLAKDVVKQRVYSE-EGISYTEFSYLLLQSYDFAHLFKEHNVVLQCGGSDQWGNITSGIDYIRRRGLG--QAYGLTYPLITDSKGK-KIGKTESG
S caatingaensis TyrRS-Z (188) -PVSQLLQREDFRNRLASGS-GLTMSPLLVPVAQGLDSVALE----CDVELGGADQLLNLQMGRRLMELRGQ--RPQLVVTMPLIEGTDGTGAKMSKSKG
P marinus TyrRS (160) -TVSQMLAKEDFNKRYNAQV-PIALHEFLVPLLQGYDSVAVQ----SDIELGGTDQKFNIAIGRDLQRHFKQ--KPQYGLLLPILTGLDGRK-KMSKSEF
Synechocystis sp TyrRS (152) -TVGQMLAKEGFAERFQKEN-PTFLHEFLVPLMQGYDSVAVN----ADVELGGTDQKFNIAVGRDLQRHFGK--TPQFGLLLPILTGTDGQQ-KMSKSLN
C aurantiacus TyrRS (138) -TLAQMLAHETFRKRYETGA-PLTILPLMVPMLQAYDSVAIK----ADVEFGGTDQKFNILAGRELMAQLGM--TPQQVFLVPLIPGTDGR--KMSKTFN
Tth TyrRS (148) -TVAQMLEREDFKKRYEAGI-PISLHPLLVPFAQAYDSVAIR----ADVEMGGTDQRFNLLVGREVQRAYGQ--SPQVCFLMPLVGLDGRE-KMSKSLD
B subtilis TyrRS-Z (157) -TVARLMERDDFEEFIAMQK-PISLHEFSVPLMQGYDSVVLE----SDIELGGTDQHFNVLMGRHFQERYNK--EKQVVILMPLLEGLDGVE-KMSKSKH
C difficile TyrRS (146) -TVARMLEREDFKKRYEGQM-PISVHEFFVPLMQAYDSIALE----ADIELGGTDQRFNLLMGRSLQREFGM--ESQIVIMMPLIEGLDGKE-KMSKSLG
C M oxyferaTyrRS (150) -TVARLLERDDFQKRYREGR-PLGVHEFLVPLAQGYDSVVLR----ADVELGGTDQKFNLLVGRELQRAYGQ--EPQVALITPLIEGTDGVQ-KMSKSLG
C jejuni TyrRS (145) -SVARMLERDDFTKRFKEQS-PISICEFLVPLLQGYDSVALK----SDIEMGGTDQKFNLLMGRQLQRVYNIG-KEQAVIMMPLLEGLDGVN-KMSKSLN
H pylori TyrRS (147) -SVARMLERDDFAKRYKENR-PISIVEFLVPLLQGYDSVAMD----ADIELGGNDQKFNLLVGRFLQRSYGLN-KEQSVITMPLLEGLDGVQ-KMSKSLG
X citri TyrRS (142) -TVARMLERDDFAKRFGSQQ-PIAIHEFLVPLVQGYDSVALK----ADVELGGTDQKFNLLMGRGLQEHYGQ--APQIVLTMPLLEGLDGVA-KMSKSLG
X fastidiosa TyrRS (142) -TVARMLERDDFAKRFASQQ-PIVIHEFLVPLLQGYDSIALR----ADVELGGTDQKFNLLMGRALQEHHGQ--PPQIVLTMPLLEGLDGVA-KMSKSLG
Pa TyrRS-Z (142) -TVARMLERDDFSKRYASNQ-PIAIHEFLVPLVQGYDSVALK----ADVELGGTDQKFNLLMGRELQRAYGQ--EAQVILTMPLLEGLDGVK-KMSKSLG
B pertussis TyrRS (154) -TVARMMERDDFTRFFKGGV-PIAVHEFLVPLLQGYDSVALK----ADIELGGTDQKFNLLVGRELQKEYGQ--EQQCILTMPLLVGTDGVE-KMSKSKG
H influenzae TyrRS (142) -TVARMLERDDFKKRFGNNQ-PIAIHEFIVPLLQGYDSVALD----ADVELGGTDQKFNLLVGRELQKSAGK--KPQVAITLPLLVGLDGEK-KMSKSLG
P multocida TyrRS (148) -TVARMLERDDFKKRFSNNQ-PIAIHEFIVPLLQGYDSVALD----ADIELGGTDQKFNLLVGRELQKSACQ--KPQVAITLPLLVGLDGEK-KMSKSLG
Vibrio sp TyrRS-Z (142) -TVARMLERDDFKKRYSSGQ-PIAIHEFMVPLLQGYDSVAME----TDVELGGTDQKFNLLMGRELQKANGQ--KPQVVLMMPLLVGLDGEK-KMSKSAH
Thiocapsa sp TyrRS (142) -TVARMLERDDFAKRYKGGQ-PIAIHEFLVPLHQGYDSVALH----ADVELGGTDQKFNLLVGRQLQDAYGQ--EPQVVITLSLLFGLDGIQ-KMSKSLG
301
400
B bacilliformis TyrRS (244) -AIWLNADMLSFYQFWQYWRNTEDADVTRFLKLYTTL--PMNEIAKLSALQGT----EINEAKKILATEITAMLHGYTVANEAAETARKTGEE-------
Mesorhizobium sp TyrRS (244) -AVWLDAEMLSFYEFWQYWRNTEDADVASFLKLYTTL--PLDEVARLEQLGGS----EINEAKKILATETTALLHGRAAEQASDTARKTGEE-------
R favelukesii TyrRS (244) -AVWLNADMLSAYDFWQYWRNTEDADVSRFLKLYTTL--PMDEVARLSALAGS----EVNEVKKILATEVTSIIHGRTAAEQAAETARKTGEE-------
C vibrioides TyrRS (247) -AVWLNADALSFYDYWQFWRNTEDADVGRFLKLFTDL--PLDKIAELEALEGA----QINDAKKVLADEATRLVHGDEAARIARATAEKAFEQ-------
Pa TyrRS-S (242) -AVWLNAERLAFDFWQFWRNCDRDVGRFLAFSEL--PMDEVRRLGALQGA----ELNEAKVVLANAATALAHGEHAARSADAARGVFAD-------
B burgdorferi TyrRS (234) -AVYLDSNLFSIYDFYQYFRNTSDSDVKTFLYLFTFL--EEPEIELISNFKGN----SLNKAKEILAFEITKIVHGEAEALKVQEASFAAFRG-------
M genitalium TyrRS (244) -ALWLDENKTSVFDFYQVWINLDDQSLKKTFLMLTML--DKKVIDELCNLKGP----KIKQTKQMLAFLITELVHGTKKAKEAQQRSELIFSNQ------
B subtilis TryRS-S (240) -AIWLDKEKTSFYEFYQFWINTDPDRDVVKKLKYFTFL--SKEEIEAYAEKTETAP--EKFRAAQKRLAEEVTSLVHGREATLEQAINISQALFSG-------
S aureus TyrRS (239) -AVWLDAEKTSFYEFYQFWINQSPEDVIKFLKYFTFL--GKEEIDRLEQSKNEAP--HLRRAQKTLAEEVTKFIHGEDALNDAIRISQALFSG-------
L fermentum TyrRS (235) -AVWLDPEKTSFYEFYQFWLNQDPRDVVKKLKYFTFL--SQPEEIAGLEEKVQTEP--WKRRAQRRLAQEVTTFVHGQAATDQAEKISAILFSG-------
S pyogenes TyrRS (237) -AVWLDADKTSFYEMYQFWLNVMDDADVRFLKIFTFL--SLDEIAEIETQFNAAR--HERIAQKTLAREVVTLVHGSEEVYKQALNITEQLFAG-------
E coli TyrRS (243) -AVWLDPKKTSFYKFYQFWINTADVYRFLKFFTFM--SIEEINALEEEDKNSG--KAPCAQYVLAEQVTRLVHGEEGLQAAKRITECLFSG-------
N meningtidis TyrRS (239) -AVWLNAKKTSFYQFYQFWLKVADADVYKFLKYFTFL--SIEEIDAIEAKDKASG--TKFPAQRILAEEVTRLIHGEAALQAAQRISESLFAE-------
Vibrio sp TyrRS-S (243) -AVWLESSKTSFYRFYQFWLNSDDADVYRFLAYYTFL--STAEIEEIRQADALHA--GKPQAQQILAEHVTRFVHGEEGLAAAQRITQALFSG-------
C hutchinsonii TyrRS (236) -NIWLDAALTSFYKFYQFWLNSADEDVSRFIRIFTLL--SKEAIEALEAEHAKAP--HERLLQKTLAKDITIRVHGSEEDYEMAIKASEILFGKSVTE---
M bovis TyrRS (239) GSLWLDPQMTSFYAWYQYFVNTADADVIRYLRWFTFL--SADELAELEQATAQRP--QQRAAQRRLASELTVLVHGEAATAAVEHASRALFSGRG------
P freudenreichii TyrRS (236) -TVWLDPELTSFYAFHQFWLNAEDAKVMDVLKIFSFR--SHDELAELAEQTEQAP--YKRTAQRALADDVTDLVHGVEARKAADEAARALFGRG------
S caatingaensis TyrRS-S (239) -TLWLALEMTTFYAFYQFWLNTDDGDISRYMRILSFK--SPPELKELEKVTEERP--QARTAQRALAEEITTLVHGADQCAAVIAASKALFGQG------
C trachomatis TyrRS (230) -TIWLDPALTPFYELFQYFLRLPDQEISKVMRTLTLL--DNPEIFALDERLTS----DPQAVKKYIAEVIVKDVHGSEGLAQAQAATESFGASEGK----
S caatingaensis TyrRS-Z (280) NYVGLT---ATADDVFGKIMSVPPRLMEFYLKAWTEWTDAEIALVLSRVEERS---LHPMDLKKVLAGEVVAALHGVEAAMAARAGFVAQFSKKS-----
P marinus TyrRS (251) NTVGLS---EDSLSMYSKLEKVPPNIIPTYFELTE-----LDLNILKDE-------NPRELQRRMALEVTSLFHGAEEALKAQSNCEKLFLG-------
Synechocystis sp TyrRS (243) NYVGLQ---EDALSMYSKLEKTPPRLLGEYFELTN-----LDLAELPE--------NPRECQKLLAKEVTAQFHGVAQAIAAQKTAEDIVTQG------
C aurantiacus TyrRS (228) NTVDIR---MPESEMYGRIMSMSPEVLPLYFEVLTDVPLAEIDEMKLA---MAAGQVNPRDLKMRLAREIVAQFHDPAAAAAAEAAFVRQFVEREI----
Tth TyrRS (239) NYIGLT---EPEEAMFKKLMRVPPPLLPSYFRLLTDLEEEEIEALLKAG-PVPAHRVLARLLTAAYALPQIPPRIDRAFYESLGYAWEAFGRDKEAGPEE
B subtilis TyrRS-Z (248) NYIGIN---EHENDMYGKTMSLPPSLMKKYIHFATD--LELPEKKQLVK-DLETGAVHPRPAKMLLAKTIVRMYHGEKAPEAAEHSFKTVFQENSL----
C difficile TyrRS (237) NYIGID---EEAGIMYQKSMEIPDELIIKYINEVTD--VHPLEVNKIES-QLKEGSVNPRDIKMNLAREIVTLYHGEEAKEAEERFKSVFQKGQI----
C M oxyferaTyrRS (241) NYIGID---EPAREVYGKAMSIPDELIVKYAEVAG--VLPEAVEAMDE-GLKLGTLHPRTAKAKVAWELVALYRGEQAAEEAALEFDRMFRDKRL----
C jejuni TyrRS (237) NYIGVT---EKANDMYAKILSISPELMFRYYELLSQ--KSLPEIAQIKK-DIEQGSLHPKPAKENLALEITERFHSKEEANNAKSEFDRIHSQNAL----
H pylori TyrRS (239) NYVGIT---EPENAMFGKIMSVSPDLMWRYYTLSA--KTLPEIEDLKH-GILNQTLHPKAVKEELAGEIVARYYDNEQAIKAKEQFSKVFSANLL----
X citri TyrRS (233) NYIGIN---EPAIDIVTKTMKIGPALTWRWIDLLSFD-ISMAEAARLKE-EVASGELHPREVKLRLAREILATRFHDAATAEQAIAGWHAVVTGQGD----
X fastidiosa TyrRS (233) NYIGIK---EPEIDIVTKTMKIGPDLMWRWIELLSFK-ISAAEAVALRE-AVAKSELNPREVKLRLAHELVSRFYDNAAEKAIAGWQAVVTGQGN----
Pa TyrRS-Z (233) NYIGIQ---EAEGVMYSKLVSIPDTLMWRYFELLSF--RSLPEIDSFRK-DVEAG-ANPRDIKIKLAEEIVARFHGEEAAASAHKSAGNRLKEGEL----
B pertussis TyrRS (245) NYIGIS---EAEESMFGKLMSISPTLMWRYYELLSF--RSLADIAALKA-EIDGG-RNPRDAKVALAQEIVVRFHSPQAAEAALAAFEARFRDGAI----
H influenzae TyrRS (233) NYIGVT---EAESDMFGKVMSISPELMWDWYNLLSF--RPLNEIAQLKS-EVENG-KNPRDVKILLAKELIARFHNEEAANAAEQEFINRFQKGAM----
P multocida TyrRS (239) NYIGVA---ESETEMFGKVMSISPELMWDWYDLLSF--RPLTEIAQLKQ-EVENG-RNPRDIKVLLAKEIIARFHSEADAAEAEQEFINRFQKGAM----
Vibrio sp TyrRS-Z (233) NYIGIN---ESEEEMFGKIMSISPELMWSYYDLLSF--RPLSEVQQFKS-DVAAG-KNPRDIKILLAKEIIARFHDEAAADGAEQEFIARFQKGAI----
Thiocapsa sp TyrRS (233) NYIGIT---DSEDDMFGKIMSISPDLMWRYFELLST--RDLSEIQAWRD-AVDEG-ANPRDIKFELGLELVSRFHDATQARQALERFVARFQRGAM----




**Supplemental Figure S3. Alignment of the amino acid sequence of TyrRS-Z and TyrRS-S from various bacterial phylogenic taxa.** (**A**) The phylogenic tree formed from the amino acid alignment of thirty-seven TyrRS-Z and TyrRS-S proteins. (**B**) The alignment of the thirty-seven TyrRS proteins. The TyrRS proteins were from: *Bartonella bacilliformis, Mesorhizobium sp, Rhizobium favelukesii, Caulobacter vibrioides, P. aeruginosa, Borrelia burgdorferi, Mycoplasma genitalium, Bacillus subtilis, Staphylococcus aureus, Lactobacillus fermentum,*

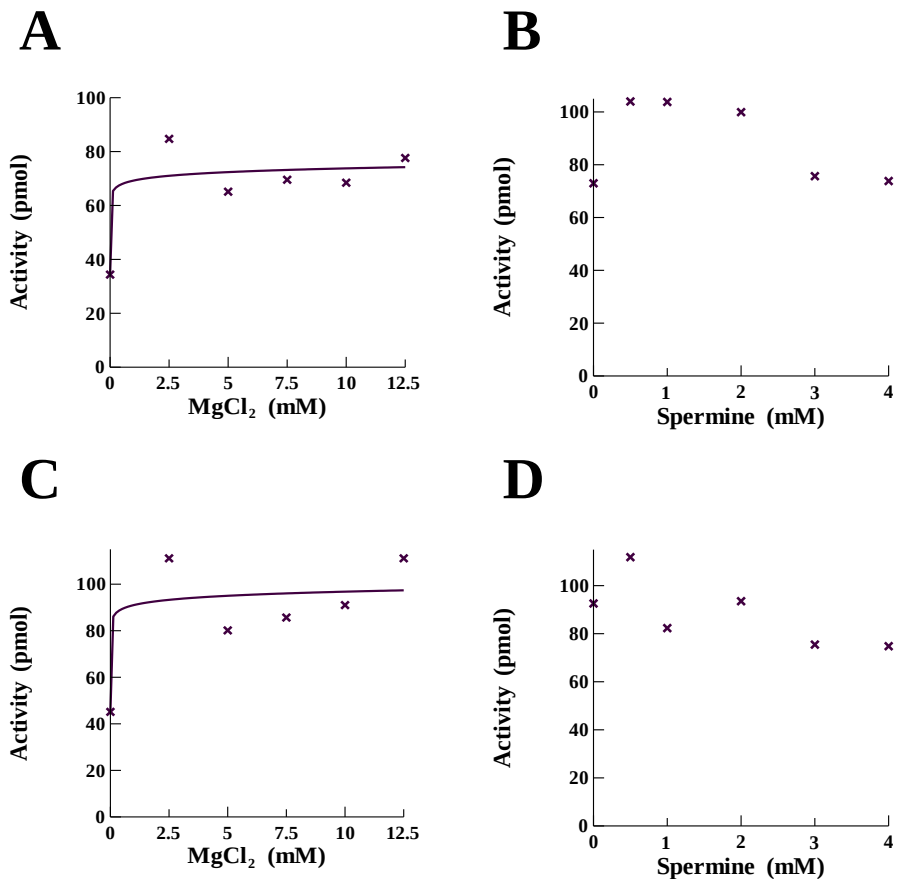
*Streptococcus pyogenes, Escherichia coli, Neisseria meningitidis, Vibrio sp, Cytophaga hutchinsonii, Mycobacterium bovis, Propionbacterium freudenreichii, Streptomyces caatingaensis, Chlamydia trachomatis, Prochlorococcus marinus, Synechocystis sp, Chloroflexus aurantiacus, Thermus thermophilus, Clostridium difficile, Candidatus methylomirabilis oxyfera, Campylobacter jejuni, Helicobacter pylori, Xanthomonas citri, Xylella fastidiosa, Bordetella pertussis, Haemophilus influenzae, Pasteurella multocida, Thiocapsa sp.* Conserved amino acid residues are shown as white letters on a black background and similar amino acid residues are shown as black letters on a grey background. Sequence alignments were performed using Vector NTI Advance (TM) 11.5.3 (Invitrogen).

*P. aeruginosa*, contain both forms of TyrRS synthetases, (TyrRS-Z and TyrRS-S). These two enzymes share only 27% sequence conservation (Fig. S4), but both are classified as class 1 aminoacyl-tRNA synthetases.



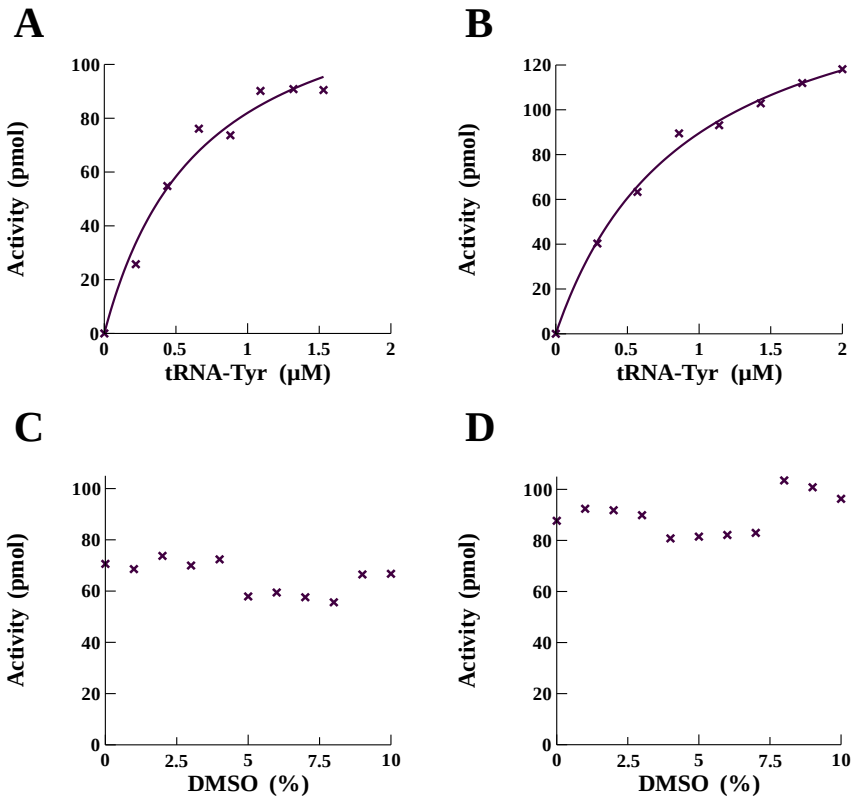

**Supplemental Figure S4. Alignment of the amino acid sequence of *P. aeruginosa* TyrRS-Z and TyrRS-S.** Conserved amino acid residues are shown as white letters on a black background and similar amino acid residues are shown as black letters on a grey background. The protein sequences were downloaded from the National Center for Biotechnology Information (NCBI). Accession numbers for *P. aeruginosa* TyrRS-Z and TyrRS-S are NP_249359 and NP_252827, respectively. Sequence alignments were performed using Vector NTI Advance (TM) 11.5.3 (Invitrogen).

The activity of both TyrRS-Z and TyrRS-S were screened against a chemical compound library composed of 2000 distinct compounds. To achieve the maximum signal in the SPA assays, first, the non-enzymatic components of the aminoacylation reaction were individually titrated into the assay to determine saturating concentrations of each (Fig S5). From these assays 10 mM $MgCl_2$ and 0.5 mM spermine were chosen for use in the screening assays.



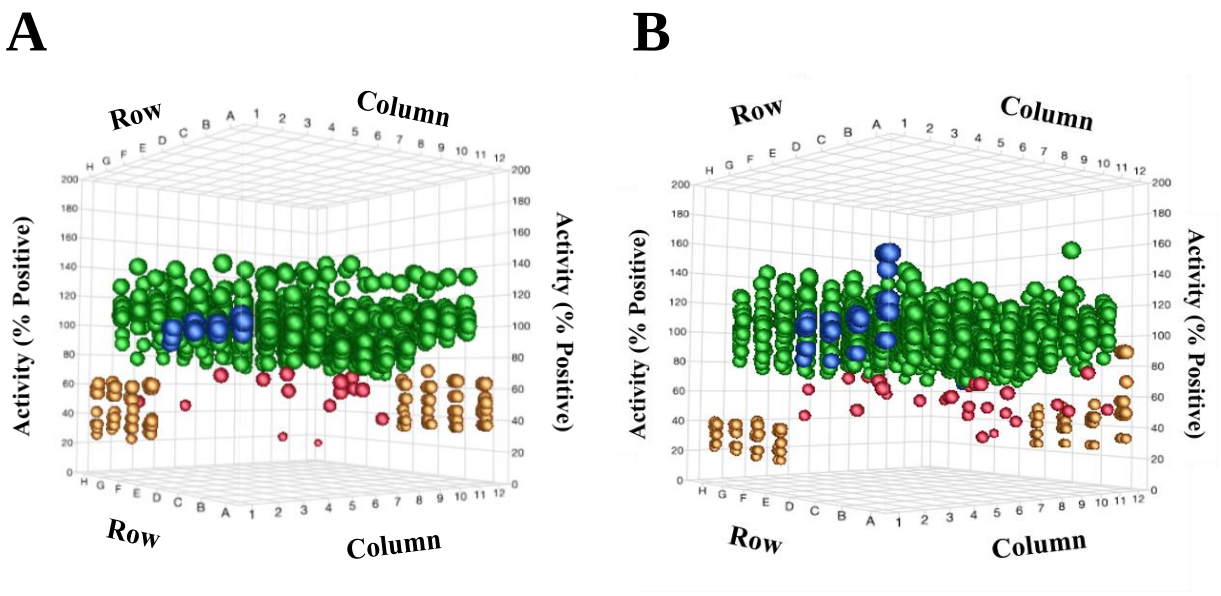

**Supplemental Figure S5. Titration of components of the amino acylation assay.** To determine optimized concentration of components, both $MgCl_2$ and spermine were titrated into the SPA aminoacylation assays containing (**A-B**) TyrRS-Z and (**C-D**) TyrRS-S.

Next, tRNA was titrated into the assay to ensure that the assay was within the linear region of the reaction-detection time and to determine the concentration of $tRNA^{Tyr}$ to be used in the screening assays (Fig. S6A-B). Chemical compounds were dissolved in 100% dimethyl sulfoxide (DMSO) resulting in final DMSO concentrations in screening assays of 4%. Therefore, the ability of *P. aeruginosa* TyrRS to function in the presence of increasing amounts of DMSO was determined (Fig. S6C-D).




**Supplemental Figure 6. Screening assay development.** To determine the tRNA concentration to be used in screening assays of (**A**) TyrRS-Z and (**B**) TyrRS-S, and to determine that the amount of $tRNA^{Tyr}$ used would be within the linear region of the reaction-detection time, tRNA was added into the aminoacylation assay described in the "Chemical Compound Screening" section of "Methods and Materials". The effect of DMSO in the (**C**) TyrRS-Z and (**D**) TyrRS-S aminoacylation assay was determined by adding DMSO (0-10%) into the aminoacylation assay described in the "Chemical Compound Screening" section of "Methods and Materials". The activity was monitored using SPA technology.

The ChemDiv Soluble Diversity Library, containing 2000 compounds, was screened for the ability to inhibit the aminoacylation activity of *P. aeruginosa* TyrRS-Z and TyrRS-S in screening platforms as described in "Materials and Methods". Positive controls for activity in both screening platforms contained DMSO in the absence of compound. Negative controls for inhibition of activity contained either no enzyme (TyrRS-Z) or EDTA (TyrRS-S). Results were converted to percent positive of the positive control and plotted using 3-D scatter graph (JMP Pro 14) (Fig. S7). Compounds were considered initial hits if 50% of the activity was inhibited. From the initial screens, 16 compounds were identified that inhibited TyrRS-Z and 30 compounds that inhibited PaTyrRS-S.




**Supplemental Figure S7. Initial single-point assay screens against the Soluble Diversity chemical compound library.** The screen was a single-point assay against the aminoacylation activity of (**A**) TyrRS-Z and (**B**) TyrRS-S from *P. aeruginosa*. Blue spheres represent positive DMSO control assays and gold spheres represent negative (EDTA/minus enzyme) control assays. Green spheres represent assays containing compounds that had no effect on the aminoacylation activity. Red spheres represent assays containing compounds that inhibited at least 50% of the aminoacylation activity.

**Table S1.** Initial velocities were determined for *P. aeruginosa* TyrRS-S and TyrRS-Z and the data were fit to a Michaelis-Menten steady-state model using XLfit 5.3 (IDBS) to determine $K_M$ and $V_{max}$.

| | **TyrRS-S** | | | **TyrRS-Z** | | |
|---|---|---|---|---|---|---|
| | **ATP** | **Tyrosine** | **tRNA$^{Tyr}$** | **ATP** | **Tyrosine** | **tRNA$^{Tyr}$** |
| $K_M$ (μM) | 204 | 172 | 1.5 | 496 | 29 | 2.1 |
| $V_{max}$ (μM/min) | 24 | 91 | 0.56 | 92 | 75 | 0.17 |
| $k_{cat}$ ($s^{-1}$) | 1.0 | 3.8 | 0.19 | 3.8 | 3.1 | 1.9 |
| $k_{cat}/K_M$ ($s^{-1}$ $\mu M^{-1}$) | 0.01 | 0.02 | 0.12 | 0.01 | 0.11 | 0.9 |

**Table S2.** MIC values for the four chemical compounds observed to inhibit the activity of *P. aeruginosa* TyrRS enzymes.

| **Compound** | **MIC (μg/ml)** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | ***E. coli*[a]** | ***E. coli Efflux*** | ***E. fae*** | ***H. flu*** | ***M. catt*** | ***P. aer*** | ***P. aer Efflux*** | ***P. aer Hyper*** | ***S. aureus*** | ***S. pneumo*** |
| BCD37H06 | 128 | 64 | 32 | 64 | 8 | 128 | 64 | 128 | 16 | 64 |
| BCD38C11 | 128 | 64 | 64 | 128 | 32 | 128 | 64 | 128 | 16 | 64 |
| BCD49D09 | >128 | 16 | >128 | 128 | 64 | 128 | 64 | 128 | 32 | 64 |
| BCD54B04 | 128 | 16 | 64 | 64 | 32 | >128 | 64 | 128 | 64 | 64 |

[a] MIC values were determined for *E. coli* (ATCC® 25922™), *E. coli tol*C efflux mutant, *Enterococcus faecalis* (ATCC® 29212™), *Haemophilus influenzae* (ATCC® 49766™), *Moraxella catarrhalis* (ATCC® 25238) *Pseudomonas aeruginosa* (ATCC® 47085™), *Pseudomonas aeruginosa* PAO200 (efflux pump mutant), *Pseudomonas aeruginosa* hypersensitive strain (ATCC® 35151™), *Staphylococcus aureus* (ATCC® 29213™), and *Streptococcus pneumonia* (ATCC® 49619™). MIC values were determined for each compound in triplicate.

**Table S3.** Standard deviation for the data points (three) for the MTT toxicity assays to access the impact of the hit compounds on human cell viability using HEK293 cells. The compounds were not found to be toxic in these assays.

| **Concentration (μg/ml)** | **0** | **25** | **50** | **100** | **200** | **300** | **400** |
|---|---|---|---|---|---|---|---|
| **Compound** | | | | | | | |
| **BCD37H06** | 0.026 | 0.061 | 0.058 | 0.070 | 0.132 | 0.283 | 0.249 |
| **BCD38C11** | 0.035 | 0.111 | 0.061 | 0.086 | 0.135 | 0.107 | 0.102 |
| **BCD49D06** | 0.075 | 0.062 | 0.080 | 0.153 | 0.027 | 0.123 | 0.053 |
| **BCD54B04** | 0.032 | 0.084 | 0.142 | 0.176 | 0.086 | 0.129 | 0.161 |

Spectral data for the four hit compounds as supplied by the vendor.