

Nylonase Genes and Proteins – Distribution, Conservation, and Possible Origins.

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ABSTRACT

Nylon comprises a family of man-made substances that were first manufactured in 1935. Nylonases are biological enzymes that can break down nylon oligomers. Although the most prominent nylonases are within the family of enzymes classified as 6-aminohexanoate hydrolases, some enzymes not formally classified as 6-aminohexanoate hydrolases also have the ability to breakdown nylons, and so can also be classified as nylonases.

Organisms that encode a nylonase enzyme do not necessarily have the ability to actually survive on a nylon substrate as their sole carbon source. Among the first documented organisms that did have this ability was the soil bacterium *Arthrobacter* KI72.

It has long been thought that nylonase genes and proteins were essentially absent from the biosphere prior to 1935. This belief led to the widespread assumption that any nylonase gene observed in the present must have emerged since 1935. Several authors developed hypothetical models of how a specific nylonase gene (the *nylB* gene found within *Arthrobacter* KI72), might have arisen very recently as a *de novo* gene.

In this paper we show that the widely-held assumption that all nylonase genes must have evolved very recently is no longer credible. This is in light of the wide-spread distribution of diverse nylonases throughout the biosphere. Likewise, we show that the early speculations regarding the possible *de novo* origin of the *nylB* nylonase gene are no longer credible.

Our review of the literature shows that a variety of nylonase-digesting bacteria have been found in extremely diverse natural environments – far removed from any synthetic nylon sources. In addition, we show there are over 1800 organisms with computationally predicted (provisional) 6-aminohexanoate hydrolase/nylonase genes in the NIH-funded UNIPROT database. These 1800 predicted nylonases are not yet experimentally confirmed to cleave nylons, but have significant homology to the experimentally confirmed nylonases.

In addition to 6-aminohexanoate hydrolases, proteases like trypsin and certain lipases have been experimentally demonstrated to have nylonase activity. If we include all proteases and lipases that may have nylonase activity, the number of organisms with nylonase activity may exceed ten thousand. The widespread distribution of nylonases and their homologs strongly suggests that nylonases were already widespread prior to 1935.

Lastly, we have carefully examined the claims that the *nylB* gene arose as a *de novo* gene very recently. The theories of Ohno (the frame shift hypothesis) and Okada (the gene duplication hypothesis), were speculative in nature, and yet were uncritically accepted. In light of new data, these early speculations no longer appear tenable.

INTRODUCTION AND SURVEY OF NYLONASES

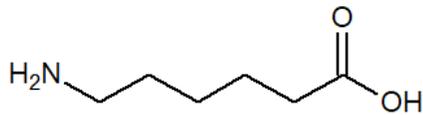
Nylons exist in a variety of chemical forms, which can cause confusion in terms of defining nylonase activity. The nylon of primary interest in this review is nylon-6, and unless otherwise stated the term “nylon” will refer to short nylon-6 oligomers that are generally a hexamer or less in length. Nylon-6’s are polymers of de-hydrated lysine-like molecules known as 6-aminohexanoic acids (figure 1).

The 6-aminohexanoic acids have the chemical formula $C_6H_{13}NO_2$, and the dehydrated form of the 6-aminohexanoic acid that constitutes the nylon-6 monomer has the chemical formula $C_6H_{11}NO$. 6-aminohexanoates are the conjugate base of 6-aminohexanoic acids. Terms like 6-aminocaproic acid or ϵ -polycaproamide are also used to describe nylons. Commercially viable nylon-6’s are composed of polymers connecting at least 100 dehydrated 6-aminohexanoic acid monomers.¹ The nylon-6 linear oligomers are terminated by a lysine-like monomer (figure 2). There also exist nylon-6 cyclic oligomers.

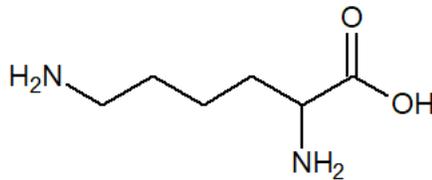
Because this paper may be of interest to a fairly wide range of scientists, we wish to clarify that when we are specifying a nylonase gene we use italicized letters, beginning with lower case (i.e., *nylB*), but when we are specifying nylonase proteins we use non-italicized letters, beginning with upper case (i.e., NylB). This is the standard convention used in this application.

Many biological nylonases (such as the NylB enzyme, which is encoded by the *nylB* gene, which is found within the bacterium *Arthrobacter* KI72), can only degrade nylon dimers and short oligomers² where the lysine-like component constitutes a noticeable fraction of the nylon oligomer. Once the oligomer is larger than a hexamer, it causes the lysine-like terminus to occupy a smaller proportion of the total oligomer, and effectively precludes enzymatic action.³ This suggests the less a nylon oligomer resembles a naturally occurring amino acid such as lysine (and/or the lysine-like terminus is physically prevented access to the enzyme’s active site by numerous non-lysine-like nylon monomers as would be the case with a large nylon polymer), the less effective a nylonase will be in cleaving the nylon.

Because most biological nylonases can only degrade short nylon polymers, it is worth emphasizing that the term “nylonase” can be misleading. The term nylonase clouds the fact that most biological nylonases cannot actually digest what are normally considered nylons (commercially viable nylons are polymers of 100 or more monomers).⁴ Most “nylonases” can only digest waste product nylons that have already been degraded into short oligomers.

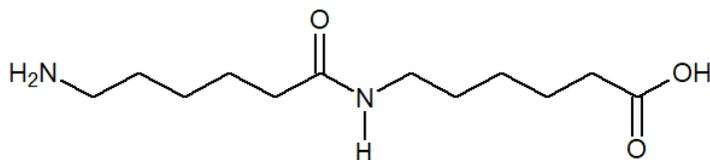


6-aminohexanoic acid

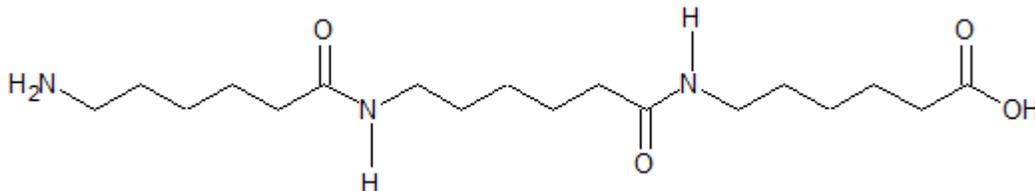


lysine

Figure 1. A man-made 6-aminohexanoic acid molecule is depicted (at the top), which is an analog of a naturally occurring biological lysine molecule (at the bottom). The molecules are shown in a neutral charge configuration. Some references may show a slightly different configuration in an ionized state.



nylon-6 linear dimer



nylon-6 linear trimer

Figure 2. A nylon-6 dimer is depicted at the top and nylon-6 trimer at the bottom. The lysine-analogous 6-aminohexanoate group is on the right side for each molecule. The lysine-like termination group will also be present for higher order nylon oligomers and polymers as well. The molecules are shown in the neutral charge configuration. Some references may show the molecules in a slightly different configuration in an ionized state.

Bonds joining the nylon-6 monomer units are similar to the peptide bonds joining amino acids in a protein. One might expect that the similarity of these bonds might enable a protease to be able to break the bonds in a short nylon oligomer, and indeed this appears to be the case. The earliest report of a nylonase appeared in 1959, when Ebata and Morita experimentally demonstrated that the protease known as trypsin can act as a nylonase.^{5,6} Trypsin is a very common protein-cleaving enzyme that appears in vertebrate eukaryotes and various prokaryotes. Unfortunately, Ebata and Morita did not specify what the organismal source was of the trypsin they used in their experiments.

While Ebata and Morita demonstrated proteases could act as nylonases, it was also found that triacylglycerol lipases could act as nylonases. In 2009, Kiumarsi and Parvinzadeh reported hydrolyzing nylon-6 using a lipase known as Lipex50T commercially available from Novozyme (formerly Novo Nordisk).⁷ Unfortunately, data on the degree, if any, that Lipex50T diverges from natural lipases was not readily available. Lipex is a commercially marketed lipase that originated from the LIP gene of

Thermomyces lanuginosus (formerly *Humicola lanuginosa*), by way of directed evolution in the laboratory.

It is important to point out that an organism having nylonase activity may still not be able to metabolize nylon as its sole carbon source. Furthermore, in some cases the nylonase-mediated breakdown of nylon creates 6-aminohexanoic acids, which can be toxic to the organism. Nevertheless, there are some organisms that not only have a nylonase, but can also live on a nylon substrate. In 1966, Fukumura reported such an organism, *Corynebacteria aurantiacum B-2*.⁸ Since that time, the inventory of bacteria that are known to digest nylon has steadily increased. One of the most recent examples of this was published in 2016, where Mahdi *et. al*, reported on a thermophilic bacteria with nylonase activity, which was found in the soils of Iraq.⁹

Although various instances of nylon-digesting bacteria have been found, the best known instance of this is *Arthrobacteria* KI72 (formerly *Flavobacteria* sp. KI72, formerly *Acromobacter gutatus* KI72).¹⁰ This bacterium was extracted from soil samples and then tested for ability to grow on nylon waste by-products.¹¹ KI72's nylon-eating ability was reported by Kinoshita in 1973 and 1975.¹² Even though Kinoshita acknowledged Fukumura's earlier 1966 work, and Fukumura acknowledged Ebata's 1959 work, the Kinoshita papers have been touted as the first discovery of nylonase activity in the biological world. Kinoshita's discovery led to the identification of the first nylonase gene.¹³ It appears that Kinoshita believed his nylonase gene was a newly evolved gene that arose due to the introduction of man-made nylon.^{14,15} In his 1977 paper, Kinoshita described a particular nylonase¹⁶ (eventually named NylA in a later paper by Okada¹⁷), as being “exceedingly rare” because other strains of the same bacteria did not have the nylonase activity. Yet by 1981 he suggested that evolution of nylonases “is not a very rare phenomenon in nature.”¹⁸

Some popular literature has claimed that KI72 was extracted from the nylon-rich environment of the waste water of a nylon factory.^{19,20,21} However, it should be pointed out the most important original papers that described nylonase in KI72 did not make that claim, but rather claimed KI72 came from soil in an unspecified geographic location.²² Furthermore, in 2007, Sudhakar *et al*. reported various marine bacteria such as *Bacillus cereus*, *Vibrio furnisii*, and *Brevundimonas vesicularis* in the Indian Ocean, which could digest nylon.²³ In all these cases it would seem difficult to argue that such nylon digesting ability evolved in response to a nylon rich environment. We will show that other environments that are far removed from human activity (i.e., arctic soils), can harbor organisms such as *Cryobacterium arcticum*, which also seem to possess nylonases as predicted by UNIPROT.^{24,25}

After Kinoshita's initial discovery of *nylA*, three other nylonase genes were discovered in the very same KI72 strain, namely *nylB*, *nylB'*, and *nylC*.²⁶ The *nylA*, *nylB*, and *nylC* genes were unrelated and non-homologous. The *nylB'* gene was partially homologous to *nylB*. Given three entirely unrelated nylonase genes in the same organism, the claim that nylonases were necessarily very new and very rare should have been challenged at that time. It was further discovered the four nylonase genes were found on the same plasmid, and that two of them were part of the same operon – sharing the same promoter.²⁷ In 2007, three of these four nylonase genes (*nylB*, *nylB'*, and *nylC*) were also reported to be present on the chromosome of a different genus, *Agromyces* KYR5.²⁸

As we will show, we have identified 162 organisms that contain computationally-predicted *nylA* genes, refuting Kinoshita's early claim that *nylA* is exceedingly rare. We have identified 193 organisms that contain computationally-predicted *nylB* genes and we have identified over 1800 organisms that contain

computationally-predicted 6-aminohexanoate hydrolase/nylonase genes. The predicted *nylB* genes are frequently seen to be non-homologous on the DNA level, even though the predicted proteins they code for are structurally homologous or functionally convergent. The existence of structurally similar and functional convergent NylB enzymes coded from non-homologous *nylB* genes precludes large scale post-1935 horizontal gene transfer as the sole explanation for the widespread distribution of *nylB*.

There is evidence that nylonase function can be acquired or enhanced through adaptive processes taking place in pre-existing nylonase homologs. The process is similar to the process of bacteria evolving antibiotic resistance through point mutations of existing genes. For example, Prijambada reported in 1995 that nylon-digesting ability could be evolved in the laboratory from bacterial strains that initially lacked such ability.²⁹ It was shown that the human pathogenic bacteria *Pseudomonas aeruginosa* evolved a nylon digesting strain in just 9 days by restricting the kinds of nutrients available to the colony. Even though Prijambada was not able to determine the exact mutations and genes responsible for the acquired ability to degrade nylon, the rapidity of the change suggests nylonase activity arises very easily.

The ease of adapting pre-existing genes via point-mutation for nylonase function was further demonstrated in 1991 when Kato reported that a mere 2-residue change out of the 392-residues of the pre-existing homolog of NylB, known as NylB', enabled it to break down nylon 1000 times more effectively than before the change.³⁰

In summary, the literature clearly indicates that nylonase activity is widely observed in nature, and that it is also very easily derived. This strongly suggests that nylonase activity is very low hanging fruit – it does not seem to require a highly constrained or specific active site, and it may be readily derived from other enzymes having similar activities.

METHODS/RESULTS – DISCOVERING NEW NYLONASES AND HOMOLOGS

Enzyme homologies and functional convergences of nylonases can be inferred in a variety of ways. The most direct method is a simple search for DNA sequences or protein sequences that are similar to known nylonases. This is not as comprehensive as structural searches since structurally similar proteins may have only 12% sequence similarity.^{31,32} We initially began with the *nylB* gene, doing BLASTN and BLASTP searches, and then contrasted those BLAST results with searches using UNIPROT.

We first did a BLASTN search of the NCBI database, looking for homologs of the *nylB* gene in *Arthrobacter* KI72. We found only one substantially different type of bacterium, *Agromyces sp KYR5*, that contained DNA homologous to the *nylB* gene.³³ However, when an amino acid sequence search for the NylB protein was made using BLASTP, many types of bacteria were found to have homologous proteins. For example, 11 different species had E-values of effectively zero, and 25 species had an E-value in the range of e^{-100} . Because such databases contain only a tiny fraction of all protein sequences in the biosphere, there must be a great number of species having proteins homologous to NylB. Therefore, there is no rational basis to presume a recent *de novo* origin of the *nylB* gene.

When the search for the *nylB* gene was expanded for *nylB* genes that coded for structurally and functionally similar proteins using the computational gene predictions from the UNIPROT database of proteins, around 193 different strains and species emerged that contained a predicted gene called *nylB*

or variations of that theme such as *nylB_1*. Such a search is easily accomplished by going to the UNIPROT website and simply typing in “nylB” in the search box.

One such organism listed from the search for *nylB* genes was the *nylB_1* gene of *Bacillus cereus*.³⁴ As mentioned earlier, the marine bacteria *Bacillus cereus* from the Indian Ocean has been experimentally confirmed to digest nylon - validating the UNIPROT prediction of a *nylB* gene in this species.

Given the observed nylonase activity in this bacterium, and the fact that it has a NylB homolog, it is reasonable to assume that the predicted *Bacillus cereus nylB* genes are active. It is interesting to point out, that the predicted the *Bacillus cereus nylB_1* gene has 94.4% sequence identity with the predicted *nylB* gene of a strain of the human pathogenic bacteria *Streptococcus pneumoniae*.³⁵

Remarkably, *Streptococcus pneumoniae* also has UNIPROT-predicted homologs of the two other well studied nylonase genes, *nylB'* and *nylA*. These results raise a profound question: “Why are bacteria from diverse environments around the globe coding for diverse families of nylonase proteins that are structurally and functionally homologous, but are encoded by non-homologs DNA sequences?” This seems to be happening in soil bacteria, marine bacteria, and human pathogenic bacteria.

Further search for organisms with predicted or confirmed *nylB'* homologs yielded approximately 125 organisms. A search for organisms with a predicted or confirmed *nylA* homolog yielded approximately 162 organisms. A search for organisms with a predicted or confirmed *nylC* homolog yielded approximately 9 organisms. These numbers are not exact, because some UNIPROT entries are for the same organism under different names and the UNIPROT database is ever-expanding.

The names used for the predicted proteins of *nylB* and *nylB'* were mostly “6-aminohexanoate dimer hydrolases”, but a few were named simply “hydrolases” or “beta-lactamases.” NylA entries for predicted proteins were mostly named “amidases” and the rest “6-aminohexanoate cyclic dimer hydrolases.” NylC predicted proteins were named “6-aminohexanoate dimer hydrolases”, “endotype6-aminohexanoate-oligomer hydrolases” and “NylC-like proteins.”

When the search for nylonases was expanded to “6-aminohexanoate hydrolase”, 1827 organisms were found with predicted proteins for such nylonases, but the majority were not listed with genes names such as *nylA*, *nylB*, *nylB'* or *nylC*. This search is very easy to carry out by simply going to the UNIPROT website and entering “6-aminohexanoate hydrolase” in the search box. It is worth noting “nylonase” is a colloquial term, so UNIPROT will not return any hits if “nylonase” is used as a search term.

The UNIPROT list of predicted 6-aminohexanoate hydrolases is at least tentatively supported by exact or related organisms confirmed to digest nylon such as those mentioned earlier such as *Bacillus cereus*, *Vibrio furnisii*, *Corynebacterium aurantiacum B-2*, *Pseudomonas aeruginosa*. It seems conservation of the protein motifs of confirmed nylonases is beyond dispute even without complete experimental demonstration of nylonase activity for every nylonase gene reported by UNIPROT.

Detailed output from the UNIPROT analyses, as well as details about the BLASTP searches, are available under “Supplemental materials.”

DISCUSSION

We began this work hoping to better understanding the various claims regarding the *de novo* origin of certain nylonase genes. The idea that nylonases would have arisen very recently, *de novo*, was based upon the widely-held assumption that nylonases would have been essentially non-existent prior to the artificial manufacture of nylon. This basic assumption would not be justified if there were any nylon-like polymers in nature, or if nylonase activity required very low specificity, such that enzymes with other functions might also possess or acquire nylonase activity.

Our analyses indicate that nylonase genes are abundant, come in many diverse forms, are found in a great number of organisms, and these organisms are found within a great number of natural environments. We also show that nylonase activity is easily acquired through mutation of other enzymes, which strongly suggests that nylonase activity has very low specificity of the active site.

These findings refute the widely held assumption that nylonases were essentially non-existent before 1935. In this light, there is no reason to believe that any nylonase emerged since 1935, and so there is no solid basis for invoking any *de novo* nylonase genes. Therefore, it seems only reasonable to re-examine the earlier claims of *de novo* genes.

Various Models Claiming Newly Evolved Enzymes

In 1977 Kinoshita reported on a nylonase enzyme that would eventually be named NylA. He hypothesized that “the enzyme has evolved by adaptation to a new synthetic substance which is a waste product of nylon-6 production.”³⁶ Kinoshita did limited testing of the enzyme, and as best as he could determine, it appeared the enzyme had lost all ability to catalyze reactions involving naturally occurring biological substances. He suggested that the enzyme was “an evolved enzyme which originally had an activity on a physiological substrate but lost it by the evolutionary mutation.” By 1981, Kinoshita was referring to the NylA and NylB enzymes as “the two newly evolved enzymes” without specifying how the enzymes evolved.

The phrase “newly evolved enzymes” came to mean different things to different theorists. Okada in 1983 indicated that as little as 1 residue change could create a nylonase from a pre-existing gene, but then in the same paper he argued for a gene duplication of a pre-existing gene (*nylB*) followed by enough point mutations to create a 47 residue changes resulting in the NylB protein consisting of 392 residues.³⁷

In 1984, Ohno was critical of Okada’s gene duplication hypothesis, and instead suggested NylB was the result of a single frame shift mutation resulting in over 400 simultaneous residue changes in an open reading frame that would then have coded for 427 residues. He declared that this hypothetical transformation was the “Birth of a Unique Enzyme”, and he specifically used the phrase “*de novo*” to describe the transformation³⁸ (as best as can be determined, Kinoshita and Okada do not actually use the phrase “*de novo*”). In fact, Ohno hinted that the identical hypothetical frame shift event as happened in *nylB* also happened independently in *nylB'* (even though *nylB'* codes for a different protein with 47 divergent amino acids).

The Kinoshita narrative about nylonase evolution led to the Okada narrative, which led to the Ohno narrative. The evolution of the narrative quickly progressed to the point that popular literature characterized Ohno’s frame shift model as a direct observation and an established fact. By 2016 the popular narrative included claims that x-ray crystallography proved that the NylB protein folds were all

de novo, and could be directly attributed to the hypothetical frame shift event.³⁹ The popular narrative also implied that the hypothetical frame shift mutation was directly observed under reproducible laboratory conditions.⁴⁰

The independent hypotheses of Ohno and Okada about the origin of the *nylB* gene appear to have been accepted uncritically, despite their speculative nature and the absence of any direct support. In retrospect, Okada and Ohno's speculations seem especially *ad hoc*, given that *nylB* was found physically linked to two other non-homologous nylonase genes on the very same plasmid (i.e., *nylA* and *nylC*). No explanation for the evolution of these other nylonases was ever attempted, nor was there any attempt to explain how all three nylonase genes could have become linked.

Strangely, no one seems to have asked the obvious question: “if the *nylB* gene evolved *de novo* very recently, then where did the linked *nylA* and *nylC* nylonase genes come from?” Nor has anyone commented on the fact that *nylB* and *nylC* were found tightly coupled, being found in the same polycistron and sharing the very same promoter. Logically, if *nylB* required a *de novo* explanation, certainly so did *nylA* and *nylC*. Furthermore, if these three *de novo* nylonase genes arose independently, how could we explain how they fortuitously landed on the same plasmid, where they were found to be co-regulated and were working in concert as part of a catabolic cascade? Indeed, during the last four decades why has no one commented on the relevance of the many newly emerging nylonase genes and related proteins listed in the literature review above? In light of all these issues, it seems remarkable that for decades no one challenged the widely accepted premise that *nylB* arose *de novo* in the very recent past.

Problems with Ohno's Frame-shift Hypothesis

In the introduction of Ohno's paper he describes his real purpose – he wished to support his broader thesis that frame-shift mutations play a major role in the evolution of new proteins. Ohno was not primarily interested in understanding the origin of the nylonase gene – his primary interest was in supporting this broader thesis. He designed his model for the origin of NylB nylonase as a hypothetical example that might illustrate how frame-shifts could routinely create new proteins instantaneously.

The speculative nature of Ohno's paper – Ohno speculated that an unknown precursor gene gave rise to NylB, via a frame shift mutation. He speculated that a specific hypothetical frameshift mutation in the hypothetical precursor gene gave rise to what is now the start codon of *nylB*. Ohno's hypothesis required that *nylB* arose from a DNA sequence encoding an unknown precursor protein consisting of a specific string of 427 amino acids. He speculated that this DNA sequence underwent a frame-shift mutation which fortuitously and instantly established an entirely new gene, encoding a totally new sequence of 392 amino acids. He hypothesized that by chance that new protein had *de novo* nylonase activity that enabled a nylonase-based metabolism.

Ohno asserted his hypothesis so forcefully that it appears that many readers thought he actually had evidence for a hypothetical frame shift event. Remarkably, Ohno's hypothesis was not critically examined, and was never rigorously tested. His hypothesis involved a series of assumptions: 1) he presumed a precursor gene/protein existed having a function totally unrelated to nylonase activity; 2) he presumed that a very specific frame-shift mutation turned a serendipitous long open reading frame into a random string of amino acids; 3) he presumed that this amino acid string serendipitously and instantly encoded a stable and fully functional nylonase enzyme.

In his paper Ohno showed the sequence that he imagined was the precursor gene. Ohno hypothesized the single nucleotide frame shift insertion shown below. The top line represents the relevant section of his imagined ancestor to the *nylB* gene, which he calls PR.C, while the bottom line is the relevant section of the actual *nylB* gene, showing the presumed insertion of a thymine residue, which would have created the start codon of the *nylB* gene:

....TCGGAGACACTCGA-GAACGCACGTTCCACC.... (Ohno hypothetical PR.C sequence)
....TCGGAGACACTCGATGAACGCACGTTCCACC.... (actual *nylB* gene seen in KI72)

By Ohno's own admission, this was speculation, but somehow his hypothesis came to be generally accepted in popular literature as if it were a documented historical event.⁴¹ Ohno's simple thought experiment became widely accepted as an empirical proof that functional proteins can routinely and instantly emerge from what are essentially random DNA sequences.⁴²

The problem of stop codons – Ohno's broader theory is now largely forgotten, but unfortunately his hypothetical illustration (the *nylB* frame-shift hypothesis) has come to be mistaken as an historical event. The reason that Ohno's broader thesis is now forgotten is because geneticists understand that frame-shift mutations consistently result in multiple stop codons, which result in the premature truncation of any hoped-for new enzymes.

In the particular example of the *nylB* gene, Ohno had speculated that there just happened to be an alternative long open reading frame in the region of the presumed precursor gene. Statistically, this would have been very improbable. Without the fortuitous pre-existence of the long alternative open reading frame, Ohno would have had no rational basis for his frame-shift model. For this reason, Ohno needed to explain the fortuitous alternative ORF. He did this by claiming that the NylB protein sequence just happens to have a great deal of internal repeats, which perhaps tended to exclude stop codons. He tried to support this idea in Figure 2 of his paper.⁴³ He shows a handful of very short amino acid strings that occur more than once within the NylB protein sequence. Ohno's claim of extreme internal redundancy is not persuasive - the actual amount of internal homology does not appear to be greater than expected, and Ohno did no statistical analysis that would support the idea that internal similarities were more abundant than expected by chance. In this light, Ohno's hypothesis requires that the long alternative reading frame (that would fortuitously and instantly give rise to an active nylonase enzyme), arose by extremely good luck - against long odds. Given this limitation, even if his NylB model were correct, it would not help support his broader thesis that new genes typically arise by frame shift mutations.

The apparent non-existence of the reputed precursor gene - If the *nylB* gene arose just a few decades ago, due to a simple frame shift mutation in a previously existing gene, it would be reasonable to expect evidence for the original precursor gene/protein (which should still be found in many places in the biosphere). Ohno predicted the exact precursor DNA sequence, and the exact protein sequence of the hypothetical precursor gene. We have looked for Ohno's hypothetical precursor gene using BLASTN and BLASTP searches and have failed to detect clear evidence for Ohno's proposed precursor DNA or protein sequences. This is direct evidence against Ohno's hypothesis.

It is important to note that alternate reading frames do exist in biology, whereby different proteins can be alternately read within the same genetic locus via frame-shift *reading* (not *mutation*). In systems where such frame-shifted *reads* are needed by the organism, such overlapping genetic elements would

be even less tolerant to frame-shift *mutations* than normal. So the existence of functional alternate reading frames does not help Ohno's frame-shift mutation hypothesis.

Given that BLASTP returned 11 organisms with predicted proteins similar to NylB and UNIPROT found 193 organisms with provisional proteins similar to NylB, it is striking that the same databases gave no proteins similar to Ohno's hypothetical PR.C sequence. The most reasonable explanation for the lack of evidence for Ohno's hypothetical ancestral PR.C sequence is that it never existed, and the post-1935 frame-shift mutation never happened.

The problem of creating a stable and functional protein from a random sequence - When Ohno published his frame shift hypothesis it appears he was not aware of the extreme improbability of establishing a random *de novo* protein sequence that can fold into a stable form and can effectively perform any specific function. It is widely understood that a random amino acid sequence is not likely to be stable in the cellular environment, and that even if it were to be stable, it would have essentially zero chance of having a specific function directly relevant to the needs of the organism. Since the time of Ohno we have learned a great deal about the nature of biological information and the daunting probabilistic constraints that limit the creation of meaningful biological information from random strings of characters.^{44,45}

The difficulty of explaining of all the other *nylB* genes and homologs - If a frame-shift mutation gave rise to the original *nylB* gene, this raises the problem of where the *nylB'* gene, and the many other *nylB* homologs came from, especially the ones having mostly structural homology in the coded proteins rather than sequence homology in the genes. The *nylB'* gene is only 88% identical to the *nylB* gene. It is not reasonable to invoke the identical frame shift mutation in both genes.

In regard to this problem, Ohno's thinking seems ambiguous:

“It is of interest to note here that this stretch of base sequence is duplicated elsewhere within the pOAD2 genome roughly 90° away and that the coding sequence for the second isozymic form of 6-AHA LOH is found in this duplicated stretch. Thus, a pair of isozymic preexisted coding sequences might have given rise independently to the coding sequences for two isozymic forms of 6-AHA LOH.”

In regard to this same problem, Thwaites of the NCSE has interpreted Ohno's comments as indicating that two identical frame shifts happened independently on the same plasmid, creating two different but homologous nylonases.⁴⁶

A similar problem arises for the *nylB* and *nylB'* genes found in the chromosome of *Agromyces KYR5* which are also each 1179 bases in length. Given this additional problem, one must invoke even more unreasonable assumptions. There has to be a very specific frame shift mutation followed by horizontal gene transfer of both new genes to another type of bacteria. Alternatively, Ohno's frame shift mutation must have happened identically but independently four times in four separate reading frames, in two different genera.

The problem of non-trivial errors - Lastly, it appears Ohno made some non-trivial errors in his PNAS publication. For example, he states in the abstract that the hypothetical precursor (PR.C) was 472 amino acids long, but in his figures caption he says (correctly), it was 427 amino acids long. A more serious error arises in the final part of his PR.C sequence, which inadvertently results in a pre-mature stop codon. He makes no mention of why he deleted a guanine from the tail end of his PR.C sequence,

thus creating a pre-mature stop codon for his hypothetical protein. The tail end of his hypothetical PR.C sequence reads "GCGGCTGA" but the Genbank entries of the RS-II_A sequence of KI72 and Okada's paper records the proper sequence as "GCGGCGTGA". (See "Supplement G" that describes in detail Ohno's errors.) On many levels, the paper by Ohno seems poorly done, which seems surprising for a publication of the National Academy of Science.

Problems with Okada's 1983 Gene Duplication Hypothesis

Ironically, Ohno's hypothesis emerged in part because of problems he perceived in Okada's hypothesis. The gene duplication hypothesis of Okada *et al.* was proposed a year before Ohno's paper in 1983.⁴⁷ Okada *et al.* assumed, as did Ohno, that the *nylB* evolved sometime after 1935. Okada *et al.* proposed that *nylB* arose from the duplication of the linked *nylB'*. But Okada's model now seems very unlikely for several reasons.

Firstly, Negoro reported in 1992 that *nylB* shares a promoter with *nylC*,⁴⁸ and this strongly suggests a pre-1935 coordinated function involving both *nylB* and *nylC*. This leaves *nylC* entirely unexplained, and strongly argues that *nylB* and *nylC* emerged as a functional unit (a polycistron), much earlier than 1935.

If there had been a gene duplication, it would be much more reasonable to invoke the hypothesis that *nylB* gave rise to *nylB'*, rather than vice versa. Otherwise a random duplicate copy of *nylB* would have to arise and fortuitously land in the *nylC* cistron at precisely the right position for the co-regulation of the active nylonase NylC and the not-yet-nylonase NylB. Then the not-yet-nylonase NylB would have to accumulate a very large number of mutations on its way to becoming the active NylB that is co-regulated with NylC. The genes *nylB* and *nylB'* differ by 140 point mutations (out of 1179 bases).⁴⁹ This very precise insertion point for the duplication event, followed by so many mutations, would have to happen in just a few decades, which seems extremely unlikely - as pointed out by Ohno.⁵⁰

A diagram in S. Negoro's 2000 paper⁵¹ suggests that the NylA and NylC nylonases work together with the NylB nylonase. This apparently involves a catabolic pathway wherein NylA and NylC degrade complex cyclic nylon oligomers to nylon linear dimers, which are then degraded by the NylB nylonase. This sort of coordinated specialization between three non-homologous nylonase genes, two of which share the same promoter, is remarkable. If this nylon degrading cascade composed of NylA, NylB and NylC evolved post-1935, it would require simultaneous evolutionary convergence and coordination from three non-homologous genes. How could all this come together in a few decades? Given the shared promoter and the 3-way cooperation of three unrelated nylonases, it is not credible that NylB arose very recently via random genetic duplication. This sophisticated genetic network must have existed long before 1935, for the purpose of catabolizing an unknown natural substance.

In addition to the above problems, Ohno's frame shift hypothesis and Okada's recent duplication scenario are now falsified because of the many organisms that we have now shown have either *nylB* genes or homologs (see literature review above). Such genes are not found in nylon-rich environments but are found in pathogens, soils and oceans. UNIPROT listed 193 organisms with predicted *nylB-like* genes and 125 organisms with predicted *nylB'-like* genes. It seems clear that we do not yet know the origin of either *nylB* or *nylB'* - just as we do not know the origin of *nylA* or *nylC*. There is no reason to think any of these genes arose *de novo* in the recent past.

CONCLUSION

We began this work hoping to better understanding the various claims of the *de novo* origin of certain nylonase genes. Kinoshita claimed the nylonases he studied were newly evolved enzymes that did not exist prior to 1935. Okada claimed the “newly evolved” NylB enzyme arose via gene duplication of NylB' followed by 47 residue substitutions. Ohno claimed the “*de novo*” NylB and NylB' enzymes arose via two independent single frame shift mutations in each of the corresponding genes which instantly created two functional *de novo* proteins.

Our analyses indicate that nylonase genes are actually abundant, come in many diverse forms, are found in a great number of organisms, and such organisms are found in a great number of natural environments. We show there is no reason to think that any of these nylonases emerged since 1935, and so there is no basis for invoking any *de novo* genes arising since 1935. Furthermore, there are numerous glaring problems with the specific *de novo* speculations of Okada and Ohno. The early claims of *de novo* nylonase genes were unsupported and speculative, and in light of new data these hypotheses now appear to be unwarranted and essentially falsified.

The discovery of numerous naturally-occurring genes having nylonase activity, along with a multitude of homologous genes and proteins that provisionally have similar activities, opens the door to further exploration of nylonases and their functions.

ENDNOTES

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- ¹ Negoro, S. Biodegradation of Nylon Oligomers. *Appl Microbiol Biotechnol* (2000) 54:461-466. Springer-Verlag 2000.
- ² Negoro, S. Biodegradation of Nylon Oligomers. *Appl Microbiol Biotechnol* (2000) 54:461-466. Springer-Verlag 2000.
- ³ Kinoshita, S. Purification and Characterization of 6-Aminohexanoic-Acid-Oligomer Hydrolase of *Flavobacterium* sp. KI72. *Eur. J. Biochem.* 116, 547-551 (1981).
- ⁴ Kinoshita, S. Purification and Characterization of 6-Aminohexanoic-Acid-Oligomer Hydrolase of *Flavobacterium* sp. KI72. *Eur. J. Biochem.* 116, 547-551 (1981). The paper says: "As regular nylon fiber is higher than dimer, this enzyme can not hydrolyze ordinary nylon."
- ⁵ Ebata, M. Morita, K. Hydrolysis of ϵ -Aminocaproic Compounds by Trypsin. *The Journal of Biochemistry*, Vol 46, No. 4, 1959.
- ⁶ Fukumura, T. Hydrolysis of Cyclic and Linear Oligomers of 6-Aminocaproic Acid by a Bacterial Cell Extract. *The Journal of Biochemistry*, Vol. 59, No. 6, 1966.
- ⁷ Kiumarsi, A. Parvinzadeh, M. "Enzymatic Hydrolysis of Nylon 6 Fiber Using Lipolytic Enzyme." *Journal of Applied Polymer Science* DOI 10.1002/app. 4 June 2009.
- ⁸ Fukumura, T. "Hydrolysis of Cyclic and Linear Oligomers of 6-Aminocaproic Acid by a Bacterial Cell Extract". *The Journal of Biochemistry*, Vol. 59, No. 6, 1966.
- ⁹ Mahdi, MS. Ameen, R. Ibrahim, H. "Study on Degradation of Nylon 6 by thermophilic bacteria *Anoxybacillus rupiensis* Ir3 (JQ912241)." *International Journal of Advanced Research in Biological Sciences*. ISSN:2348-8069. DOI: 10.22192/ijarbs. Coden:IJARQG(USA) Volume 3, Issue 9- 2016. www.ijarbs.com
- ¹⁰ The re-naming history is complex. The first name change was noted by Ohno in 1984 in "Birth of a unique enzyme from an alternative reading frame of the preexisted, internally repetitive coding sequence. *Proc Natl. Acad. Sci. USA* Vol 81, pp. 2421-2425, April 1984. Evolution." The second name change was noted in "Genetic Organization of Nylon-Oligomer-Degrading Enzymes from Alkalophilic Bacteria, *Agromyces* sp. KYR5. *Journal of Bioscience and Bioengineering*. Vol. 104, No. 6, 521-524, 2007. DOI: 10.1263/jbb.104.521."
- ¹¹ Kinoshita, S. Kageyama, S. Iba, K. Yamada, Y. Okada, H. "Utilization of a Cyclic Dimer and Linear Oligomers of ϵ -Aminocaproic Acid by *Achromobacter guttatus* KI 72." *Agr. Biol. Chem.*, 39 (6), 1219-1223, 1975.
- ¹² The reference for 1973 discovery was in the end note of Kinoshita's 1975 "Utilization of Cyclic Dimer and Linear Oligomers of ϵ -Aminocaproic Acid by *Achromobacter guttatus* KI 72" paper. The end note said "Kinoshita, S. Kobayashi, E. Okada, H. *J Ferment. Technol.*, 51, 719(1973)." That Journal appears to be out of print and is not readily available.
- ¹³ Kinoshita, S. Kageyama, S. Iba, K. Yamada, Y. Okada, H. "Utilization of a Cyclic Dimer and Linear Oligomers of ϵ -Aminocaproic Acid by *Achromobacter guttatus* KI 72." *Agr. Biol. Chem.*, 39 (6), 1219-1223, 1975.
- ¹⁴ Kinoshita, S. Negoro, S. Muramatsu, M. Bisaria, V. Sawada, S. Okada, H. "6-Aminohexanoic Acid Cyclic Dimer Hydrolase. A New Cyclic Amide Hydrolase Produced by *Achromobacter guttatus* KI72." *Eur. J. Biochem.* 80,489-495 (1977).
- ¹⁵ Kinoshita, S. Terada, T. Taniguchi, T. Takene, Y. Masuda, S. "Purification and Characterization of 6-Aminohexanoic-Acid-Oligomer Hydrolase of *Flavobacterium* sp. KI72.
- ¹⁶ Kinoshita, S. Negoro, S. Muramatsu, M. Bisaria, V. Sawada, S. Okada, H. "6-Aminohexanoic Acid Cyclic Dimer Hydrolase. A New Cyclic Amide Hydrolase Produced by *Achromobacter guttatus* KI72." *Eur. J. Biochem.* 80,489-495 (1977).
- ¹⁷ Okada, H. Negoro, S. Kimura, H. Nakamura, S. "Evolutionary adaptation of plasmid-encoded enzymes for degrading nylon oligomers." *Nature* Vol. 306 November 1983.
- ¹⁸ Kinoshita, S. Purification and Characterization of 6-Aminohexanoic-Acid-Oligomer Hydrolase of *Flavobacterium* sp. KI72. *Eur. J. Biochem.* 116, 547-551 (1981).
- ¹⁹ <http://biologos.org/blogs/dennis-venema-letters-to-the-duchess/intelligent-design-and-nylon-eating-bacteria/>
- ²⁰ <https://ncse.com/cej/5/2/new-proteins-without-gods-help>
- ²¹ Miller, K. Only a Theory: Evolution and the Battle for America's Soul. Penguin June 12, 2008.
- ²² Negoro, S. Shinagawa, H. Nakata, A. Kinoshita, S. Hatozaki, T. Okada H. "Plasmid Control of 6-Aminohexanoic Acid Cyclic Dimer Degradation of Enzymes of *Flavobacterium* sp KI72." *Journal of Bacteriology*, July 1980, p. 238-245. 0021-9193/80/00-238/08\$02.00/0.
- ²³ Sudhakar, M. Priyadarshini, C. Doble, M. Murthy, P. Venkatesan, R. "Marine bacteria mediated degradation of nylon 66 and 6." *International Biodeterioration & Biodegradation*. 60 (2007) 144-151.
- ²⁴ The UNIPROT number for the 6-aminohexanoate hydrolase in *Cryobacteria arcticum*, an organism found in Arctic is A0A1B1BHD0.

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- ²⁵ Bajerski, F. Ganzer, L. Mangelsdorf, K. Lipski, A. Wagner, D. “Cryobacterium arcticum sp. nov., a psychrotolerant bacterium from an Arctic soil”. 01 August 2011, *International Journal of Systematic and Evolutionary Microbiology*. Microbiology 61:1849-1853, doi:10.1099/ijs.0.027128-0.
- ²⁶ Negoro, S. Kakudo, S. Urabe, I. Okada, H. “A New Nylon Oligomer Degradation Gene (nylC) on Plasmid pOAD2 from *Flavobacterium* sp.” *Journal of Bacteriology*, Dec. 1992, p 7948-7953 0021-9193/92/247948-06\$02.00/0.
- ²⁷ Negoro, S. Kakudo, S. Urabe, I. Okada, H. “A New Nylon Oligomer Degradation Gene (nylC) on Plasmid pOAD2 from *Flavobacterium* sp.” *Journal of Bacteriology*, Dec. 1992, p 7948-7953 0021-9193/92/247948-06\$02.00/0.
- ²⁸ Negoro, S. Kakudo, S. Urabe, I. Okada, H. “Genetic Organization of Nylon-Oligomer-Degrading Enzymes from Alkalophilic Bacteria, *Agromyces* sp. KYR5.” *Journal of Bioscience and Bioengineering*. Vol. 104, No. 6, 521-524, 2007. DOI: 10.1263/jbb.104.521.
- ²⁹ Prijambada, I. Negoro, S. Yomo, T. Urabae, I. “Emergence of Nylon Oligomer Degradation Enzymes in *Pseudomonas aeruginosa* PAO through Experimental Evolution.” *Applied and Environmental Microbiology*, May 1995, p 2020-2022. 0099-2240/95/\$04.00+0.
- ³⁰ Kato, K. Fujiyama, K. Hatanaka, H. Priyambada, I. Negoro, S. Urabe, I. “Amino acid alterations essential for increasing the catalytic activity of the nylon-oligomer-degradation enzyme of *Flavobacterium* sp.” *Eur. J. Biochem*. 200, 165-169(1991). 0014295691000522N.
- ³¹ Rost, B. “Twilight zone of protein sequence alignments.” *Protein Engineering* vol. 12 no.2 pp85-94, 1999.
- ³² Rost, B. “Protein structures sustain evolutionary drift.” *Fold Des*. 1997;2(3):219-24.
- ³³ The GenBank accession number is D10686 for *nylB* *Flavobacteria* sp. Plasmid pOAD2. The *Flavobacteria* strain was renamed to *Arthrobacteria*, and was formerly named *Acromobacter guttatus*
- ³⁴ The UNIPROT entry for *nylB_1* for *Bacillus cereus* is A0A0K6IYF0
- ³⁵ The UNIPROT entry for *nylB* for *Streptococcus pneumoniae* is A0A0u0EFW2.
- ³⁶ Kinoshita, S. Negoro, S. Muramatsu, M. Bisaria, V. Sawada, S. Okada, H. “6-Aminohexanoic Acid Cyclic Dimer Hydrolase. A New Cyclic Amide Hydrolase Produced by *Acromobacter guttatus* KI72.” *Eur. J. Biochem*. 80,489-495 (1977).
- ³⁷ Okada, H. Negoro, S. Kimura, H. Nakamura, S. “Evolutionary adaptation of plasmid-encoded enzymes for degrading nylon oligomers.” *Nature* Vol. 306 November 1983.
- ³⁸ Ohno, S. “Birth of a unique enzyme from an alternative reading frame of the preexisted, internally repetitious coding sequence.” *Proc Natl. Acad. Sci. USA*. Vol. 81, pp. 2421-2425, April 1984. Evolution.
- ³⁹ <http://biologos.org/blogs/dennis-venema-letters-to-the-duchess/intelligent-design-and-nylon-eating-bacteria/>
- ⁴⁰ Miller, K. Only a Theory: Evolution and the Battle for America’s Soul. Penguin June 12, 2008.
- ⁴¹ <http://biologos.org/blogs/dennis-venema-letters-to-the-duchess/intelligent-design-and-nylon-eating-bacteria/>
- ⁴² <https://ncse.com/cej/5/2/new-proteins-without-gods-help>
- ⁴³ Ohno, S. “Birth of a unique enzyme from an alternative reading frame of the preexisted, internally repetitious coding sequence.” *Proc Natl. Acad. Sci. USA*. Vol. 81, pp. 2421-2425, April 1984. Evolution.
- ⁴⁴ Marks, R. Dembski, W. Sanford, J. Behe, M. Gordon, M. *Biological Information: New Perspectives*. <http://www.biologicalinformationnewperspectives.org/>
- ⁴⁵ Axe, D. “Estimating the Prevalence of Protein Sequences Adopting Functional Enzyme Folds” (*Axe, J Mol Biol* 341, 1295-1315, 2004).
- ⁴⁶ <https://ncse.com/cej/5/2/new-proteins-without-gods-help>
- ⁴⁷ Okada, H. Negoro, S. Kimura, H. Nakamura, S. “Evolutionary adaptation of plasmid-encoded enzymes for degrading nylon oligomers.” *Nature* Vol. 306 November 1983.
- ⁴⁸ Negoro, S. Kakudo, S. Urabe, I. Okada, H. “A New Nylon Oligomer Degradation Gene (nylC) on Plasmid pOAD2 from *Flavobacterium* sp.” *Journal of Bacteriology*, Dec. 1992, p 7948-7953 0021-9193/92/247948-06\$02.00/0.
- ⁴⁹ Okada, H. Negoro, S. Kimura, H. Nakamura, S. “Evolutionary adaptation of plasmid-encoded enzymes for degrading nylon oligomers.” *Nature* Vol. 306 November 1983.
- ⁵⁰ Ohno, S. “Birth of a unique enzyme from an alternative reading frame of the preexisted, internally repetitious coding sequence.” *Proc Natl. Acad. Sci. USA*. Vol. 81, pp. 2421-2425, April 1984. Evolution.
- ⁵¹ Negoro, S. Biodegradation of Nylon Oligomers. *Appl Microbiol Biotechnol* (2000) 54:461-466. Springer-Verlag 2000.

SUPPLEMENTAL MATERIALS

Supplement A

List of organisms with predicted or actual
nylA genes from UNIPROT

METHODS

The raw list is simply generated by going to www.UNIPROT.org and typing “nylA” into the search box. As of this writing it will generate 399 entries. Care was taken to remove spurious search hits such as those that contain words like “nylandria” which is a species of ants since the word “nylandria” contains the string “nyla”.

RESULTS

The following list took those 399 entries and removed most of the duplicates and spurious hits. Around 162 species or strains were listed to have actual or predicted *nylA* genes or homologs.

Achromobacter piechaudii ATCC 43553
Achromobacter sp.
Acidiphilium sp. JA12-A1
Acidithrix ferrooxidans
Acinetobacter johnsonii
Acinetobacter venetianus
Aerococcus viridans ATCC 11563 = CCUG 4311
Agrobacterium sp. DSM 25559
Alcaligenes xylosoxydans xylosoxydans (Achromobacter xylosoxidans)
Altererythrobacter dongtanensis
Amycolatopsis sp. M39
Anaeromyxobacter sp. PSR-1
Aneurinibacillus soli
Bacillus beveridgei
Bacillus pumilus (Bacillus mesentericus)
Bacillus subtilis BEST7613
Bacillus thermoamylovorans
bacterium ADurb.BinA028
bacterium YEK0313
Blastococcus saxobsidens (strain DD2)
Bordetella ansorpii
Bordetella hinzii
Bordetella pertussis
Bordetella trematum
Burkholderia mallei (strain NCTC 10229)
Burkholderia mallei (strain ATCC 23344)
Burkholderia pseudomallei (Pseudomonas pseudomallei)
Burkholderia pseudomallei (strain 1710b)
Candidatus Endolissoclinum faulkneri L5
Carnobacterium maltaromaticum LMA28
Chlamydia trachomatis
Cupriavidus necator (strain ATCC 43291 / DSM 13513 / N-1) (Ralstonia eutropha)

Deltaproteobacteria bacterium ADurb.Bin002
Deltaproteobacteria bacterium ADurb.Bin022
Deltaproteobacteria bacterium ADurb.Bin072
Deltaproteobacteria bacterium ADurb.Bin151
Deltaproteobacteria bacterium ADurb.BinA179
Desmospora sp. 8437
Endozoicomonas sp. S-B4-1U
Enterococcus faecalis TX0104
Enterococcus faecium (Streptococcus faecium)
Ferrimicrobium acidiphilum DSM 19497
Flavobacterium sp. (strain K172)
Granulicatella adiacens ATCC 49175
Halobacillus karajensis
Janthinobacterium lividum
Janthinobacterium sp. HH106
Janthinobacterium sp. KBS0711
Janthinobacterium sp. MP5059B
Jeotgalibaca dankookensis
Komagataeibacter rhaeticus
Labrenzia alba
Lactobacillus brevis subsp. gravesensis ATCC 27305
Lactobacillus buchneri ATCC 11577
Lactobacillus buchneri CD034
Lactobacillus fermentum ATCC 14931
Lactobacillus fermentum
Lactobacillus hilgardii DSM 20176 = ATCC 8290
Lactobacillus iners DSM 13335
Lactobacillus paracasei subsp. paracasei ATCC 25302
Lactobacillus ruminis ATCC 25644
Lactobacillus sunkii
Lactobacillus vaginalis DSM 5837 = ATCC 49540
Lactococcus garvieae
Legionella massiliensis
Legionella pneumophila
Leptolyngbya sp. O-77
Leptospira interrogans serogroup Icterohaemorrhagiae serovar copenhageni (strain Fiocruz L1-130)
Limnohabitans sp. 63ED37-2
Listeria monocytogenes
Marinobacter adhaerens (strain HP15)
Marinobacter hydrocarbonoclasticus ATCC 49840
Marinobacter salarius
Marinomonas gallaica
Marinomonas spartinae
Mesorhizobium huakuii 7653R
Mesorhizobium plurifarum
Mesorhizobium sp. ORS3359
Mesorhizobium sp. SOD10
Methylobrevis pamukkalensis
Microbacterium azadirachtae

mine drainage metagenome
Mycobacterium abscessus subsp. abscessus
Mycobacterium abscessus subsp. massiliense
Mycobacterium abscessus subsp. bolletii
Mycobacterium abscessus
Mycobacterium chlorophenicum
Mycobacterium chubuense
Mycobacterium kansasii 824
Mycobacterium kansasii
Mycobacterium obuense
Mycobacterium parascrofulaceum ATCC BAA-614
Mycobacterium smegmatis (strain MKD8)
Mycobacterium smegmatis
Mycobacterium tuberculosis
Mycobacterium ulcerans str. Harvey
Mycobacterium xenopi 3993
Myxococcus xanthus (strain DK 1622)
Neisseria macacae ATCC 33926
Neisseria shayeganii 871
Nitrincola nitratireducens
Nocardia farcinica
Nocardioides dokdonensis FR1436
Oceanobacillus oncorhynchi
Oceanobacillus picturae
Oleispira antarctica RB-8
Paenibacillus riograndensis SBR5
Paenibacillus sp. AD87
Paraliobacillus sp. PM-2
Phaeobacter sp. CECT 5382
Planktothrix agardhii NIVA-CYA 126/8
Providencia burhodogranaria DSM 19968
Pseudarthrobacter siccitolerans
Pseudomonas citronellolis
Pseudomonas putida (Arthrobacter siderocapsulatus)
Pseudomonas sp. 1 R 17
Pseudomonas sp. 22 E 5
Pseudomonas sp. 58 R 3
Pseudomonas sp. (strain NK87)
Pseudomonas sp. TAD18
Pseudomonas syringae pv. tomato
Pseudonocardia autotrophica (Amycolata autotrophica) (Nocardia autotrophica)
Pseudovibrio sp. Ad26
Psychrobacter sp. 1501(2011)
Psychrobacter sp. CIP 110853
Psychrobacter sp. CIP 110854
Psychrobacter sp. DAB_AL43B
Rathayibacter tanaceti
Rhizobium etli CIAT 894
Rhizobium tibeticum

Rhodococcus erythropolis (Arthrobacter picolinophilus)
Rhodococcus fascians D188
Rhodococcus sp. AD45
Rhodococcus sp. Br-6
Rhodococcus sp. PBTS 2
Rhodococcus sp. PBTS 1
Roseomonas sp. TAS13
Ruegeria atlantica
Ruegeria pomeroyi (strain ATCC 700808 / DSM 15171 / DSS-3) (Silicibacter pomeroyi)
Ruegeria sp. CECT 5091
Serratia
Shinella sp. DD12
Sinorhizobium meliloti (strain SM11)
Spirochaetes bacterium ADurb.BinA120
Streptococcus equi subsp. equi
Streptococcus mutans serotype c (strain ATCC 700610 / UA159)
Streptococcus mutans
Streptococcus pneumoniae
Streptococcus salivarius (strain CCHSS3)
Streptococcus salivarius (strain JIM8777)
Streptococcus suis
Streptomyces reticuli
Streptomyces scabiei
Streptomyces sp. AVP053U2
Streptomyces sp. F-1
Synechocystis sp. (strain PCC 6714) (Aphanocapsa sp. (strain PCC 6714))
Syntrophaceae bacterium PtaB.Bin095
Tistrella mobilis (strain KA081020-065)
Variovorax paradoxus
Vibrio celticus
Vibrio hemicentroti
Virgibacillus massiliensis

Supplement B

List of organisms with predicted or actual
nylB genes from UNIPROT

METHODS

The raw list is simply generated by going to www.UNIPROT.org and typing “nylB” into the search box. As of this writing it will generate 478 entries.

RESULTS

The following list took those 478 entries and removed most of the duplicates. Unfortunately there were many *nylB'* entries returned by the search, and to create the following list, the *nylB'* entries were manually removed.

Around 193 species or strains were listed to have actual or predicted *nylB* genes or homologs.

[Eubacterium] angustum
[Eubacterium] contortum
[Eubacterium] eligens
[Ruminococcus] torques
Acetobacterium wieringae
Achromobacter sp.
Acinetobacter baumannii
Actinobacteria bacterium ADurb.BinA094
Agromyces sp. KY5R
Agromyces sp. NDB4Y10
alpha proteobacterium Q-1
Alphaproteobacteria bacterium ADurb.Bin100
Alphaproteobacteria bacterium ADurb.BinA280
Altererythrobacter atlanticus
Altererythrobacter dongtanensis
Altererythrobacter namhicola
Amantichitinum ursilacus
Amycolatopsis sp. M39
Arenibacter sp. C-21
Arthrobacter sp. Rue61a
Bacillus anthracis
Bacillus cereus
Bacillus subtilis
Bacillus thuringiensis Bt18247
Bacillus thuringiensis
Bacillus toyonensis
Bacillus weihenstephanensis
bacterium ADurb.Bin478
bacterium YEK0313
Bacteroidales bacterium Barb4
Bacteroides stercoris
Bacteroides uniformis
Bacteroidetes bacterium ADurb.Bin008

Bacteroidetes bacterium ADurb.Bin035
Bacteroidetes bacterium ADurb.Bin041
Bacteroidetes bacterium ADurb.Bin123
Bacteroidetes bacterium ADurb.Bin141
Bacteroidetes bacterium ADurb.Bin145
Bacteroidetes bacterium ADurb.BinA012
Bacteroidetes bacterium ADurb.BinA174
Betaproteobacteria bacterium ADurb.Bin341
Blautia obeum
Blautia wexlerae
blood disease bacterium R229
Bradyrhizobium japonicum SEMIA 5079
Burkholderia gladioli (Pseudomonas marginata) (Phytomonas marginata)
Candidatus Accumolibacter sp. SK-02
Candidatus Accumolibacter sp. BA-92
Candidatus Accumolibacter sp. SK-01
Candidatus Aminicenantes bacterium ADurb.Bin147
Candidatus Hydrogenedentes bacterium ADurb.Bin101
Candidatus Thorarchaeota archaeon AB_25
Catenibacterium mitsuokai
Cecembia lonarensis LW9
Chlamydia abortus (Chlamydophila abortus)
Chloroflexi bacterium ADurb.Bin222
Chloroflexi bacterium ADurb.Bin325
Chryseobacterium sp. MOF25P
Clostridium innocuum
Clostridium ragsdalei P11
Clostridium sp. C105KSO15
Clostridium sp. FS41
Coproccoccus comes
Coralloccoccus coralloides (strain ATCC 25202 / DSM 2259 / NBRC 100086 / M2) (Myxococcus coralloides)
Cupriavidus metallidurans (strain ATCC 43123 / DSM 2839 / NBRC 102507 / CH34) (Ralstonia metallidurans)
Cupriavidus necator (strain ATCC 43291 / DSM 13513 / N-1) (Ralstonia eutropha)
Cupriavidus taiwanensis (strain DSM 17343 / BCRC 17206 / CIP 107171 / LMG 19424 / R1) (Ralstonia taiwanensis (strain LMG 1944))
Deltaproteobacteria bacterium ADurb.Bin072
Deltaproteobacteria bacterium ADurb.Bin151
Deltaproteobacteria bacterium ADurb.BinA014
Eisenbergiella tayi
Endozoicomonas sp. S-B4-1U
Enterobacter cloacae EcWSU1
Enterobacter cloacae
Enterobacter kobei
Escherichia coli
Fibrella aestuarina BUZ 2
Firmicutes bacterium ADurb.Bin182
Firmicutes bacterium ADurb.Bin248

Firmicutes bacterium ADurb.Bin262
Flavobacteriaceae bacterium UJ101
Flavobacterium sp. (strain K172)
Fusicatenibacter saccharivorans
Fusicatenibacter
Gammaproteobacteria bacterium MOLA455
Isoptericola dokdonensis DS-3
Jannaschia rubra
Janthinobacterium lividum
Janthinobacterium sp. HH106
Janthinobacterium sp. KBS0711
Janthinobacterium sp. MP5059B
Kineosphaera limosa NBRC 100340
Klebsiella aerogenes (Enterobacter aerogenes)
Klebsiella oxytoca
Klebsiella pneumoniae
Komagataeibacter europaeus (Gluconacetobacter europaeus)
Labrenzia alba
Limnohabitans sp. 63ED37-2
Marinobacter hydrocarbonoclasticus ATCC 49840
Marinomonas spartinae
Mesorhizobium huakuii 7653R
Mesorhizobium prunaredense
Mesorhizobium sp. STM 4661
Methylobacterium radiotolerans
Microbacterium azadirachtae
Microbacterium ketosireducens
Microbacterium oxydans
Microbacterium sp. HM58-2
Microbacterium sp. SA39
Microbacterium sp. TNHR37B
Microbacterium trichothecenolyticum
Microlunatus phosphovorius (strain ATCC 700054 / DSM 10555 / JCM 9379 / NBRC 101784 / NCIMB 13414 / VKM Ac-1990 / NM-1)
mine drainage metagenome
Mucilaginibacter gotjawali
Mycobacterium abscessus subsp. abscessus
Mycobacterium chlorophenolicum
Mycobacterium tuberculosis
Nocardia asteroides NBRC 15531
Nocardia seriola
Paenarthrobacter aurescens (strain TC1)
Paenibacillus sp. P1XP2
Paenibacillus sp. P1XP2
Paenibacillus sp. TI45-13ar
Paeniclostridium sordellii (Clostridium sordellii)
Paeniglutamicibacter gangotriensis Lz1y
Paludisphaera borealis
Parabacteroides distasonis

Phaeobacter sp. CECT 5382
Photorhabdus asymbiotica subsp. australis
Planctomyces sp. SH-PL14
Planctomyces sp. SH-PL62
Planctomycetes bacterium ADurb.Bin126
Planktotalea frisia
Pseudomonas aeruginosa DK1
Pseudomonas aeruginosa
Pseudomonas citronellolis
Pseudomonas fluorescens
Pseudomonas putida (Arthrobacter siderocapsulatus)
Pseudomonas sp. 22 E 5
Pseudomonas sp. 37 R 15
Pseudomonas sp. 44 R 15
Pseudomonas sp. TAD18
Pseudomonas sp. URMO17WK12:I11
Pseudomonas sp.
Pseudomonas syringae pv. actinidiae
Pseudovibrio axinellae
Pseudovibrio sp. (strain FO-BEG1)
Psychrobacter sp. DAB_AL43B
Ralstonia solanacearum (strain Po82)
Ralstonia solanacearum CFBP2957
Ralstonia solanacearum CMR15
Ralstonia solanacearum K60-1
Ralstonia solanacearum PSI07
Ralstonia syzygii R24
Rhizobium etli CIAT 894
Rhizobium favelukesii
Rhizobium tibeticum
Rhodobacter capsulatus (strain ATCC BAA-309 / NBRC 16581 / SB1003)
Rhodococcus wratislaviensis NBRC 100605
Roseburia intestinalis
Roseivivax jejudonensis
Roseobacter denitrificans (strain ATCC 33942 / OCh 114) (Erythrobacter sp. (strain OCh 114))
(Roseobacter denitrificans)
Roseovarius gaetbuli
Ruegeria atlantica
Ruegeria sp. CECT 5091
Serratia marcescens
Shimia marina
Shimia sp. SK013
Shinella sp. DD12
Sinorhizobium meliloti (strain SM11)
Sphingobium sp. EP60837
Spirochaetes bacterium ADurb.Bin215
Streptococcus pneumoniae
Streptomyces acidiscabies
Streptomyces sp. AVP053U2

Streptomyces sp. F-1
 Streptomyces sp. PTY087I2
 Tannerella forsythia (Bacteroides forsythus)
 Thalassobius gelatinovorius (Ruegeria gelatinovora)
 Thalassobius mediterraneus
 Tistrella mobilis (strain KA081020-065)
 uncultured Anaerotruncus sp.
 uncultured Blautia sp.
 uncultured Clostridium sp.
 uncultured Coprococcus sp.
 uncultured Eubacterium sp.
 uncultured Ruminococcus sp.
 Variovorax paradoxus
 Verrucomicrobia bacterium ADurb.Bin006
 Verrucomicrobia bacterium ADurb.Bin018
 Vibrio azureus NBRC 104587
 Vibrio celticus
 Vibrio scophthalmi

NOTE some sample screen shots of the entries:

UniProtKB - A0A0K6IYF0 (A0A0K6IYF0_BACCE)

Display
 Entry
 Publications
 Feature viewer
 Feature table

All None
 Function
 Names & Taxonomy
 Subcellular location
 Pathology & Biotech
 PTM / Processing
 Expression
 Interaction
 Structure
 Family & Domains
 Sequence
 Similar proteins
 Cross-references
 Entry information
 Miscellaneous

Protein Submitted name: **6-aminohexanoate-dimer hydrolase**
Gene **nylB_1**
Organism *Bacillus cereus*
Status Unreviewed - Annotation score: ●○○○○ - Protein predictedⁱ

Functionⁱ
GO - Molecular functionⁱ
 6-aminohexanoate-dimer hydrolase activity Source: UniProtKB-EC

Keywordsⁱ
 Molecular function Hydrolase Imported

Names & Taxonomyⁱ

Protein names ¹	Submitted name: 6-aminohexanoate-dimer hydrolase Imported (EC:3.5.1.46 Imported)
Gene names ¹	Name: nylB_1 Imported ORF Names: BN2127_JRS1_00149 Imported
Organism ¹	Bacillus cereus Imported
Taxonomic identifier ¹	1396 [NCBI]
Taxonomic lineage ¹	Bacteria > Firmicutes > Bacilli > Bacillales > Bacillaceae > Bacillus > Bacillus cereus group
Proteomes ¹	UP000182087 Component ¹ : Unassembled WGS sequence

Family & Domainsⁱ

UniProtKB - A0A0U0EFW2 (A0A0U0EFW2_STREE)

- Display
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 - Feature table

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- All None
- Function
 - Names & Taxonomy
 - Subcellular location
 - Pathology & Biotech
 - PTM / Processing
 - Expression
 - Interaction
 - Structure
 - Family & Domains
 - Sequence
 - Similar proteins
 - Cross-references
 - Entry information
 - Miscellaneous
- Top

Protein Submitted name: **6-aminohexanoate-dimer hydrolase**
Gene **nylB**
Organism *Streptococcus pneumoniae*
Status Unreviewed - Annotation score: ●○○○○ - Protein predicted¹

Function¹

GO - Molecular function¹
6-aminohexanoate-dimer hydrolase activity Source: UniProtKB-EC

Complete GO annotation...

Keywords¹

Molecular function	Hydrolase Imported
--------------------	--------------------

Names & Taxonomy¹

Protein names ¹	Submitted name: 6-aminohexanoate-dimer hydrolase Imported (EC:3.5.1.46 Imported)
Gene names ¹	Name: nylB Imported ORF Names: ERS020515_01316 Imported
Organism ¹	Streptococcus pneumoniae Imported
Taxonomic identifier ¹	1313 [NCBI]
Taxonomic lineage ¹	Bacteria > Firmicutes > Bacilli > Lactobacillales > Streptococcaceae > Streptococcus
Proteomes ¹	UP000039704 Component ¹ : Unassembled WGS sequence

Family & Domains¹

Supplement C

List of organisms with predicted or actual *nylC* genes from UNIPROT

METHODS

The raw list is simply generated by going to www.UNIPROT.org and typing “nylC” into the search box. As of this writing it will generate 11 entries.

RESULTS

The following list took those 11 entries and removed the obvious duplicates. Around 9 species or strains were listed to have actual or predicted *nylC* genes or homologs.

Agromyces sp. KY5R

Brachyspira hyodysenteriae (strain ATCC 49526 / WA1)

Brachyspira intermedia (strain ATCC 51140 / PWS/A) (Serpulina intermedia)

Flavobacterium sp.

Flavobacterium sp. KI723T1

Flavobacterium sp. (strain K172)

Fundulus heteroclitus (Killifish) (Mummichog)

Kocuria sp. KY2

Microbacterium sp. TS-1

Supplement D

List of organisms with predicted or actual
nylB' genes from UNIPROT

METHODS

The raw list is simply generated by going to www.UNIPROT.org and typing “nylB” into the search box. As of this writing it will generate 478 entries. After the list was generated, entries with *nylB* genes were removed and the remainder were *nylB*' genes.

Duplicates were removed.

RESULTS

Around 125 species or strains were listed to have actual or predicted *nylB*' genes or homologs.

[Clostridium] clostridioforme
[Clostridium] neopropionicum
Achromobacter sp.
Acidobacteria bacterium DSM 100886
Acinetobacter baumannii
Agrobacterium sp. DSM 25559
Agrobacterium sp. RAC06
Agromyces sp. KY5R
Alcaligenes xylosoxydans xylosoxydans (Achromobacter xylosoxidans)
Altererythrobacter dongtanensis
Amantichitinum ursilacus
Arenibacter sp. C-21
Bacillus toyonensis
bacterium ADurb.BinA028
bacterium YEK0313
Bacteroidales bacterium Barb6
Bacteroidales bacterium Barb6XT
Bacteroides cellulosilyticus
Bacteroides xylanisolvens
Bacteroidetes bacterium ADurb.Bin123
Bacteroidetes bacterium ADurb.Bin145
Bacteroidetes bacterium ADurb.Bin397
Bifidobacterium pseudocatenulatum
candidate division BRC1 bacterium ADurb.BinA364
Candidatus Accumulibacter sp. BA-94
Candidatus Accumulibacter sp. SK-01
Candidatus Thorarchaeota archaeon AB_25
Cesiribacter andamanensis AMV16
Chlamydia abortus (Chlamydophila abortus)
Chloroflexi bacterium ADurb.Bin344
Chloroflexi bacterium ADurb.Bin360
Chryseobacterium sp. MOF25P
Clostridiales bacterium CHKCI001
Clostridioides difficile (Peptoclostridium difficile)
Clostridium Ijungdahlii

Clostridium oryzae
Clostridium sp. C105KSO14
Clostridium sp. FS41
Cupriavidus metallidurans
Deltaproteobacteria bacterium ADurb.BinA014
Dorea longicatena
Duganella sp. HH101
Duganella sp. HH105
Elizabethkingia miricola (Chryseobacterium miricola)
Enterobacter cloacae
Firmicutes bacterium ADurb.Bin146
Flavobacterium sp. (strain K172)
Flavobacterium sp. KI723T1
Fusicatenibacter saccharivorans
Gammaproteobacteria bacterium MOLA455
Grimontia celer
Hungatella hathewayi
Jannaschia aquimarina
Jannaschia donghaensis
Jannaschia rubra
Janthinobacterium lividum
Janthinobacterium sp. HH106
Janthinobacterium sp. KBS0711
Janthinobacterium sp. MP5059B
Janthinobacterium sp. MP5059B
Klebsiella oxytoca
Labrenzia aggregata
Lachnospira pectinoschiza
Lacunisphaera limnophila
Legionella gratiana
Lentisphaerae bacterium ADurb.Bin242
Limnohabitans sp. 63ED37-2
lostridium puniceum
Marinomonas gallaica
Microbacterium sp. TNHR37B
Microbulbifer sp. CCB-MM1
mine drainage metagenome
Mycobacterium abscessus subsp. abscessus
Mycobacterium parascrofulaceum ATCC BAA-614
Nautella italica
Nereida ignava
Nocardia farcinica
Nocardioides dokdonensis FR1436
Octadecabacter temperatus
Paenibacillus sp. AD87
Paenibacillus sp. P1XP2
Phaeobacter sp. CECT 7735
Pseudomonas aeruginosa DK1
Pseudomonas aeruginosa

Pseudomonas fluorescens
Pseudomonas putida (*Arthrobacter siderocapsulatus*)
Pseudomonas sp. 1 R 17
Pseudomonas sp. 22 E 5
Pseudomonas sp. 24 E 1
Pseudomonas sp. 44 R 15
Pseudomonas sp. 58 R 3
Pseudomonas sp. URMO17WK12:I11
Pseudomonas syringae pv. *actinidiae*
Pseudomonas syringae pv. *tomato*
Pseudovibrio axinellae
Pseudovibrio sp. Ad26
Pseudovibrio sp. W74
Pseudovibrio sp. WM33
Roseovarius aestuarii
Roseovarius gaetbuli
Roseovarius halotolerans
Roseovarius mucosus
Roseovarius sp. A-2
Roseovarius tolerans
Ruegeria meonggei
Ruegeria sp. CECT 5091
Salinivirga cyanobacteriivorans
Serratia marcescens
Shimia sp. SK013
Spirochaetes bacterium ADurb.BinA120
Streptococcus pneumoniae
Tardiphaga robiniae
Thalassobius gelatinovorans (*Ruegeria gelatinovora*)
Thalassobius mediterraneus
Tropicibacter multivorans
uncultured *Bacteroides* sp.
uncultured *Blautia* sp.
uncultured *Clostridium* sp.
uncultured *Dorea* sp.
uncultured *Lachnospira* sp.
uncultured *Ruminococcus* sp.
Variibacter gotjawalensis
Verrucomicrobia bacterium ADurb.Bin070
Vibrio alginolyticus
Vibrio hemicentroti

Supplement E
List of organisms with predicted or actual
nylonases of the 6-aminohexanoate hydrolase variety
from UNIPROT

METHODS

The raw list is simply generated by going to www.UNIPROT.org and typing “6-aminohexanoate hydrolase” into the search box. As of this writing it will generate 3,502 entries.

RESULTS

The following list took those 3,502 entries and removed most of the duplicates. 1827 species or strains are listed. There are a variety of 6-aminohexanoate hydrolases such as the dimer, cyclic dimer, or oligomer variety. All these varieties are reflected in the list below.

Acetobacterium wieringae
Acholeplasma laidlawii (strain PG-8A)
Achromobacter denitrificans (Alcaligenes denitrificans)
Achromobacter piechaudii
Achromobacter piechaudii ATCC 43553
Achromobacter ruhlandii
Achromobacter sp.
Achromobacter sp. AR476-2
Achromobacter sp. KAs 3-5
Achromobacter sp. LC458
Achromobacter sp. Root170
Achromobacter sp. Root565
Achromobacter sp. Root83
Achromobacter sp. RTa
Achromobacter spanius
Acidiphilium sp. JA12-A1
Acidiphilium sp. PM
Acidithrix ferrooxidans
Acidobacteria bacterium DSM 100886
Acidovorax sp. GW101-3H11
Acidovorax sp. KKS102
Acidovorax sp. Leaf76
Acidovorax sp. Leaf78
Acidovorax sp. NO-1
Acidovorax sp. Root217
Acidovorax sp. Root219
Acidovorax sp. Root275
Acidovorax sp. Root402
Acidovorax sp. Root70
Acidovorax sp. SCN 65-28
Acidovorax sp. SD340
Acinetobacter baumannii
Acinetobacter johnsonii

Acinetobacter junii MTCC 11364
Acinetobacter venetianus
Actinobacteria bacterium ADurb.BinA094
Actinokineospora spheciospongiae
Actinomadura sp. CNU-125
Actinoplanes friuliensis DSM 7358
Actinoplanes sp. (strain ATCC 31044 / CBS 674.73 / SE50/110)
Afipia massiliensis
Afipia sp. 62-7
Afipia sp. GAS231
Afipia sp. Root123D2
Agarivorans albus MKT 106
Agrobacterium albertimagni AOL15
Agrobacterium arsenijevicei
Agrobacterium fabrum str. J-07
Agrobacterium genomosp. 1 str. TT111
Agrobacterium genomosp. 2 str. CFBP 5494
Agrobacterium radiobacter (strain K84 / ATCC BAA-868)
Agrobacterium rhizogenes
Agrobacterium sp. DSM 25559
Agrobacterium sp. RAC06
Agrobacterium sp. SCN 61-19
Agrobacterium sp. (strain H13-3) (Rhizobium lupini (strain H13-3))
Agrobacterium tumefaciens 5A
Agrobacterium vitis (Rhizobium vitis)
Agromyces sp. KY5R
Agromyces sp. NDB4Y10
Ahrensia marina
Alcaligenes faecalis
Alcaligenes faecalis subsp. faecalis NBRC 13111
Alcaligenes xylosoxydans xylosoxydans (Achromobacter xylosoxidans)
Alcanivorax dieselolei
Alcanivorax pacificus W11-5
Alcanivorax sp. KX64203
Alcanivorax xenomutans
Algibacter lectus
Alicyclobacillus acidocaldarius LAA1
Alicyclobacillus ferrooxydans
Aliiroseovarius crassostreae
Aliivibrio sp. 1S175
Alkalibacterium sp. AK22
Alkanindiges sp. H1
alpha proteobacterium AAP38
alpha proteobacterium Q-1
alpha proteobacterium U9-1i
Alphaproteobacteria bacterium 64-6
Alphaproteobacteria bacterium 65-7
Alphaproteobacteria bacterium ADurb.Bin100
Alphaproteobacteria bacterium ADurb.BinA280

Alphaproteobacteria bacterium AO1-B
Alphaproteobacteria bacterium RIFCSPHIGHO2_12_FULL_63_12
Altererythrobacter atlanticus
Altererythrobacter dongtanensis
Altererythrobacter epoxidivorans
Altererythrobacter namhicola
Altererythrobacter sp. Root672
Amantichitinum ursilacus
Aminobacter aminovorans (Chelatobacter heintzii)
Aminobacter sp. Root100
Amycolatopsis sp. M39
Anabaena sp. LE011-02
Anabaena sp. MDT14b
Anaeromyxobacter sp. PSR-1
Anaeromyxobacter sp. (strain Fw109-5)
Aneurinibacillus soli
Aquamicrobium defluvii
Aquimarina atlantica
archaeon 13_1_20CM_2_54_9
Archangium sp. Cb G35
Arenibacter sp. C-21
Arthrobacter crystallopoietes BAB-32
Arthrobacter sp. Hiyo1
Arthrobacter sp. Hiyo4
Arthrobacter sp. Hiyo8
Arthrobacter sp. IHBB 11108
Arthrobacter sp. Rue61a
Arthrobacter sp. SW1
Aspergillus niger (strain CBS 513.88 / FGSC A1513)
Asticcacaulis sp. AC402
Asticcacaulis sp. AC460
Aurantimonas sp. Leaf443
Aureimonas altamirensis
Aureimonas sp. Leaf324
Aureimonas sp. Leaf427
Aureimonas sp. Leaf454
Aureimonas sp. Leaf460
Aureimonas ureilytica
Auxenochlorella protothecoides (Green microalga) (Chlorella protothecoides)
Azospirillum brasilense
Azospirillum sp. (strain B510)
Azospirillum thiophilum
Bacillus anthracis
Bacillus anthracis str. H9401
Bacillus aquimaris
Bacillus beberidgei
Bacillus bombysepticus str. Wang
Bacillus cereus
Bacillus cereus 172560W

Bacillus cereus 95/8201
Bacillus cereus AH1134
Bacillus cereus AH1271
Bacillus cereus AH1272
Bacillus cereus AH603
Bacillus cereus AH621
Bacillus cereus ATCC 10876
Bacillus cereus ATCC 4342
Bacillus cereus B5-2
Bacillus cereus BAG10-1
Bacillus cereus BAG10-3
Bacillus cereus BAG1X2-1
Bacillus cereus BAG1X2-2
Bacillus cereus BAG1X2-3
Bacillus cereus BAG20-1
Bacillus cereus BAG20-3
Bacillus cereus BAG30-1
Bacillus cereus BDRD-Cer4
Bacillus cereus BDRD-ST196
Bacillus cereus BDRD-ST26
Bacillus cereus BGSC 6E1
Bacillus cereus F65185
Bacillus cereus HuA2-9
Bacillus cereus HuA3-9
Bacillus cereus HuB13-1
Bacillus cereus HuB4-4
Bacillus cereus ISP2954
Bacillus cereus K-5975c
Bacillus cereus m1293
Bacillus cereus m1550
Bacillus cereus MC118
Bacillus cereus MM3
Bacillus cereus NVH0597-99
Bacillus cereus R309803
Bacillus cereus Rock3-28
Bacillus cereus Rock3-29
Bacillus cereus Rock3-42
Bacillus cereus Rock3-44
Bacillus cereus Rock4-18
Bacillus cereus Rock4-2
Bacillus cereus str. Schrouff
Bacillus cereus (strain 03BB102)
Bacillus cereus (strain AH187)
Bacillus cereus (strain AH820)
Bacillus cereus (strain ATCC 10987 / NRS 248)
Bacillus cereus (strain ATCC 14579 / DSM 31 / JCM 2152 / NBRC 15305 / NCIMB 9373 / NRRL B-3711)
Bacillus cereus (strain B4264)
Bacillus cereus (strain G9842)

Bacillus cereus (strain Q1)
Bacillus cereus (strain ZK / E33L)
Bacillus cereus TIAC219
Bacillus cereus var. anthracis (strain CI)
Bacillus cereus VD021
Bacillus cereus VD118
Bacillus cereus VD131
Bacillus cereus VD133
Bacillus cereus VD136
Bacillus cereus VD142
Bacillus cereus VD146
Bacillus cereus VD184
Bacillus cereus VD196
Bacillus cereus VDM006
Bacillus cereus VDM019
Bacillus cereus VDM021
Bacillus cereus VDM053
Bacillus coagulans
Bacillus cytotoxicus
Bacillus cytotoxicus (strain DSM 22905 / CIP 110041 / 391-98 / NVH 391-98)
Bacillus gaemokensis
Bacillus glycinifermentans
Bacillus hemicellulosilyticus JCM 9152
Bacillus intestinalis
Bacillus licheniformis
Bacillus malacitensis
Bacillus manliponensis
Bacillus mycoides
Bacillus mycoides Rock1-4
Bacillus obstructivus
Bacillus pseudomycoides
Bacillus pseudomycoides DSM 12442
Bacillus pumilus (Bacillus mesentericus)
Bacillus solani
Bacillus sp. 0711P9-1
Bacillus sp. CDB3
Bacillus sp. CHD6a
Bacillus sp. EB422
Bacillus sp. FJAT-18017
Bacillus sp. FJAT-18019
Bacillus sp. FJAT-21945
Bacillus sp. FJAT-26390
Bacillus sp. FJAT-27225
Bacillus sp. FJAT-27264
Bacillus sp. G3(2015)
Bacillus sp. GeD10
Bacillus sp. GZT
Bacillus sp. JCM 19045
Bacillus sp. JCM 19046

Bacillus sp. JCM 19047
Bacillus sp. JH7
Bacillus sp. LK2
Bacillus sp. LL01
Bacillus sp. MB366
Bacillus sp. MN5
Bacillus sp. N35-10-2
Bacillus sp. N35-10-4
Bacillus sp. NH24A2
Bacillus sp. NRRL B-14911
Bacillus sp. Root11
Bacillus sp. Root131
Bacillus sp. RZ2MS9
Bacillus sp. SG-1
Bacillus sp. TD41
Bacillus sp. TD42
Bacillus sp. UMTAT18
Bacillus sporothermodurans
Bacillus subterraneus
Bacillus subtilis
Bacillus subtilis BEST7613
Bacillus thermoamylovorans
Bacillus thuringiensis
Bacillus thuringiensis Bt18247
Bacillus thuringiensis HD-771
Bacillus thuringiensis IBL 200
Bacillus thuringiensis MC28
Bacillus thuringiensis serovar andalousiensis BGSC 4AW1
Bacillus thuringiensis serovar coreanensis
Bacillus thuringiensis serovar huazhongensis BGSC 4BD1
Bacillus thuringiensis serovar israelensis ATCC 35646
Bacillus thuringiensis serovar monterrey BGSC 4AJ1
Bacillus thuringiensis serovar pakistani str. T13001
Bacillus thuringiensis serovar pondicheriensis BGSC 4BA1
Bacillus thuringiensis serovar pulsiensis BGSC 4CC1
Bacillus thuringiensis serovar sotto str. T04001
Bacillus thuringiensis serovar thuringiensis str. IS5056
Bacillus thuringiensis serovar tochiensis BGSC 4Y1
Bacillus thuringiensis (strain Al Hakam)
Bacillus thuringiensis subsp. aizawai
Bacillus thuringiensis subsp. finitimus (strain YBT-020)
Bacillus thuringiensis subsp. indiana
Bacillus thuringiensis subsp. israelensis
Bacillus thuringiensis subsp. konkukian (strain 97-27)
Bacillus thuringiensis subsp. tolworthi
Bacillus thuringiensis T01-328
Bacillus toyonensis
Bacillus weihenstephanensis
Bacillus weihenstephanensis FSL H7-687

Bacillus weihenstephanensis (strain KBAB4)
Bacillus wiedmannii
bacterium ADurb.Bin478
bacterium ADurb.BinA028
bacterium YEK0313
Bacteroidales bacterium Barb4
Bacteroidales bacterium Barb6
Bacteroidales bacterium Barb6XT
Bacteroides cellulosilyticus
Bacteroides stercoris
Bacteroides uniformis
Bacteroides xylanisolvens
Bacteroidetes bacterium ADurb.Bin008
Bacteroidetes bacterium ADurb.Bin035
Bacteroidetes bacterium ADurb.Bin041
Bacteroidetes bacterium ADurb.Bin123
Bacteroidetes bacterium ADurb.Bin141
Bacteroidetes bacterium ADurb.Bin145
Bacteroidetes bacterium ADurb.Bin397
Bacteroidetes bacterium ADurb.BinA012
Bacteroidetes bacterium ADurb.BinA174
Balneola sp. EhC07
beta proteobacterium AAP99
Betaproteobacteria bacterium ADurb.Bin341
Bifidobacterium dentium (strain ATCC 27534 / DSM 20436 / JCM 1195 / Bd1)
Bifidobacterium pseudocatenulatum
Bifidobacterium pullorum
Bifidobacterium saeculare DSM 6531 = LMG 14934
Blastococcus saxobsidens (strain DD2)
Blastopirellula marina DSM 3645
Blautia obeum
Blautia wexlerae
blood disease bacterium A2-HR MARDI
blood disease bacterium R229
Bordetella ansorpii
Bordetella hinzii
Bordetella pertussis
Bordetella petrii (strain ATCC BAA-461 / DSM 12804 / CCUG 43448)
Bordetella sp. SCN 67-23
Bordetella trematum
Bradyrhizobiaceae bacterium SG-6C
Bradyrhizobium canariense
Bradyrhizobium diazoefficiens
Bradyrhizobium elkanii
Bradyrhizobium erythrophlei
Bradyrhizobium icense
Bradyrhizobium japonicum
Bradyrhizobium japonicum SEMIA 5079
Bradyrhizobium jicamae

Bradyrhizobium lablabi
Bradyrhizobium liaoningense
Bradyrhizobium lupini HPC(L)
Bradyrhizobium manausense
Bradyrhizobium ottawaense
Bradyrhizobium pachyrhizi
Bradyrhizobium paxllaeri
Bradyrhizobium retamae
Bradyrhizobium sp. AS23.2
Bradyrhizobium sp. AT1
Bradyrhizobium sp. BR 10245
Bradyrhizobium sp. BR 10303
Bradyrhizobium sp. BR10280
Bradyrhizobium sp. CCGE-LA001
Bradyrhizobium sp. DFCI-1
Bradyrhizobium sp. DOA9
Bradyrhizobium sp. Leaf396
Bradyrhizobium sp. LMTR 3
Bradyrhizobium sp. LTSP849
Bradyrhizobium sp. LTSP885
Bradyrhizobium sp. LTSPM299
Bradyrhizobium sp. NAS80.1
Bradyrhizobium sp. NAS96.2
Bradyrhizobium sp. ORS 285
Bradyrhizobium sp. S23321
Bradyrhizobium sp. STM 3809
Bradyrhizobium sp. STM 3843
Bradyrhizobium sp. (strain BTAi1 / ATCC BAA-1182)
Bradyrhizobium sp. (strain ORS 278)
Bradyrhizobium sp. (strain ORS 375)
Bradyrhizobium sp. UASWS1016
Bradyrhizobium sp. UFLA 03-321
Bradyrhizobium valentinum
Bradyrhizobium yuanmingense
Brevibacillus brevis (Bacillus brevis)
Brevibacillus formosus
Brevibacillus laterosporus (Bacillus laterosporus)
Brevibacillus laterosporus LMG 15441
Brevibacillus reuszeri
Brevibacillus sp. SKDU10
[Brevibacterium] halotolerans
Brevibacterium linens
Brevundimonas sp. LM2
Brucella abortus
Brucella abortus bv. 5 str. B3196
Brucella abortus str. 2308 A
Brucella abortus (strain 2308)
Brucella canis (strain ATCC 23365 / NCTC 10854)
Brucella ceti str. Cudo

Brucella melitensis
Brucella melitensis biotype 1 (strain 16M / ATCC 23456 / NCTC 10094)
Brucella melitensis biotype 2 (strain ATCC 23457)
Brucella microti (strain CCM 4915)
Brucella neotomae 5K33
Brucella pinnipedialis M292/94/1
Brucella sp. 09RB8910
Brucella sp. 10RB9215
Brucella sp. 141012304
Brucella sp. 83/13
Brucella sp. BO2
Brucella sp. NVSL 07-0026
Brucella suis bv. 5 str. 513
Brucella vulpis
Burkholderia ambifaria (strain ATCC BAA-244 / AMMD) (*Burkholderia cepacia* (strain AMMD))
Burkholderia anthina
Burkholderia cenocepacia
Burkholderia cenocepacia (strain MC0-3)
Burkholderia cepacia JBK9
Burkholderia cepacia (*Pseudomonas cepacia*)
Burkholderia contaminans
Burkholderia diffusa
Burkholderia gladioli (*Pseudomonas marginata*) (*Phytomonas marginata*)
Burkholderia glumae PG1
Burkholderia hypogea
Burkholderia lata (strain ATCC 17760 / LMG 22485 / NCIMB 9086 / R18194 / 383)
Burkholderia latens
Burkholderia mallei GB8 horse 4
Burkholderia mallei (*Pseudomonas mallei*)
Burkholderia mallei (strain ATCC 23344)
Burkholderia mallei (strain NCTC 10229)
Burkholderia metallica
Burkholderia multivorans
Burkholderia multivorans (strain ATCC 17616 / 249)
Burkholderia oklahomensis C6786
Burkholderia oklahomensis EO147
Burkholderia plantarii
Burkholderia pseudomallei (*Pseudomonas pseudomallei*)
Burkholderia pseudomallei (strain 1026b)
Burkholderia pseudomallei (strain 1710b)
Burkholderia pseudomultivorans
Burkholderia pyrrocinia (*Pseudomonas pyrrocinia*)
Burkholderia seminalis
Burkholderia sp. A2
Burkholderia sp. A27
Burkholderia sp. ABCPW 11
Burkholderia sp. ABCPW 14
Burkholderia sp. BDU6
Burkholderia sp. BDU8

Burkholderia sp. Bp7605
Burkholderia sp. Bp7605
Burkholderia sp. CAMPA 1040
Burkholderia sp. DNA89
Burkholderia sp. FL-7-2-10-S1-D7
Burkholderia sp. GAS332
Burkholderia sp. HB1
Burkholderia sp. LA-2-3-30-S1-D2
Burkholderia sp. Leaf177
Burkholderia sp. LK4
Burkholderia sp. MSh2
Burkholderia sp. MSMB0852
Burkholderia sp. MSMB0856
Burkholderia sp. MSMB1552
Burkholderia sp. MSMB1835
Burkholderia sp. MSMB617WGS
Burkholderia sp. NRF60-BP8
Burkholderia sp. OLGA172
Burkholderia sp. PAMC 26561
Burkholderia sp. RF2-non_BP3
Burkholderia sp. SRS-W-2-2016
Burkholderia sp. ST111
Burkholderia sp. TSV85
Burkholderia sp. TSV86
Burkholderia sp. USM B20
Burkholderia stabilis
Burkholderia stagnalis
Burkholderia territorii
Burkholderia thailandensis
Burkholderia thailandensis (strain ATCC 700388 / DSM 13276 / CIP 106301 / E264)
Burkholderia ubonensis
Burkholderia vietnamiensis
Burkholderiaceae bacterium 16
Burkholderiales bacterium 64-34
Burkholderiales bacterium 67-32
Burkholderiales bacterium RIFCSPHIGHO2_01_FULL_64_960
Burkholderiales bacterium RIFCSPHIGHO2_12_FULL_65_48
Burkholderiales bacterium RIFCSLOWO2_02_FULL_57_36
Caballeronia glathei PML1(12)
candidate division BRC1 bacterium ADurb.BinA364
Candidatus Accumulibacter sp. BA-92
Candidatus Accumulibacter sp. BA-94
Candidatus Accumulibacter sp. SK-01
Candidatus Accumulibacter sp. SK-02
Candidatus Aminicenantes bacterium ADurb.Bin147
Candidatus Endolissoclinum faulkneri L5
Candidatus Hydrogenedentes bacterium ADurb.Bin101
Candidatus Phaeomarinobacter ectocarpi
Candidatus Rhodobacter lobularis

Candidatus Thorarchaeota archaeon AB_25
Capnocytophaga canimorsus
Capnocytophaga canimorsus (strain 5)
Capnocytophaga canis
Capnocytophaga cynodegmi
Carnobacterium maltaromaticum (Carnobacterium piscicola)
Carnobacterium maltaromaticum DSM 20342
Carnobacterium maltaromaticum LMA28
Carnobacterium sp. CP1
Catabacter hongkongensis
Catenibacterium mitsuokai
Caulobacter crescentus (strain ATCC 19089 / CB15)
Caulobacter crescentus (strain NA1000 / CB15N)
Caulobacter henricii
Caulobacter sp. Root1455
Caulobacter sp. Root1472
Caulobacter sp. Root342
Caulobacter sp. Root343
Caulobacter sp. Root655
Caulobacter sp. Root656
Caulobacteraceae bacterium OTSz_A_272
Caulobacterales bacterium 68-7
Cecembia lonarensis LW9
Cesiribacter andamanensis AMV16
Chelatococcus sp. CO-6
Chitinophaga pinensis (strain ATCC 43595 / DSM 2588 / NCIB 11800 / UQM 2034)
Chlamydia abortus (Chlamydophila abortus)
Chlamydia trachomatis
Chlorobi bacterium OLB4
Chlorobi bacterium OLB7
Chloroflexi bacterium ADurb.Bin222
Chloroflexi bacterium ADurb.Bin325
Chloroflexi bacterium ADurb.Bin344
Chloroflexi bacterium ADurb.Bin360
Chromohalobacter japonicus
Chryseobacterium antarcticum
Chryseobacterium sp. MOF25P
Chryseobacterium sp. StRB126
Citricella sp. SE45
Clostridiales bacterium CHKCI001
Clostridiales bacterium CHKCI001
Clostridioides difficile (Peptoclostridium difficile)
Clostridium argentinense CDC 2741
[Clostridium] clostridioforme
Clostridium innocuum
Clostridium ljungdahlii
[Clostridium] neopropionicum
Clostridium oryzae
Clostridium pasteurianum

Clostridium puniceum
Clostridium ragsdalei P11
Clostridium sp. C105KSO14
Clostridium sp. C105KSO15
Clostridium sp. FS41
Clostridium sp. HMSC19B10
Clostridium tyrobutyricum DIVETGP
Coccidioides immitis RMSCC 3703
Coccidioides posadasii RMSCC 3488
Coccidioides posadasii (strain RMSCC 757 / Silveira) (Valley fever fungus)
Collimonas fungivorans (strain Ter331)
Comamonas sp. E6
Comamonas testosteroni (*Pseudomonas testosteroni*)
Comamonas testosteroni (strain DSM 14576 / KF-1)
Comamonas testosteroni TK102
Comamonas thiooxydans
Commensalibacter sp. MX01
Confluentimicrobium sp. EMB200-NS6
Coprococcus comes
Corallococcus coralloides (strain ATCC 25202 / DSM 2259 / NBRC 100086 / M2) (*Myxococcus coralloides*)
Corchorus capsularis
Corynebacterium marinum DSM 44953
Crenarchaeota archaeon 13_1_40CM_3_53_5
Cryobacterium arcticum
Cupriavidus basilensis OR16
Cupriavidus gilardii CR3
Cupriavidus metallidurans
Cupriavidus metallidurans (strain ATCC 43123 / DSM 2839 / NBRC 102507 / CH34) (*Ralstonia metallidurans*)
Cupriavidus nantongensis
Cupriavidus necator (*Alcaligenes eutrophus*) (*Ralstonia eutropha*)
Cupriavidus necator (strain ATCC 43291 / DSM 13513 / N-1) (*Ralstonia eutropha*)
Cupriavidus necator (strain JMP 134 / LMG 1197) (*Ralstonia eutropha* (strain JMP 134))
Cupriavidus sp. HMR-1
Cupriavidus sp. HPC(L)
Cupriavidus sp. SK-3
Cupriavidus sp. SK-4
Cupriavidus sp. USMAA2-4
Cupriavidus taiwanensis (strain DSM 17343 / BCRC 17206 / CIP 107171 / LMG 19424 / R1) (*Ralstonia taiwanensis* (strain LMG 19424))
Cutibacterium avidum
Cutibacterium avidum ATCC 25577
Cutibacterium granulosum DSM 20700
Cutibacterium granulosum TM11
Defluviimonas alba
Defluviimonas sp. 20V17
Deinococcus marmoris

Deinococcus radiodurans (strain ATCC 13939 / DSM 20539 / JCM 16871 / LMG 4051 / NBRC 15346 / NCIMB 9279 / R1 / VKM B-1422)
Delftia acidovorans (strain DSM 14801 / SPH-1)
Delftia sp. 670
Delftia sp. JD2
Delftia sp. RIT313
Delftia sp. (strain Cs1-4)
Delftia tsuruhatensis
Deltaproteobacteria bacterium ADurb.Bin002
Deltaproteobacteria bacterium ADurb.Bin022
Deltaproteobacteria bacterium ADurb.Bin072
Deltaproteobacteria bacterium ADurb.Bin151
Deltaproteobacteria bacterium ADurb.BinA014
Deltaproteobacteria bacterium ADurb.BinA179
Deltaproteobacteria bacterium SG8_13
Desulfovibrio piger
Devosia geojensis
Devosia limi DSM 17137
Devosia soli
Devosia sp. 66-22
Devosia sp. Leaf64
Devosia sp. S37
Dietzia cinnamea
Dietzia cinnamea P4
Dietzia sp. 111N12-1
Dietzia sp. HMSC21D01
Dokdonella koreensis DS-123
Dorea longicatena
Drosophila ficusphila (Fruit fly)
Duganella sp. HH101
Duganella sp. HH105
Duganella sp. Leaf126
Duganella sp. Leaf61
Eisenbergiella tayi
Elizabethkingia miricola (*Chryseobacterium miricola*)
Endozoicomonas sp. S-B4-1U
Ensifer adhaerens OV14
Ensifer adhaerens (*Sinorhizobium morelense*)
Ensifer glycinis
Ensifer sp. LC14
Ensifer sp. LC54
Ensifer sp. LCM 4579
Ensifer sp. Root1312
Ensifer sp. Root142
Ensifer sp. Root278
Ensifer sp. YIC4027
Enterobacter cloacae
Enterobacter cloacae EcWSU1
Enterobacter kobei

Enterococcus faecalis ATCC 6055
Enterococcus faecalis EnGen0302
Enterococcus faecalis EnGen0354
Enterococcus faecalis EnGen0359
Enterococcus faecalis EnGen0426
Enterococcus faecalis PF3
Enterococcus faecalis (strain ATCC 700802 / V583)
Enterococcus faecalis (Streptococcus faecalis)
Enterococcus faecalis TX0104
Enterococcus faecium (Streptococcus faecium)
Enterococcus gallinarum
Enterococcus sp. C1
Enterococcus sp. HSIEG1
Enterovibrio corallii
Enterovibrio pacificus
Erythrobacter sp. HI0063
Erythrobacter sp. JL475
Erythrobacter sp. SG61-1L
Escherichia coli
[Eubacterium] angustum
[Eubacterium] contortum
[Eubacterium] eligens
Ferrimicrobium acidiphilum DSM 19497
Fibrella aestuarina BUZ 2
Fibrisoma limi BUZ 3
Fictibacillus phosphorivorans
Filimonas lacunae
Firmicutes bacterium ADurb.Bin146
Firmicutes bacterium ADurb.Bin182
Firmicutes bacterium ADurb.Bin248
Firmicutes bacterium ADurb.Bin262
Firmicutes bacterium CAG:95
Flavobacteria bacterium BAL38
Flavobacteriaceae bacterium UJ101
Flavobacterium hibernum
Flavobacterium hydatis (Cytophaga aquatilis)
Flavobacterium sp.
Flavobacterium sp. 38-13
Flavobacterium sp. KI723T1
Flavobacterium sp. KMS
Flavobacterium sp. Leaf359
Flavobacterium sp. (strain K172)
[Flexibacter] sp. ATCC 35208
Frateuria sp. Soil773
Fusarium langsethiae
Fusarium oxysporum f. sp. cubense (strain race 1) (Panama disease fungus)
Fusarium oxysporum f. sp. cubense (strain race 4) (Panama disease fungus)
Fusicatenibacter
Fusicatenibacter saccharivorans

gamma proteobacterium HTCC2207
Gammaproteobacteria bacterium MOLA455
Gammaproteobacteria bacterium RIFCSPLOWO2_12_FULL_52_10
Gammaproteobacteria bacterium SG8_11
Gammaproteobacteria bacterium SG8_47
Geomicrobium sp. JCM 19037
Geomicrobium sp. JCM 19038
Geomicrobium sp. JCM 19039
Geomicrobium sp. JCM 19055
Gluconacetobacter sp. SXCC-1
Gordonia amarae NBRC 15530
Gordonia paraffinivorans NBRC 108238
Granulicatella adiacens ATCC 49175
Grimontia celer
Haematobacter missouriensis
Halanaerobium saccharolyticum subsp. saccharolyticum DSM 6643
Halobacillus karajensis
Halocynthiibacter arcticus
Halolamina sediminis
Halomonas boliviensis LC1
Halomonas campaniensis
Halomonas hydrothermalis
Halomonas salina
Halomonas sp. 1513
Halomonas sp. 54_146
Halomonas sp. A3H3
Halomonas sp. ALS9
Halomonas sp. G11
Halomonas sp. KHS3
Halomonas sp. QHL1
Halomonas sp. Soap Lake #7'
Halotalea alkalilenta
Herbaspirillum seropedicae
Herbaspirillum seropedicae (strain SmR1)
Herminiimonas arsenicoxydans
Herpetosiphon aurantiacus (strain ATCC 23779 / DSM 785)
Herpetosiphon geysericola
Hirschia baltica (strain ATCC 49814 / DSM 5838 / IFAM 1418)
Hoeflea olei
Hoyosella subflava (strain DSM 45089 / JCM 17490 / NBRC 109087 / DQS3-9A1) (Amycolicoccus subflavus)
human gut metagenome
Hungatella hathewayi
Hyalangium minutum
hydrocarbon metagenome
Hydrogenophaga intermedia
Hydrogenophaga sp. 70-12
Hydrogenophaga sp. PBC
hydrothermal vent metagenome

Hyphomicrobium sp. SCN 65-11
Hyphomonadaceae bacterium BRH_c29
Hyphomonas adhaerens MHS-3
Hyphomonas hirschiana VP5
Hyphomonas jannaschiana VP2
Hyphomonas johnsonii MHS-2
Hyphomonas neptunium (strain ATCC 15444)
Hyphomonas oceanitis SCH89
Hyphomonas polymorpha PS728
Hyphomonas sp. BRH_c22
Ideonella sakaiensis (strain 201-F6)
Inquilinus limosus MP06
Isoptericola dokdonensis DS-3
Jannaschia aquimarina
Jannaschia donghaensis
Jannaschia rubra
Janthinobacterium lividum
Janthinobacterium sp. 1_2014MBL_MicDiv
Janthinobacterium sp. CG23_2
Janthinobacterium sp. HH106
Janthinobacterium sp. KBS0711
Janthinobacterium sp. LM6
Janthinobacterium sp. MP5059B
Janthinobacterium sp. (strain Marseille) (Minibacterium massiliensis)
Jeotgalibaca dankookensis
Jeotgalibacillus marinus
Jhaorihella thermophila
Ketogulonicigenium vulgare (strain WSH-001)
Kineosphaera limosa NBRC 100340
Klebsiella aerogenes (Enterobacter aerogenes)
Klebsiella oxytoca
Klebsiella pneumoniae
Klebsiella pneumoniae 30660/NJST258_1
Klebsiella pneumoniae 30684/NJST258_2
Kocuria sp. KY2
Komagataeibacter europaeus (Gluconacetobacter europaeus)
Komagataeibacter rhaeticus
Komagataeibacter rhaeticus AF1
Komagataeibacter xylinus E25
Ktedonobacter racemifer DSM 44963
Kutzneria sp. 744
Labrenzia aggregata
Labrenzia alba
Lachnospira pectinoschiza
Lactobacillus acidifarinae DSM 19394
Lactobacillus acidipiscis
Lactobacillus brevis KB290
Lactobacillus brevis subsp. gravesensis ATCC 27305
Lactobacillus buchneri ATCC 11577

Lactobacillus buchneri CD034
Lactobacillus casei 21/1
Lactobacillus casei 32G
Lactobacillus casei A2-362
Lactobacillus casei LOCK919
Lactobacillus casei M36
Lactobacillus casei (strain BD-II)
Lactobacillus casei UCD174
Lactobacillus casei UW4
Lactobacillus casei W56
Lactobacillus diolivorans DSM 14421
Lactobacillus farraginis DSM 18382 = JCM 14108
Lactobacillus fermentum
Lactobacillus fermentum ATCC 14931
Lactobacillus fermentum (strain CECT 5716)
Lactobacillus frumenti DSM 13145
Lactobacillus hilgardii DSM 20176 = ATCC 8290
Lactobacillus iners DSM 13335
Lactobacillus mucosae DSM 13345
Lactobacillus nodensis DSM 19682 = JCM 14932 = NBRC 107160
Lactobacillus oligofermentans DSM 15707 = LMG 22743
Lactobacillus oryzae JCM 18671
Lactobacillus otakiensis DSM 19908 = JCM 15040
Lactobacillus parabuchneri
Lactobacillus paracasei subsp. paracasei ATCC 25302
Lactobacillus paracasei subsp. paracasei Lpp49
Lactobacillus paracasei subsp. paracasei Lpp74
Lactobacillus rhamnosus LRHMDP2
Lactobacillus ruminis CAG:367
Lactobacillus ruminis SPM0211
Lactobacillus spicheri DSM 15429
Lactobacillus sunkii
Lactobacillus sunkii DSM 19904
Lactobacillus tuceti DSM 20183
Lactobacillus vaginalis DSM 5837 = ATCC 49540
Lactobacillus zymae DSM 19395
Lactococcus garvieae
Lactococcus garvieae DCC43
Lactococcus lactis subsp. cremoris A76
Lactococcus lactis subsp. cremoris (Streptococcus cremoris)
Lactococcus lactis subsp. lactis Dephy 1
Lactococcus lactis subsp. lactis (Streptococcus lactis)
Lactococcus piscium
Lactococcus piscium MKFS47
Lactococcus raffinolactis 4877
Lactococcus sp. DD01
Lacunisphaera limnophila
Leeuwenhoekiella blandensis (strain CECT 7118 / CCUG 51940 / MED217) (Flavobacterium sp. (strain MED217))

Legionella gratiana
Legionella massiliensis
Legionella pneumophila
Legionella pneumophila subsp. *pneumophila* ATCC 43290
Legionella pneumophila subsp. *pneumophila* str. Mississauga
Legionella pneumophila subsp. *pneumophila* (strain Philadelphia 1 / ATCC 33152 / DSM 7513)
Leifsonia rubra CMS 76R
Leisingera sp. JC1
Lentisphaerae bacterium ADurb.Bin242
Leptolyngbya sp. Heron Island J
Leptolyngbya sp. O-77
Leptolyngbya valderiana BDU 20041
Leptospira alstonii
Leptospira interrogans serogroup *Icterohaemorrhagiae* serovar *copenhageni* (strain Fiocruz L1-130)
Leptospira interrogans serovar *Canicola*
Leptospira interrogans serovar *Canicola* str. Gui44
Leptospira interrogans serovar *Lai*
Leptospira sp. ZV016
Leucobacter chromiirestans
Leucobacter komagatae
Leucobacter sp. Ag1
Leucobacter sp. UCD-THU
Limnobacter sp. MED105
Limnohabitans planktonicus II-D5
Limnohabitans sp. 63ED37-2
Listeria monocytogenes
Loktanella atrilutea
Loktanella sp. 1ANDIMAR09
Loktanella sp. 3ANDIMAR09
Loktanella sp. 5RATIMAR09
Loktanella sp. S4079
Luteibacter yeojuensis
Luteimonas sp. FCS-9
Luteimonas sp. JM171
Lutibaculum baratangense AMV1
Lysinibacillus manganicus DSM 26584
Lysinibacillus sinduriensis BLB-1 = JCM 15800
Lysinibacillus sp. FJAT-14222
Lysinibacillus sp. FJAT-14745
Lysinibacillus sp. ZYM-1
Lysinibacillus xylanilyticus
Lysobacter capsici AZ78
Lysobacter concretionis Ko07 = DSM 16239
Lysobacter defluvii IMMIB APB-9 = DSM 18482
Magnetospira sp. QH-2
Mameliella alba
marine gamma proteobacterium HTCC2080
Marinilactibacillus psychrotolerans 42ea
Marinobacter adhaerens

Marinobacter adhaerens (strain HP15)
Marinobacter algicola DG893
Marinobacter excellens LAMA 842
Marinobacter hydrocarbonoclasticus ATCC 49840
Marinobacter hydrocarbonoclasticus (Pseudomonas nautica)
Marinobacter hydrocarbonoclasticus (strain ATCC 700491 / DSM 11845 / VT8)
Marinobacter manganoxydans Mni7-9
Marinobacter nitratireducens
Marinobacter salarius
Marinobacter salinus
Marinobacter sp. C1S70
Marinobacter sp. CP1
Marinobacter sp. EN3
Marinobacter sp. EVN1
Marinobacter sp. LQ44
Marinobacter sp. T13-3
Marinobacter sp. X15-166B
Marinomonas gallaica
Marinomonas sp. (strain MWYL1)
Marinomonas spartinae
Maritimibacter alkaliphilus HTCC2654
Marivita cryptomonadis
Marivita geojedonensis
Massilia sp. JS1662
Massilia sp. LC238
Melioribacter roseus (strain JCM 17771 / P3M-2)
Mesorhizobium alhagi CCNWXJ12-2
Mesorhizobium amorphae CCNWGS0123
Mesorhizobium ciceri
Mesorhizobium ciceri biovar biserrulae (strain HAMBI 2942 / LMG 23838 / WSM1271)
Mesorhizobium huakuii 7653R
Mesorhizobium metallidurans STM 2683
Mesorhizobium opportunistum (strain LMG 24607 / HAMBI 3007 / WSM2075)
Mesorhizobium plurifarum
Mesorhizobium prunedense
Mesorhizobium sp. 61-13
Mesorhizobium sp. 65-26
Mesorhizobium sp. AA22
Mesorhizobium sp. AA23
Mesorhizobium sp. B7
Mesorhizobium sp. L103C105A0
Mesorhizobium sp. L103C119B0
Mesorhizobium sp. L103C120A0
Mesorhizobium sp. L103C131B0
Mesorhizobium sp. L103C565B0
Mesorhizobium sp. L2C054A000
Mesorhizobium sp. L2C066B000
Mesorhizobium sp. L2C084A000
Mesorhizobium sp. L2C089B000

Mesorhizobium sp. L48C026A00
Mesorhizobium sp. LC103
Mesorhizobium sp. LCM 4576
Mesorhizobium sp. LNHC209A00
Mesorhizobium sp. LNHC220B00
Mesorhizobium sp. LNHC221B00
Mesorhizobium sp. LNHC229A00
Mesorhizobium sp. LNHC232B00
Mesorhizobium sp. LNHC252B00
Mesorhizobium sp. LNJ372A00
Mesorhizobium sp. LNJ386A00
Mesorhizobium sp. LNJ391B00
Mesorhizobium sp. LNJ394B00
Mesorhizobium sp. LNJ395A00
Mesorhizobium sp. LNJ399B00
Mesorhizobium sp. LNJ405B00
Mesorhizobium sp. LSHC412B00
Mesorhizobium sp. LSHC414A00
Mesorhizobium sp. LSHC420B00
Mesorhizobium sp. LSHC422A00
Mesorhizobium sp. LSHC426A00
Mesorhizobium sp. LSJC264A00
Mesorhizobium sp. LSJC265A00
Mesorhizobium sp. LSJC269B00
Mesorhizobium sp. LSJC277A00
Mesorhizobium sp. LSJC280B00
Mesorhizobium sp. LSJC285A00
Mesorhizobium sp. ORS3324
Mesorhizobium sp. ORS3359
Mesorhizobium sp. ORS3428
Mesorhizobium sp. Root102
Mesorhizobium sp. Root157
Mesorhizobium sp. Root172
Mesorhizobium sp. Root552
Mesorhizobium sp. Root554
Mesorhizobium sp. SCN 65-20
Mesorhizobium sp. SEMIA 3007
Mesorhizobium sp. SOD10
Mesorhizobium sp. STM 4661
Mesorhizobium sp. UASWS1009
Mesorhizobium sp. WSM1497
Mesorhizobium sp. WSM3873
Methanosarcina mazei C16
Methanosarcina mazei LYC
Methanosarcina mazei (Methanosarcina frisia)
Methanosarcina mazei S-6
Methanosarcina mazei SarPi
Methanosarcina mazei (strain ATCC BAA-159 / DSM 3647 / Goe1 / Go1 / JCM 11833 / OCM 88)
(Methanosarcina frisia)

Methanosarcina mazei Tuc01
Methanosarcina mazei WWM610
Methanosarcina sp. 1.H.A.2.2
Methanosarcina sp. 1.H.T.1A.1
Methanosarcina sp. 2.H.A.1B.4
Methanosarcina sp. 2.H.T.1A.15
Methanosarcina sp. 2.H.T.1A.3
Methanosarcina sp. 2.H.T.1A.6
Methanosarcina sp. 2.H.T.1A.8
Methanosarcina sp. MTP4
Methylobacterium radiotolerans
Methylobrevia pamukkalensis
Microbacterium azadirachtae
Microbacterium azadirachtae
Microbacterium ketosireducens
Microbacterium mangrovi
Microbacterium oxydans
Microbacterium sp. HM58-2
Microbacterium sp. Leaf159
Microbacterium sp. Leaf320
Microbacterium sp. Root180
Microbacterium sp. SA39
Microbacterium sp. TNHR37B
Microbacterium sp. TS-1
Microbacterium trichothecenolyticum
Microbulbifer sp. CCB-MM1
Microbulbifer sp. ZGT114
Microlunatus phosphovorius (strain ATCC 700054 / DSM 10555 / JCM 9379 / NBRC 101784 / NCIMB 13414 / VKM Ac-1990 / NM-1)
Micromonospora rifamycinica
Micromonospora sp. Rc5
Microscilla marina ATCC 23134
Microvirga ossetica
Microvirga vignae
mine drainage metagenome
Moraxella bovoculi 237
Moraxella ovis
Mucilaginibacter gotjawali
Mumia flava
Mycobacterium abscessus
Mycobacterium abscessus subsp. abscessus
Mycobacterium abscessus subsp. bolletii
Mycobacterium abscessus subsp. bolletii 50594
Mycobacterium abscessus subsp. massiliense
Mycobacterium avium (strain 104)
Mycobacterium avium subsp. hominissuis (strain TH135)
Mycobacterium brisbanense
Mycobacterium caprae
Mycobacterium chlorophenolicum

Mycobacterium chubuense
Mycobacterium farcinogenes
Mycobacterium fortuitum
Mycobacterium fortuitum subsp. *fortuitum* DSM 46621 = ATCC 6841
Mycobacterium kansasii
Mycobacterium kansasii 824
Mycobacterium mageritense DSM 44476 = CIP 104973
Mycobacterium neworleansense
Mycobacterium obuense
Mycobacterium parascrofulaceum ATCC BAA-614
Mycobacterium pseudoshottsii
Mycobacterium smegmatis
Mycobacterium smegmatis (strain ATCC 700084 / mc(2)155)
Mycobacterium smegmatis (strain MKD8)
Mycobacterium sp. 852002-51759_SCH5129042
Mycobacterium sp. 852013-50091_SCH5140682
Mycobacterium sp. QIA-37
Mycobacterium sp. (strain KMS)
Mycobacterium sp. VKM Ac-1817D
Mycobacterium tuberculosis
Mycobacterium tuberculosis (strain CDC 1551 / Oshkosh)
Mycobacterium ulcerans str. Harvey
Mycobacterium vaccae ATCC 25954
Mycobacterium vulneris
Mycobacterium xenopi 3993
Mycobacterium xenopi 4042
Myxococcus fulvus 124B02
Myxococcus fulvus (strain ATCC BAA-855 / HW-1)
Myxococcus hansupus
Myxococcus stipitatus (strain DSM 14675 / JCM 12634 / Mx s8)
Myxococcus xanthus (strain DK 1622)
Nakamurella multipartita (strain ATCC 700099 / DSM 44233 / CIP 104796 / JCM 9543 / NBRC 105858 / Y-104) (*Microsphaera multipartita*)
Nautella italica
Neisseria arctica
Neisseria elongata subsp. *glycolytica* ATCC 29315
Neisseria flavescens
Neisseria flavescens SK114
Neisseria macacae ATCC 33926
Neisseria mucosa C102
Neisseria shayeganii 871
Neisseria sp. 74A18
Neisseria sp. HMSC03D10
Neisseria sp. HMSC055H02
Neisseria sp. HMSC056A03
Neisseria sp. HMSC064E01
Neisseria sp. HMSC067G11
Neisseria sp. HMSC068C04
Neisseria sp. HMSC069H12

Neisseria sp. HMSC070A01
Neisseria sp. HMSC073G10
Neisseria sp. HMSC077D05
Neisseria sp. HMSC31F04
Neisseria sp. HMSC70E02
Neorhizobium galegae bv. officinalis
Neorhizobium galegae bv. officinalis bv. officinalis str. HAMBI 1141
Neorhizobium galegae bv. orientalis
Neorhizobium galegae bv. orientalis str. HAMBI 540
Nereida ignava
Nesterenkonia sp. PF2B19
Nitratireductor basaltis
Nitrincola nitratireducens
Nitrosomonas eutropha (strain C91)
Nitrospira bacterium SG8_35_4
Nocardia asteroides NBRC 15531
Nocardia farcinica
Nocardia seriolae
Nocardia sp. 852002-20019_SCH5090214
Nocardioidaceae bacterium Broad-1
Nocardioides dokdonensis FR1436
Nocardioides sp. Root140
Nocardioides sp. Root151
Nocardioides sp. Root190
Nocardioides sp. Root240
Nocardioides sp. Root614
Nocardioides sp. Root682
Nocardioides sp. Root79
Nocardioides sp. Soil796
Nocardioides sp. Soil797
Nonomuraea sp. ATCC 39727
Nonomuraea sp. ATCC 55076
Novosphingobium aromaticivorans (strain ATCC 700278 / DSM 12444 / CIP 105152 / NBRC 16084 / F199)
Novosphingobium fuchskuhlense
Novosphingobium mathurense
Novosphingobium nitrogenifigens DSM 19370
Novosphingobium sp. AAP1
Novosphingobium sp. AAP93
Novosphingobium sp. FSW06-99
Novosphingobium sp. Fuku2-ISO-50
Novosphingobium sp. SCN 63-17
Novosphingobium sp. SCN 66-18
Novosphingobium subterraneum
Novosphingobium tardaugens NBRC 16725
Oceanibaculum pacificum
Oceanibulbus indolifex HEL-45
Oceanicaulis sp. HLUCCA04
Oceanicola litoreus

Oceanimonas sp. (strain GK1)
Oceanobacillus iheyensis (strain DSM 14371 / CIP 107618 / JCM 11309 / KCTC 3954 / HTE831)
Oceanobacillus oncorhynchi
Oceanobacillus picturae
Ochrobactrum anthropi
Ochrobactrum anthropi (strain ATCC 49188 / DSM 6882 / JCM 21032 / NBRC 15819 / NCTC 12168)
Ochrobactrum cytisi
Ochrobactrum intermedium 229E
Ochrobactrum intermedium LMG 3301
Ochrobactrum intermedium M86
Ochrobactrum pseudogrignonense
Ochrobactrum sp. EGD-AQ16
Ochrobactrum sp. P6BS-III
Octadecabacter temperatus
Oleiphilus sp. HI0065
Oleispira antarctica RB-8
Paenarthrobacter aurescens (strain TC1)
Paenibacillus alvei DSM 29
Paenibacillus antarcticus
Paenibacillus borealis
Paenibacillus durus (Paenibacillus azotofixans)
Paenibacillus elgii
Paenibacillus etheri
Paenibacillus ferrarius
Paenibacillus glacialis
Paenibacillus glucanolyticus
Paenibacillus lautus (Bacillus lautus)
Paenibacillus macerans (Bacillus macerans)
Paenibacillus macquariensis (Bacillus macquariensis)
Paenibacillus macquariensis subsp. defensor
Paenibacillus odorifer
Paenibacillus selenitireducens
Paenibacillus sp. A3
Paenibacillus sp. AD87
Paenibacillus sp. E194
Paenibacillus sp. FJAT-22460
Paenibacillus sp. FSL A5-0031
Paenibacillus sp. FSL H7-0357
Paenibacillus sp. FSL H7-0737
Paenibacillus sp. IHBB 10380
Paenibacillus sp. KS1
Paenibacillus sp. LC231
Paenibacillus sp. P1XP2
Paenibacillus sp. TI45-13ar
Paenibacillus tyrfis
Paeniclostridium sordellii (Clostridium sordellii)
Paeniglutamicibacter gangotriensis Lz1y
Paenirhodobacter enshiensis
Paludisphaera borealis

Pandoraea norimbergensis
Pandoraea sp. SD6-2
Pannonibacter phragmitetus
Pantoea sp. 3.5.1
Parabacteroides distasonis
Parabacteroides distasonis (strain ATCC 8503 / DSM 20701 / CIP 104284 / JCM 5825 / NCTC 11152)
Paraburkholderia caribensis
Paraburkholderia fungorum
Paraburkholderia ginsengiterrae
Paraburkholderia monticola
Paraburkholderia ribeironis
Paraburkholderia sacchari
Paraburkholderia sp. SOS3
Paraburkholderia sprentiae WSM5005
Paraburkholderia susongensis
Paraburkholderia terricola
Paraburkholderia tropica
Paraburkholderia xenovorans (strain LB400)
Paracoccus aminophilus JCM 7686
Paracoccus halophilus
Paracoccus sanguinis
Paracoccus sp. MKU1
Paracoccus sp. SM22M-07
Paracoccus versutus (Thiobacillus versutus)
Paracoccus yeei
Paraliobacillus sp. PM-2
Paramesorhizobium deserti
Pararhizobium polonicum
Patulibacter medicamentivorans
Pediococcus acidilactici
Pedobacter heparinus (strain ATCC 13125 / DSM 2366 / CIP 104194 / JCM 7457 / NBRC 12017 / NCIMB 9290 / NRRL B-14731 / HIM 762-3)
Pelagibacterium sp. SCN 63-23
Peptoclostridium litorale DSM 5388
Phaeobacter gallaeciensis
Phaeobacter inhibens
Phaeobacter sp. 11ANDIMAR09
Phaeobacter sp. CECT 5382
Phaeobacter sp. CECT 7735
Phaeobacter sp. S26
Phenylobacterium sp. RIFCSPHIGHO2_01_FULL_69_31
Phenylobacterium sp. RIFCSPHIGHO2_01_FULL_70_10
Phenylobacterium sp. Root1277
Phenylobacterium sp. Root1290
Phenylobacterium sp. Root700
Phenylobacterium sp. Root77
Phenylobacterium sp. SCN 70-31
Phenylobacterium zucineum (strain HLK1)
Photobacterium gaetbulicola

Photobacterium gaetbulicola Gung47
Photobacterium phosphoreum
Photorhabdus asymbiotica subsp. australis
Planctomyces sp. SCGC AG-212-M04
Planctomyces sp. SH-PL14
Planctomyces sp. SH-PL62
Planctomycetes bacterium ADurb.Bin126
Planktotalea frisia
Pleomorphobacterium xiamenense
Polaribacter irgensii 23-P
Polaribacter sp. MED152
Polysphondylium pallidum (strain ATCC 26659 / Pp 5 / PN500)
Ponticoccus sp. SJ5A-1
Porphyrobacter mercurialis
Porphyromonas cangingivalis
Powai lake megavirus
Propionibacterium acnes JCM 18909
Propionibacterium acnes JCM 18916
Propionibacterium acnes JCM 18918
Propionibacterium acnes JCM 18920
Propionibacterium acnes (strain KPA171202 / DSM 16379)
[Propionibacterium] humerusii P08
Propionibacterium sp. 4572_24
Propionibacterium sp. CG1_02_60_36
Proteobacteria bacterium HN_bin10
Proteobacteria bacterium SG_bin9
Proteobacteria bacterium SG_bin9
Providencia burhodogranariae DSM 19968
Pseudaminobacter manganicus
Pseudoalteromonas phenolica
Pseudoalteromonas sp. H105
Pseudohalaea rubra DSM 19751
Pseudomonadales bacterium GWC2_63_15
Pseudomonas aeruginosa
Neorhizobium galegae bv. officinalis bv. officinalis str. HAMBI 1141
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Nocardioidaceae bacterium Broad-1
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Nocardioides sp. Root140
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Paenibacillus ferrarius
Paenibacillus glacialis
Paenibacillus glucanolyticus
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Paenibacillus macquariensis (Bacillus macquariensis)
Paenibacillus macquariensis subsp. defensor
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Paenirhodobacter enshiensis
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Pandoraea sp. SD6-2
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Paraburkholderia fungorum
Paraburkholderia ginsengiterrae
Paraburkholderia monticola
Paraburkholderia ribeironis
Paraburkholderia sacchari
Paraburkholderia sp. SOS3
Paraburkholderia sprentiae WSM5005
Paraburkholderia susongensis
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Paraburkholderia tropica
Paraburkholderia tropica
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Paracoccus halophilus

Paracoccus sanguinis
Paracoccus sp. MKU1
Paracoccus sp. SM22M-07
Paracoccus versutus (Thiobacillus versutus)
Paracoccus yeei
Paraliobacillus sp. PM-2
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Pararhizobium polonicum
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Phaeobacter sp. CECT 5382
Phaeobacter sp. CECT 7735
Phaeobacter sp. S26
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Phenylobacterium sp. RIFCSPHIGHO2_01_FULLL_70_10
Phenylobacterium sp. Root1277
Phenylobacterium sp. Root1290
Phenylobacterium sp. Root700
Phenylobacterium sp. Root77
Phenylobacterium sp. SCN 70-31
Phenylobacterium zucineum (strain HLK1)
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Photobacterium gaetbulicola Gung47
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Planctomyces sp. SCGC AG-212-M04
Planctomyces sp. SH-PL14
Planctomyces sp. SH-PL62
Planctomycetes bacterium ADurb.Bin126
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Pleomorphobacterium xiamenense
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Polaribacter sp. MED152
Polysphondylium pallidum (strain ATCC 26659 / Pp 5 / PN500)
Ponticoccus sp. SJ5A-1
Porphyrobacter mercurialis
Porphyromonas cangingivalis
Powai lake megavirus
Propionibacterium acnes JCM 18909
Propionibacterium acnes JCM 18916
Propionibacterium acnes JCM 18918
Propionibacterium acnes JCM 18920

Propionibacterium acnes (strain KPA171202 / DSM 16379)
[Propionibacterium] humerusii P08
Propionibacterium sp. 4572_24
Propionibacterium sp. CG1_02_60_36
Proteobacteria bacterium HN_bin10
Proteobacteria bacterium SG_bin9
Providencia burhodogranariae DSM 19968
Pseudaminobacter manganicus
Pseudoalteromonas phenolica
Pseudoalteromonas sp. H105
Pseudohalaea rubra DSM 19751
Pseudomonadales bacterium GWC2_63_15
Pseudomonas aeruginosa
Ralstonia solanacearum UW551
Ralstonia sp. A12
Ralstonia sp. AU12-08
Ralstonia sp. MD27
Ralstonia sp. NT80
Ralstonia syzygii R24
Ramlibacter tataouinensis
Rathayibacter tanacetii
Rhizobacter sp. Root404
Rhizobiales bacterium 62-47
Rhizobiales bacterium 63-7
Rhizobiales bacterium 65-79
Rhizobium acidisoli
Rhizobium etli CIAT 894
Rhizobium etli CNPAF512
Rhizobium etli (strain CFN 42 / ATCC 51251)
Rhizobium etli (strain CIAT 652)
Rhizobium favelukesii
Rhizobium fredii (strain HH103) (Sinorhizobium fredii)
Rhizobium freirei PRF 81
Rhizobium laguerreae
Rhizobium leguminosarum
Rhizobium leguminosarum bv. trifolii
Rhizobium leguminosarum bv. trifolii WSM1689
Rhizobium leguminosarum bv. viciae
Rhizobium leguminosarum bv. viciae (strain 3841)
Rhizobium leguminosarum bv. viciae USDA 2370
Rhizobium leguminosarum bv. viciae USDA 2370
Rhizobium loti (Mesorhizobium loti)
Rhizobium loti (strain MAFF303099) (Mesorhizobium loti)
Rhizobium meliloti (strain 1021) (Ensifer meliloti) (Sinorhizobium meliloti)
Rhizobium oryzae
Rhizobium oryzae
Rhizobium phaseoli Ch24-10
Rhizobium pusense
Rhizobium radiobacter (Agrobacterium tumefaciens) (Agrobacterium radiobacter)

Rhizobium rhizogenes NBRC 13257
Rhizobium rhizosphaerae
Rhizobium sophorae
Rhizobium sp. 58
Rhizobium sp. 60-20
Rhizobium sp. 63-7
Rhizobium sp. AAP116
Rhizobium sp. AAP43
Rhizobium sp. AC27/96
Rhizobium sp. AC44/96
Rhizobium sp. BR10423
Rhizobium sp. CF080
Rhizobium sp. H41
Rhizobium sp. LC145
Rhizobium sp. LCM 4573
Rhizobium sp. Leaf306
Rhizobium sp. Leaf371
Rhizobium sp. Leaf453
Rhizobium sp. NT-26
Rhizobium sp. P44RR-XXIV
Rhizobium sp. P44RR-XXIV
Rhizobium sp. Root1203
Rhizobium sp. Root1204
Rhizobium sp. Root1212
Rhizobium sp. Root1220
Rhizobium sp. Root1240
Rhizobium sp. Root149
Rhizobium sp. Root482
Rhizobium sp. Root483D2
Rhizobium sp. Root708
Rhizobium sp. RSm-3
Rhizobium sp. UR51a
Rhizobium sp. WYCCWR10014
Rhizobium sp. WYCCWR10015
Rhizobium sp. YK2
Rhizobium sp. YS-1r
Rhizobium taibaishanense
Rhizobium tibeticum
Rhizobium tropici CIAT 899
Rhizomicrobium sp. SCGC AG-212-E05
Rhodanobacter sp. C05
Rhodanobacter thiooxydans
Rhodobacter capsulatus (strain ATCC BAA-309 / NBRC 16581 / SB1003)
Rhodobacter capsulatus Y262
Rhodobacter johrii
Rhodobacter sp. BAACL10 MAG-120419-bin15
Rhodobacter sp. BAACL10 MAG-120910-bin24
Rhodobacter sp. BAACL10 MAG-121220-bin24
Rhodobacter sp. CACIA14H1

Rhodobacter sp. LPB0142
Rhodobacter sphaeroides (strain ATCC 17023 / 2.4.1 / NCIB 8253 / DSM 158)
Rhodobacter vinaykumarii
Rhodobacteraceae bacterium EhC02
Rhodobacteraceae bacterium GWE1_64_9
Rhodobacteraceae bacterium GWF1_65_7
Rhodobacterales bacterium 65-51
Rhodobacterales bacterium RIFCSPHIGHO2_02_FULL_62_130
Rhodococcus erythropolis (Arthrobacter picolinophilus)
Rhodococcus erythropolis DN1
Rhodococcus erythropolis R138
Rhodococcus erythropolis SK121
Rhodococcus erythropolis (strain PR4 / NBRC 100887)
Rhodococcus fascians D188
Rhodococcus imtechensis RKJ300 = JCM 13270
Rhodococcus jostii (strain RHA1)
Rhodococcus opacus (Nocardia opaca)
Rhodococcus opacus PD630
Rhodococcus qingshengii
Rhodococcus sp. AD45
Rhodococcus sp. Br-6
Rhodococcus sp. EsD8
Rhodococcus sp. JVH1
Rhodococcus sp. LB1
Rhodococcus sp. P27
Rhodococcus sp. PBTS 1
Rhodococcus sp. PBTS 2
Rhodococcus sp. SC4
Rhodococcus wratislaviensis IFP 2016
Rhodococcus wratislaviensis NBRC 100605
Rhodomicrobium udaipurense JA643
Rhodopseudomonas palustris
Rhodopseudomonas palustris (strain ATCC BAA-98 / CGA009)
Rhodopseudomonas palustris (strain BisA53)
Rhodopseudomonas palustris (strain BisB5)
Rhodopseudomonas palustris (strain DX-1)
Rhodopseudomonas palustris (strain TIE-1)
Rhodopseudomonas sp. AAP120
Rhodovulum sp. NI22
Roseburia intestinalis
Roseivivax atlanticus
Roseivivax halodurans JCM 10272
Roseivivax isopora LMG 25204
Roseivivax jejudonensis
Roseobacter denitrificans (strain ATCC 33942 / OCh 114) (Erythrobacter sp. (strain OCh 114))
(Roseobacter denitrificans)
Roseobacter sp. MED193
Roseobacter sp. MedPE-SWchi
Roseobacter sp. SK209-2-6

Roseomonas gilardii
Roseomonas mucosa
Roseomonas sp. M3
Roseomonas sp. TAS13
Roseovarius aestuarii
Roseovarius atlanticus
Roseovarius gaetbuli
Roseovarius halotolerans
Roseovarius indicus
Roseovarius mucosus
Roseovarius sp. A-2
Roseovarius sp. HI0049
Roseovarius sp. TM1035
Roseovarius tolerans
Rubellimicrobium mesophilum DSM 19309
Rubellimicrobium thermophilum DSM 16684
Rubrivivax benzoatilyticus JA2 = ATCC BAA-35
Ruegeria atlantica
Ruegeria meonggei
Ruegeria pomeroyi (strain ATCC 700808 / DSM 15171 / DSS-3) (Silicibacter pomeroyi)
Ruegeria sp. ANG-R
Ruegeria sp. ANG-S4
Ruegeria sp. CECT 5091
Ruegeria sp. PBVC088
Ruegeria sp. (strain TM1040) (Silicibacter sp.)
Ruegeria sp. ZGT118
[Ruminococcus] torques
Saccharicrinis fermentans DSM 9555 = JCM 21142
Sagittula stellata E-37
Salinicola socius
Salinicola sp. MH3R3-1
Salinicola sp. MIT1003
Salinivirga cyanobacteriivorans
SAR92 bacterium BAACL16 MAG-120619-bin48
SAR92 bacterium BAACL26 MAG-121220-bin70
Scytonema hofmannii PCC 7110
Sedimentitalea nanhaiensis
Serratia
Serratia grimesii
Serratia liquefaciens
Serratia liquefaciens FK01
Serratia marcescens
Serratia sp. S119
Shewanella frigidimarina (strain NCIMB 400)
Shewanella piezotolerans (strain WP3 / JCM 13877)
Shewanella sp. cp20
Shimia marina
Shimia sp. SK013
Shinella sp. DD12

Shinella sp. HZN7
Sinorhizobium americanum
Sinorhizobium americanum CCGM7
Sinorhizobium fredii (strain NBRC 101917 / NGR234)
Sinorhizobium fredii USDA 205
Sinorhizobium fredii USDA 257
Sinorhizobium meliloti CCNWSX0020
Sinorhizobium meliloti (strain SM11)
Sinorhizobium saheli
Sinorhizobium sp. A49
Sinorhizobium sp. GL28
Sinorhizobium sp. Sb3
Skermanella aerolata KACC 11604
Skermanella stibioresistens SB22
Sphingobacterium faecium PCAi_F2.5
Sphingobacterium sp. CZ-UAM
Sphingobacterium sp. PM2-P1-29
Sphingobium japonicum (strain NBRC 101211 / UT26S)
Sphingobium sp. Ant17
Sphingobium sp. EP60837
Sphingobium sp. Leaf26
Sphingomonadales bacterium 63-6
Sphingomonadales bacterium GWF1_63_6
Sphingomonadales bacterium RIFCSPLOWO2_12_FULL_63_15
Sphingomonas hengshuiensis
Sphingomonas paucimobilis (Pseudomonas paucimobilis)
Sphingomonas sanguinis
Sphingomonas sp. 66-10
Sphingomonas sp. Ag1
Sphingomonas sp. Leaf10
Sphingomonas sp. Leaf20
Sphingomonas sp. Leaf208
Sphingomonas sp. Leaf22
Sphingomonas sp. Leaf231
Sphingomonas sp. Leaf34
Sphingomonas sp. Leaf343
Sphingomonas sp. Leaf38
Sphingomonas sp. Leaf412
Sphingomonas sp. Leaf67
Sphingomonas sp. TDK1
Sphingomonas sp. WG
Sphingopyxis granuli
Spirochaetes bacterium ADurb.Bin215
Spirochaetes bacterium ADurb.BinA120
Sporomusa sp. An4
Stenotrophomonas rhizophila
Stenotrophomonas sp. LM091
Stenotrophomonas sp. SKA14
Stigmatella aurantiaca (strain DW4/3-1)

Streptococcus agalactiae
Streptococcus equi subsp. *equi*
Streptococcus equi subsp. *zooepidemicus* (strain MGCS10565)
Streptococcus mutans serotype c (strain ATCC 700610 / UA159)
Streptococcus parauberis KRS-02083
Streptococcus pneumoniae
Streptococcus salivarius (strain CCHSS3)
Streptococcus salivarius (strain JIM8777)
Streptococcus sp. DD11
Streptococcus sp. DD12
Streptococcus sp. DD13
Streptococcus sp. HSISS1
Streptococcus sp. HSISS2
Streptococcus sp. HSISS3
Streptococcus suis
Streptococcus thermophilus CAG:236
Streptomyces acidiscabies
Streptomyces albus PD-1
Streptomyces albus subsp. *albus*
Streptomyces aurantiacus JA 4570
Streptomyces aureofaciens
Streptomyces caatingaensis
Streptomyces cyaneogriseus subsp. *noncyanogenus*
Streptomyces fulvissimus DSM 40593
Streptomyces gandocaensis
Streptomyces griseochromogenes
Streptomyces griseoflavus
Streptomyces griseoflavus Tu4000
Streptomyces griseus subsp. *griseus*
Streptomyces himastatinicus ATCC 53653
Streptomyces leeuwenhoekii
Streptomyces luteus
Streptomyces lydicus
Streptomyces nanshensis
Streptomyces niveus NCIMB 11891
Streptomyces oceani
Streptomyces parvulus
Streptomyces qinglanensis
Streptomyces rapamycinicus NRRL 5491
Streptomyces reticuli
Streptomyces rimosus subsp. *pseudoverticillatus*
Streptomyces rimosus subsp. *rimosus*
Streptomyces scabiei
Streptomyces silaceus
Streptomyces silvensis
Streptomyces sp. AVP053U2
Streptomyces sp. CB01249
Streptomyces sp. CB02414
Streptomyces sp. CB02488

Streptomyces sp. CB02923
Streptomyces sp. CB03578
Streptomyces sp. CC71
Streptomyces sp. CCM_MD2014
Streptomyces sp. F-1
Streptomyces sp. FR-008
Streptomyces sp. H036
Streptomyces sp. Mg1
Streptomyces sp. MP131-18
Streptomyces sp. NRRL B-3648
Streptomyces sp. NRRL F-4489
Streptomyces sp. NRRL F-5755
Streptomyces sp. NRRL F-6602
Streptomyces sp. NRRL F-7442
Streptomyces sp. NRRL S-1521
Streptomyces sp. NRRL WC-3753
Streptomyces sp. NTK 937
Streptomyces sp. PTY087I2
Streptomyces sp. SAT1
Streptomyces sp. Tu 6176
Streptomyces sp. WM4235
Streptomyces sp. WM6368
Streptomyces sp. WM6372
Streptomyces varsoviensis
Streptomyces virginiae
Streptomyces yokosukanensis
Sulfitobacter donghicola DSW-25 = KCTC 12864 = JCM 14565
Sulfitobacter geojensis
Sulfitobacter mediterraneus
Sulfitobacter pseudonitzschiae
Sulfitobacter sp. AM1-D1
Sulfitobacter sp. CB2047
Sulfitobacter sp. EhC04
Sulfitobacter sp. HI0023
Sulfitobacter sp. HI0054
Sulfitobacter sp. HI0076
Sulfitobacter sp. HI0082
Sulfitobacter sp. (strain NAS-14.1)
Sunxiuqinia dokdonensis
Syntrophaceae bacterium PtaB.Bin095
Tannerella forsythia (Bacteroides forsythus)
Tardiphaga robiniae
Tateyamaria omphalii
Tateyamaria sp. ANG-S1
Tatlockia micdadei (Legionella micdadei)
Tetrasphaera australiensis Ben110
Tetrasphaera japonica T1-X7
Thalassobius gelatinovorius (Ruegeria gelatinovora)
Thalassobius mediterraneus

Thalassospira lucentensis
Thalassospira permensis NBRC 106175
Thalassospira profundimaris
Thalassospira sp. HJ
Thalassospira sp. MCCC 1A01148
Thalassospira sp. MCCC 1A01428
Thalassospira sp. MCCC 1A02898
Thalassospira sp. MCCC 1A03138
Thalassospira sp. Nap_22
Thalassospira sp. TSL5-1
Thalassospira tepidiphila MCCC 1A03514
Thalassospira xiamenensis
Thermobrachium celere DSM 8682
Thermus scotoductus (strain ATCC 700910 / SA-01)
Thermus sp. 2.9
Thermus sp. CCB_US3_UF1
Thermus sp. NMX2.A1
Thermus thermophilus (strain HB27 / ATCC BAA-163 / DSM 7039)
Thioalkalivibrio sulfidiphilus (strain HL-EbGR7)
Thioclava pacifica DSM 10166
Thioclava sp. 11.10-0-13
Thioclava sp. 25B10_4
Thioclava sp. DLFJ4-1
Thioclava sp. DLFJ5-1
Thioclava sp. EIOx9
Thioclava sp. F28-4
Thioclava sp. L04-15
Thiomicrospira crunogena (strain XCL-2)
Thiomonas arsenitoxydans (strain DSM 22701 / CIP 110005 / 3As)
Thiomonas sp. CB2
Thiomonas sp. CB3
Tistrella mobilis
Tistrella mobilis (strain KA081020-065)
Treponema succinifaciens (strain ATCC 33096 / DSM 2489 / 6091)
Tropicibacter multivorans
uncultured *Anaerotruncus* sp.
uncultured *Bacteroides* sp.
uncultured *Blautia* sp.
uncultured *Clostridium* sp.
uncultured *Coprococcus* sp.
uncultured *Dorea* sp.
uncultured *Eubacterium* sp.
uncultured *Lachnospira* sp.
uncultured marine group II/III euryarchaeote KM3_177_C07
uncultured marine group II/III euryarchaeote KM3_35_H09
uncultured *Ruminococcus* sp.
Vagococcus fluvialis bH819
Variibacter gotjawalensis
Variovorax boronicumulans

Variovorax paradoxus
Variovorax paradoxus B4
Variovorax paradoxus (strain S110)
Variovorax sp. PAMC 28711
Variovorax sp. Root318D1
Variovorax sp. Root411
Variovorax sp. Root434
Variovorax sp. Root473
Variovorax sp. SCN 67-85
Variovorax sp. WDL1
Verrucomicrobia bacterium ADurb.Bin006
Verrucomicrobia bacterium ADurb.Bin018
Verrucomicrobia bacterium ADurb.Bin070
Vibrio alginolyticus
Vibrio azureus NBRC 104587
Vibrio breoganii
Vibrio celticus
Vibrio cyclitrophicus FF160
Vibrio fortis
Vibrio hemicentroti
Vibrio ishigakensis
Vibrio lentus
Vibrio maritimus
Vibrio nigripulchritudo
Vibrio ponticus
Vibrio renipiscarius
Vibrio scophthalmi
Vibrio shilonii AK1
Vibrio sp. JB196
Vibrio sp. JCM 19236
Vibrio splendidus
Vibrio splendidus 12E03
Vibrio splendidus 1F-157
Vibrio splendidus ZS-139
Vibrio toranzoniae
Vibrio tubiashii ATCC 19109
Vibrio variabilis
Vibrio vulnificus
Virgibacillus massiliensis
Viridibacillus arenosi FSL R5-213
Vulgatibacter incomptus
Weissella jogaejeotgali
Wenzhouxiangella marina
Xanthomonas sp. Mitacek01
Yangia sp. CCB-MM3
Zymoseptoria brevis

SUPPLEMENT F

Nomenclature Relevant to Okada and Ohno's Hypotheses and Miscellaneous Reference and Identification Data

ORGANISM NAMES AND MISCELLANEOUS INFORMATION

Acromobacter gutatus KI72 (1977)
renamed to Flavobacteria sp. KI72 (1983)
renamed to Arthrobacter sp. KI72 (2007)

Flavobacteria Sp. KI725 has pOAD21 plasmid which lacks NylA but contains NylC
Flavobacteria Sp. KI725R has pOAD21 plasmid which lacks NylA but contains NylC

Databases tend to have genes recorded under Flavobacteria sp.

PROTEIN AND GENE AND TRANSCRIPT NAMES

Gene names : NylA, NylB, NylB', NylC

EI (coded by *nylA*) = 6-aminohexanoic acid cyclic dimer hydrolase (1983 Okada)
= 6-AHA CDH (1984 Ohno)

EII (coded by *nylB*) =
6-aminohexanoic acid linear oligomer hydrolase (1983 Okada)
= 6-AHA LOH (1984 Ohno)

EII' (coded by *nylB'*) (1983 Okada)

EIII (coded by *nylC* in KI725 and KI725R) (1992 Negoro)

RS-IIA = contains *nylB* gene
RS-IIB = contains *nylB'* gene

A-nylB = homolog of *nylB* on Agromyces KYR5
A-nylB' = homolog of *nylB'* on Agromyces KYR5
A-nylC = homolog of *nylC* on Agromyces KYR5

PLASMID NAMES

pOAD2 associated with KI723T1
pOAD21 associated with KI725 and KI725R

NUCLEOTIDE ACCESSION NUMBERS

X00046.1 Flavobacterium sp. KI72 EII (*nylB*) gene coding for an enzyme
degrading nylon oligomers.

D26094.1 Flavobacterium sp. KI723T1 plasmid pOAD2 DNA, complete sequence

X02864.1 Flavobacterium sp. K172 pOAD2 RS-IIB repeat with nylB' gene coding for enzyme EII' degrading nylon oligomers

AB264778.2 Agromyces sp. KY5R *ftsX*, *smpB*, *nylB*, *nylC*, *oppF*, *oppD*, *oppC*, *oppB*, *oppA*, *penDE*, *nylC'*, *nylB'*, *smpB*, genes, complete cds

PROTEIN ACCESSION NUMBERS

P07061.1 RecName: Full=6-aminohexanoate-dimer hydrolase; AltName: Full=Nylon oligomers-degrading enzyme EII ...SOURCE Flavobacterium sp. K172

[my note: this is listed as coded by *nylB*]

BAE97621.1 6-aminohexanoate-dimer hydrolase [Agromyces sp. KY5R]

[my note: this is listed as coded by *nylB*]

P07062.1 RecName: Full=6-aminohexanoate-dimer hydrolase; AltName: Full=Nylon oligomers-degrading enzyme EII'

[my note: this is listed as coded by *nylB'*]

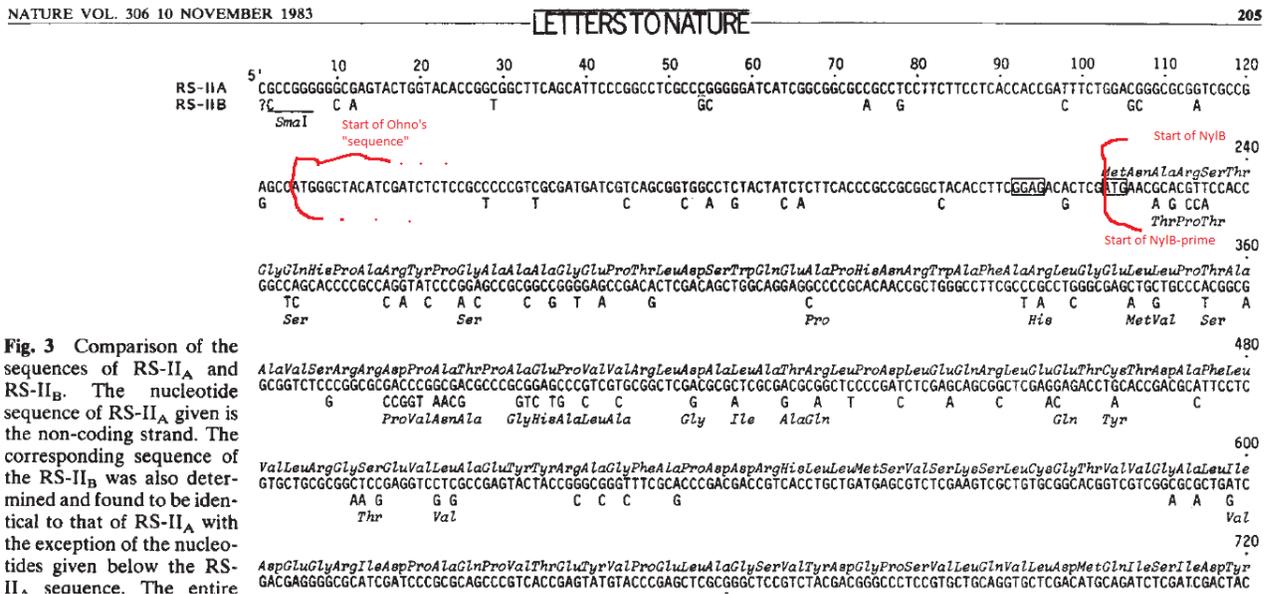
WP_012476894.1 6-aminohexanoate-dimer hydrolase [Flavobacterium sp. KI723T1]

SUPPLEMENT G

Description, Analysis and Problems with Ohno's PR.C Sequence

Because Ohno's PR.C sequence doesn't cover the entire sequence published by Okada in the 1983 paper, this supplement provides information on how to relate Ohno's PR.C sequence to Okada's RS-II_A sequence.

Okada's sequence is also recorded in Genbank under accession number X00046.1. Provided below is the section from Okada's paper that Ohno used to create the start of his PR.C sequence. The red marking and text were added for clarity to suggest how to take the sequence from X00046.1 and modify it to the PR.C sequence. Constructing PR.C in this manner ensures accuracy of creating Ohno's PR.C sequence rather than manually retyping Ohno's PR.C sequence from Ohno's 1984 paper.



There is one problem, however. Ohno reported the actual end of his sequence as "GCGGCTGA" without explaining why it deviates from the sequence reported by Okada (and thus genbank). According to genbank, the end sequence of PR.C should be "GCGGC**G**TGA" not "GCGGCTGA", where the red letter "G" indicates the guanine base Ohno omitted without any explanation.

To illustrate the problem, here is the end sequence in Okada's 1983 paper with the end sequence circled in green.

1spAlaVal BamHI 1440
 3ACGCGGTGTAGCGGGCGTGACCGGGCTCGGCACCCGGCCGGGATCCTCTA
 A C ACG CG TCCGC C CGGGCCT CCCC GG

See how this contrasts with the end sequence in Ohno's paper, with the mistake circled in red.

PR.C.	393	ARG	GLN	ALA	LEU	VAL	VAL	ALA	ARG	SER	ARG	HIS	PRO	ALA	LEU	ALA	ARG	ALA	ALA	410																																			
R-IIA	361	VAL	LYS	LEU	SER	SER	TRP	PRO	ASP	PRO	ASP	THR	ARG	HIS	TRP	HIS	GLY	LEU	GLN	378																																			
		G	T	C	A	A	G	C	T	C	T	C	G	T	C	G	T	G	G	C	C	C	G	A	T	C	C	C	G	A	C	A	C	C	C	G	G	C	A	C	T	G	G	C	A	C	G	G	G	C	T	G	C	A	G
PR.C.	411	GLU	ARG	ASP	PRO	ALA	ARG	ARG	GLN	PRO	CYS	PRO	ARG	ARG	GLY	VAL	GLY	GLY	████	427																																			
R-IIA	379	SER	GLY	ILE	LEU	LEU	ASP	VAL	SER	ARG	ALA	LEU	ASP	ALA	VAL	████	████	████	████	392																																			
		A	G	C	G	G	G	A	T	C	T	C	T	G	C	T	C	G	A	C	G	T	C	A	G	C	C	G	T	G	C	C	T	C	G	A	C	G	C	G	G	T	G	T	A	G	G	C	G	G	C	T	G	A	

Because there are the RS-II_A (of *nylB*) and RS-II_B (of *nylB'*) sequences, Ohno's typo is also an implicit mistake on the supposed corresponding frame shift mutation that must take place in RS-II_B. Ohno made a correspondence between PR.C and *nylB* but there should be some sort of PR.C' that corresponds to *nylB'* as well, so his typo effectively creates two problems, not just one.

Below is Ohno's PR.C sequence. As mentioned, the sequence can be somewhat reconstructed by taking the Genbank sequence (with the accession number X00046.1) and editing it to match Ohno's sequence. That was the technique used to reproduce Ohno's PR.C sequence since it was deemed to be more accurate than retyping the sequence from Ohno's paper. The position of the yet-to-be thymine is marked in red. The lower case letters indicate the location of actual *nylB* gene locus. Spacing was added for clarity.

ATGGGCTACATCGATCTCTCCGCCCCCGTCGCGATGATCGTCAGC
 GGTGGCCTCTACTATCTCTTACCCCGCCGCGGCTACACCTTCGGAGACACT
 CG **aga**acgcacgtccacc
 ggcagcaccgccaggtatcccggagccgcggccggggagccgacactcgacagctgg
 caggaggccccgcacaaccgctgggcttcgcccgctgggcgagctgctgcccacggcg
 gcggttccccggcgcgacccggcgacgcccgcggagcccgtctgctggctcgacgcgctc
 gcgacgcggctccccgatctcgagcagcggctcgaggagacctgcaccgacgattcctc
 gtgctgcgcggctccgaggtctcgcgagtagtactaccggcggggttcgacccgacgac
 cgtaacctgctgatgagcgtctcgaagtcgctgtgcggcacggctgctggcgcgctgatc
 gacgaggggcgcatcgatcccgcgcagcccgtcaccgagtagtaccgagctcgcgggc
 tccgtctacgacgggcccctcgtgctgcaggtgctcgacatgcagatctcgatcgactac
 aacgaggactacgctgatccggcctcggaggtgcagaccacgatcgtccgccggctgg
 cgcacgcggcgagacggggaccgccgacacctacgagttcctcaccacctccgcggc
 gacggcgaccggcgagttccagtactgctcggcgaacaccgacgtgctcgcctggatc
 gtcgagcgggtcaccggtctgcgctacgtcgaagcgtctccacgtacctgtggcggaag
 ctgacgccgatcgggatcgcaccatcacggctgaccagaccggcttcggcttcgcaac
 gggggcgtctcctgcaccgcgggatctcgcacgcgtgggcccgatgatgctcgacggc
 ggcgtcgtccccggcgacgggtcgtatcgagggctgggtgaaagcgtgctggccggc
 ggctcccgcgaagccatgaccgacgagggttcacctccgattccccgagggcagctac
 acgcgccagtgggtgctcagcgggcaacgagcgcggcaacgtgagcggcatcggcatccac
 ggccagaacctctggctcgatccgcgaccgactcggctgatcgtcaagctctcgtcgtgg
 cccgatccgacaccggcactggcaggggtcagagcgggatctgctcgacgtcagc
 cgtgccctcgacgcggtgtag **G**CGGCTGA

OHNO'S PR.C POLYPEPTIDE

The polypeptide hypothetically coded by PR.C can be generated by taking PR.C and entering in the ExPASy website:

<http://web.expasy.org/translate/>

Here is the resulting polypeptide sequence:

MGYIDLSAPVAMIVSGGLYYLFTRRGYTFGDTRERTFHRPAPRQVSRSRGRGADTRQLAG
GPAQPLGLRPPGRAAAHGGGLPARPGDARGARRAARRARDAAPRSRAAARGDLHRRIPRA
ARLRGPRRVLPGGFRTRRPSPADERLEVAVRHGRRRADRRGAHRSRAARHRVCTRARGLR
LRRALRAAGARHADLDRLQRGLRRSGLGGADPRSLRRLAHAARRGPRRHRLRVPHHPPRRR
RHRRVPVLLGEHRRARLDRRAGHRSALRRSALHVPVGEARRRSGCDHHGRPDRLRLRERG
RLLHRAGSRTRGPHDARRRRRSRRTGRIAGLGGKRAGRRLPRSHDRRGFHLRIPRGQLHA
PVVVHGRARQRERHRHPRPEPLARSAHRLGDRQALVVARSRHPALARAAERDPARRQPC
PRRGVGG

Lastly, Ohno's abstract and one footnote lists the number of residues as "472". This appears to be a typo of "472" instead of "427" since the above polypeptide is 427 residues long.

The above sequences can then be used for BLASTN and BLASTP searches. The results of such searches was described in Supplement H.

SUPPLEMENT H

Elaboration of BLASTN and BLASTP

Results on Ohno's PR.C Sequence

Supplement G has Ohno's PR.C nucleotide sequence and the amino acid sequence it hypothetically codes for.

A BLASTN search on the the PR.C nucleotide sequence can be achieved by typing in the PR.C sequence (see supplement G) into this website URL:

<https://blast.ncbi.nlm.nih.gov/Blast.cgi>

A screen shot of the result is show below:

The screenshot displays the NCBI BLASTN results interface. At the top, the job title is "Nucleotide Sequence (1284 letters)". Below this, search parameters are listed: RID T92MH2DD015, Query ID Id|Query_110575, Description None, Molecule type nucleic acid, and Query Length 1284. The database used is "nr" (Nucleotide collection) with program "BLASTN 2.6.1+".

A "Graphic Summary" section shows a bar chart titled "Distribution of the top 6 Blast Hits on 4 subject sequences". The x-axis represents the query length from 1 to 1250. A color key for alignment scores is provided: <40 (black), 40-50 (blue), 50-80 (green), 80-200 (magenta), and >=200 (red). The chart shows high alignment scores (red bars) across the entire query length for the top hits.

The "Descriptions" section lists "Sequences producing significant alignments:" with a table of results:

Description	Max score	Total score	Query cover	E value	Ident	Accession
Flavobacterium sp. K172 EII (nvlB) gene coding for an enzyme degrading nvlon oligomers	2362	2362	100%	0.0	99%	X00046.1
Flavobacterium sp. K1723T1 plasmid pOAD2 DNA, complete sequence	2357	3928	100%	0.0	99%	D26094.1
Agromyces sp. KY5RftsX_smpB_nvlB_nvlC_oppF_oppD_oppC_oppB_oppA_penDE_nvlC_nvlF_smpB_genes_complete cds	2257	4127	100%	0.0	99%	AB264778.2
Flavobacterium sp. K172 pOAD2 RS-IIB repeat with nvlB' gene coding for enzyme EII' degrading nvlon oligomers	1565	1565	99%	0.0	89%	X02864.1

Note that there are only 4 entries returned, and a zoomed in view of the entries is further provided:

Description	Max score	Total score	Query cover	E value	Ident	Accession
Flavobacterium sp. K172 EII (nvlB) gene coding for an enzyme degrading nvlon oligomers	2362	2362	100%	0.0	99%	X00046.1
Flavobacterium sp. K1723T1 plasmid pOAD2 DNA, complete sequence	2357	3928	100%	0.0	99%	D26094.1
Agromyces sp. KY5RftsX_smpB_nvlB_nvlC_oppF_oppD_oppC_oppB_oppA_penDE_nvlC_nvlF_smpB_genes_complete cds	2257	4127	100%	0.0	99%	AB264778.2
Flavobacterium sp. K172 pOAD2 RS-IIB repeat with nvlB' gene coding for enzyme EII' degrading nvlon oligomers	1565	1565	99%	0.0	89%	X02864.1

When the actual nucleotide alignments are shown, Ohno's missing thymine location that he predicted to exist in the PR.C is noticeably absent in all of the listed bacteria found in the NCBI databases. How can this be? Either all bacteria on Earth with PR.C went extinct after 1935, the sample of bacteria in Genbank is too small, or Ohno's hypothetical PR.C sequence didn't exist immediately before 1935 and thus Ohno's hypothesis is incorrect.

A sample nucleotide level comparison is provided between the PR.C sequence (marked as "Query") and the actual *nylB* gene (marked as "Sbjct") as reported by Okada and deposited in the NCBI databases. Circled in red is the location where Ohno claims thymine was inserted into PR.C but is noticeably absent in all BLASTN hits. If Ohno's sequence were real and the sampling in Genbank is sufficiently representative of the relevant sequences, then it would be expected that PR.C shows up in one of the BLASTN searches. It does not show up in any of the entries.

The BLASTN search did not find any organism with its *nylB* homolog configured like PR.C. Unsurprisingly, BLASTN only found *nylB* homologs configured like the actual functioning *nylB* homologs.

Flavobacterium sp. K172 E11 (nylB) gene coding for an enzyme degrading nylon oligomers
 Sequence ID: [X00046.1](#) Length: 1457 Number of Matches: 1

Range 1: 125 to 1410 [GenBank](#) [Graphics](#) ▼ Next ▲ Previous

Score	Expect	Identities	Gaps	Strand
2362 bits(1279)	0.0	1284/1286(99%)	2/1286(0%)	Plus/Plus

Query	Score	Subject	Score
1	60	125	184
61	119	185	244
120	179	245	304
180	239	305	364
240	299	365	424
300	359	425	484

Screen shots of the nucleotide level comparisons in the other three bacterial sequences are omitted since it is expected the research can be duplicated following the methods just described.

Similar searches and reports can easily be carried out. For example, if we take the first 175 bases of PR.C which include the location of the supposed thymine insertion that created the supposed frame shift, we can do a BLASTN search on it. Here is the sequence:

ATGGGCTACATCGATCTCTCCGCCCCCGTCGCGATGATCGTCAGC
 GGTGGCCTCTACTATCTCTTACCCGCCGCGGCTACACCTTCGGAGAACT
 CG **agaacgcacgttcacc**
 ggccagcaccgccaggtatcccgagccgcgccggggagccgacactcgacagctgg

Below are sample screen shots of the results. One will notice the few organisms that are returned by BLASTN and the complete absence of the hypothetical PR.C sequence that should be saliently indicated by the region circled in red but is noticeably absent in all the results returned by BLASTN:

BLAST » blastn suite » RID-TARD5JBP014

BLAST Results

Job title: Nucleotide Sequence (175 letters)

RID TARD5JBP014 (Expires on 08-18 17:11 pm)

Query ID |cl|Query_98279
 Description None
 Molecule type nucleic acid
 Query Length 175

Database Name nr
 Description Nucleotide collection (nt)
 Program BLASTN 2.6.1+ > Citation

Other reports: > Search Summary [Taxonomy reports] [Distance tree of results] [MSA viewer]

Graphic Summary

Distribution of the top 4 Blast Hits on 3 subject sequences

Mouse over to see the title, click to show alignments

Color key for alignment scores

- <40
- 40-50
- 50-80
- 80-200
- >=200

Query

1 30 60 90 120 150

Descriptions

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/> Flavobacterium sp. K1723T1 plasmid pOAD2 DNA, complete sequence	318	318	100%	2e-83	99%	D26094.1
<input type="checkbox"/> Flavobacterium sp. K172 E11 (nvlB) gene coding for an enzyme degrading nylon oligomers	318	318	100%	2e-83	99%	X00046.1
<input type="checkbox"/> Agromyces sp. KY5R ftsX, smpB, nvlB, nvlC, oppF, oppD, oppC, oppB, oppA, penDE, nvlC', nvlB', smpB, genes, complete cds	302	532	100%	2e-78	98%	AB264778.2

5:27 AM 8/17/2017

https://blast.ncbi.nlm.nih.gov/Blast.cgi

NCBI Blast:Nucleotide Sequ...

File Edit View Favorites Tools Help

Biochemistry I & II (B... 124

Find: ATGAACGCAGTCCAC Previous Next Options

Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/> Flavobacterium sp. KI723T1 plasmid pOAD2 DNA, complete sequence	318	318	100%	2e-83	99%	D26094.1
<input type="checkbox"/> Flavobacterium sp. KI72 EII (nylB) gene coding for an enzyme degrading nylon oligomers	318	318	100%	2e-83	99%	X00046.1
<input type="checkbox"/> Agromyces sp. KY5R ftsX, smpB, nylB, nylC, oppF, oppD, oppC, oppB, oppA, penDE, nylC', nylB'	302	532	100%	2e-78	98%	AB264778.2

Alignments

Download GenBank Graphics Next Previous Descriptions

Flavobacterium sp. KI723T1 plasmid pOAD2 DNA, complete sequence
Sequence ID: [D26094.1](#) Length: 45519 Number of Matches: 1

Range 1: 3912 to 4087 [GenBank](#) [Graphics](#) Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
318 bits(172)	2e-83	175/176(99%)	1/176(0%)	Plus/Minus

Related Information

Query 1 ATGGGCTACATCGATCTCTCCGCCCCCGTCGCGATGATCGTCAGCGGTGGCCTCTACTAT 60
Sbjct 4087 ATGGGCTACATCGATCTCTCCGCCCCCGTCGCGATGATCGTCAGCGGTGGCCTCTACTAT 4028

Query 61 CTCTTCACCCGCCGCGGTACACCTTCGGAGACACTCGA-GAACGCACGTTCCACCGGCC 119
Sbjct 4027 CTCTTCACCCGCCGCGGTACACCTTCGGAGACACTCGATGAACGCACGTTCCACCGGCC 3968

Query 120 AGCACCCCGCCAGGTATCCCGGAGCCGCGGGGAGCCGACACTCGACAGCTGG 175
Sbjct 3967 AGCACCCCGCCAGGTATCCCGGAGCCGCGGGGAGCCGACACTCGACAGCTGG 3912

A BLASTN can be executed on the following sequence which is first 115 bases of PR.C that include the location of the supposed eventual thymine insertion in PR.C.

ATGGGCTACATCGATCTCTCCGCCCCCGTCGCGATGATCGTCAGC
GGTGGCCTCTACTATCTCTTCACCCGCCGCGGCTACACCTTCGGAGACACT
CG **ag**aacgcacgttcacc

The results are consistent with the slightly longer string of 175 bases at the start of PR.C.

For completeness a BLASTP can be run on the hypothetical protein coded by PR.C. The sequence is available from Supplement G. Going to the BLAST URL provided above and clicking the appropriate buttons, one can do a BLASTP on the hypothetical protein coded by PR.C's.

The results are as follows, which provides evidence against Ohno's sequence ever existing. The results were reported as “no significant similarity found.”

The screenshot shows a web browser window displaying the NCBI BLAST results page. The address bar shows the URL <https://blast.ncbi.nlm.nih.gov/Blast.cgi>. The search query entered is `ATGAACGCACGTCAC`. The page header includes the NIH logo and the text "U.S. National Library of Medicine" and "NCBI National Center for Biotechnology Information". The main content area is titled "BLAST Results" and shows the job title "Protein Sequence (427 letters)". The query details are: RID `TAT0DJNV015` (Expires on 08-18 17:38 pm), Query ID `lcl|Query_44336`, Description `None`, Molecule type `amino acid`, and Query Length `427`. The database used is `nr` (All non-redundant GenBank CDS translations+PDB+SwissProt+PIR+PRF excluding environmental samples from WGS projects) and the program is `BLASTP 2.6.1+`. A prominent message states: "No significant similarity found. For reasons why, click here". The footer includes the NCBI logo, address (8600 Rockville Pike, Bethesda MD, 20894 USA), and various links like "Policies and Guidelines | Contact", "Support center", and "Mailing list". The Windows taskbar at the bottom shows the date and time as 5:39 AM on 8/17/2017.

To be fair, one could take the unshifted portion of PR.C, which is the first 33 residues, and see if the predicted amino acid sequences would match anything. They of course will return search hits because they are the hypothetical amino acids from the unshifted portion of PR.C sequence and correspond exactly to Okada's original sequence.

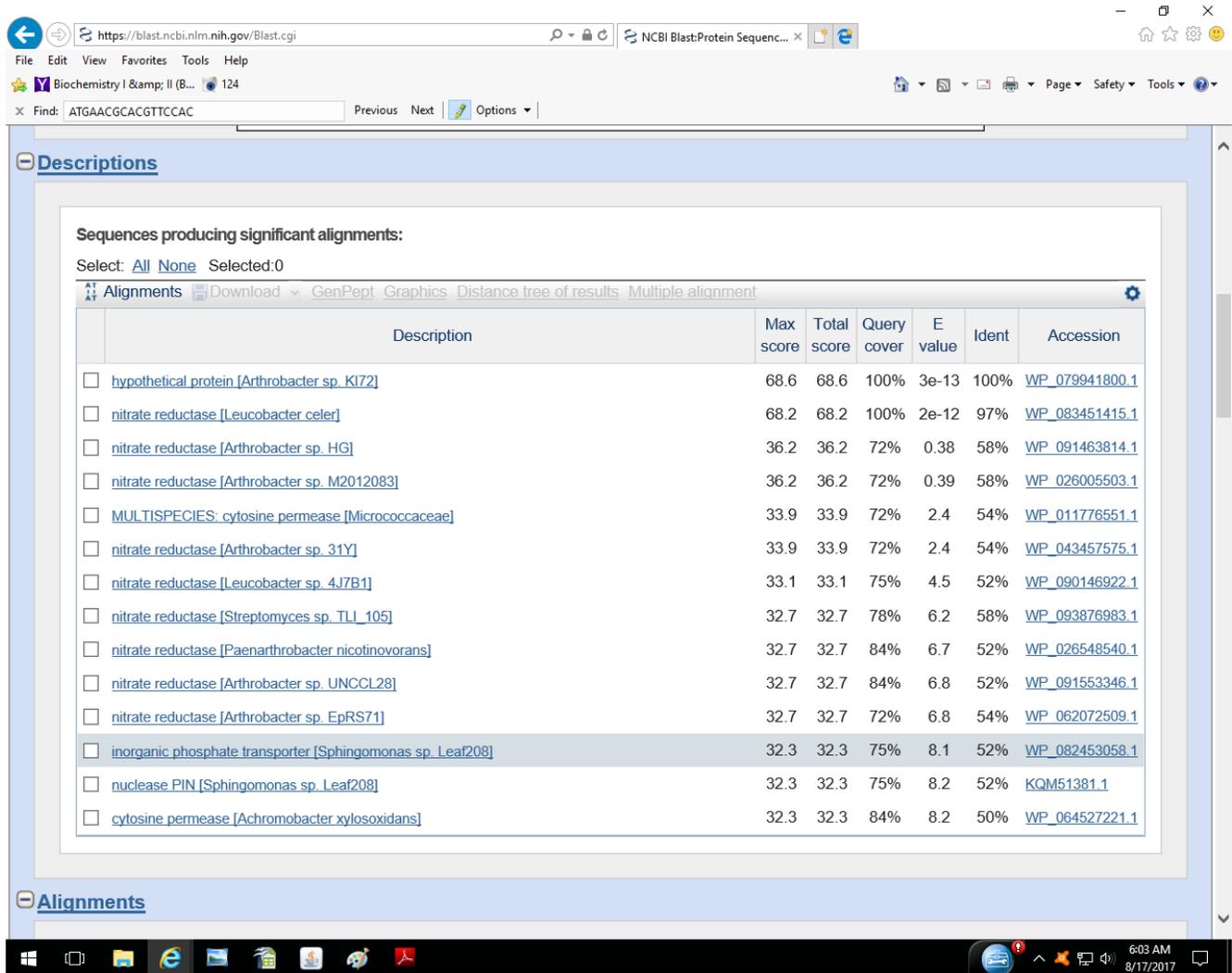
The sequence is:

MGYIDLSAPVAMIVSGGLYYLFTRRGYTFGDTR

BLASTP returned hits which the authors of this paper determined are not significant, but are only reported here for completeness. It is deemed insignificant by the authors because this sequence is the unshifted portion and would appear in searches even if Ohno's sequence existed or not, and therefore doesn't confirm Ohno's PR.C sequence.

However, the search on this sequence is reported anyway since a hostile reviewer running BLASTP got hits on the unshifted portion and raised an objection.

A screen shot is provided immediately below of the result.



The screenshot shows a web browser window displaying the NCBI BLAST search results for the query sequence ATGAACGCACGTTCCAC. The page is titled "Descriptions" and lists "Sequences producing significant alignments:". The search parameters are: Select: All, None, Selected: 0. The results are presented in a table with columns: Description, Max score, Total score, Query cover, E value, Ident, and Accession. The top hit is a hypothetical protein from *Arthrobacter sp.* K172 with a Max score of 68.6, Total score of 68.6, Query cover of 100%, and E value of 3e-13. Other hits include nitrate reductase from *Leucobacter celer* and *Arthrobacter sp.* HG, and inorganic phosphate transporter from *Sphingomonas sp.* Leaf208.

	Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/>	hypothetical protein [Arthrobacter sp. K172]	68.6	68.6	100%	3e-13	100%	WP_079941800.1
<input type="checkbox"/>	nitrate reductase [Leucobacter celer]	68.2	68.2	100%	2e-12	97%	WP_083451415.1
<input type="checkbox"/>	nitrate reductase [Arthrobacter sp. HG]	36.2	36.2	72%	0.38	58%	WP_091463814.1
<input type="checkbox"/>	nitrate reductase [Arthrobacter sp. M2012083]	36.2	36.2	72%	0.39	58%	WP_026005503.1
<input type="checkbox"/>	MULTISPECIES: cytosine permease [Micrococcaceae]	33.9	33.9	72%	2.4	54%	WP_011776551.1
<input type="checkbox"/>	nitrate reductase [Arthrobacter sp. 31Y]	33.9	33.9	72%	2.4	54%	WP_043457575.1
<input type="checkbox"/>	nitrate reductase [Leucobacter sp. 4J7B1]	33.1	33.1	75%	4.5	52%	WP_090146922.1
<input type="checkbox"/>	nitrate reductase [Streptomyces sp. TLI_105]	32.7	32.7	78%	6.2	58%	WP_093876983.1
<input type="checkbox"/>	nitrate reductase [Paenarthrobacter nicotinovorans]	32.7	32.7	84%	6.7	52%	WP_026548540.1
<input type="checkbox"/>	nitrate reductase [Arthrobacter sp. UNCCL28]	32.7	32.7	84%	6.8	52%	WP_091553346.1
<input type="checkbox"/>	nitrate reductase [Arthrobacter sp. EpRS71]	32.7	32.7	72%	6.8	54%	WP_062072509.1
<input type="checkbox"/>	inorganic phosphate transporter [Sphingomonas sp. Leaf208]	32.3	32.3	75%	8.1	52%	WP_082453058.1
<input type="checkbox"/>	nuclease PIN [Sphingomonas sp. Leaf208]	32.3	32.3	75%	8.2	52%	KQM51381.1
<input type="checkbox"/>	cytosine permease [Achromobacter xylosoxidans]	32.3	32.3	84%	8.2	50%	WP_064527221.1