

# 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

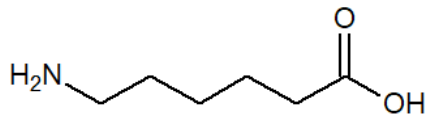
Nylons exist in a variety of chemical forms, which can cause confusion in terms of defining nylonase activity. The nylon of primary interest in this review is nylon-6, and unless otherwise stated the term “nylon” will refer to short nylon-6 oligomers that are generally a hexamer or less in length. Nylon-6's 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  $\epsilon$ -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.<sup>1</sup> The nylon-6 linear oligomers are terminated by a lysine-like monomer (figure 2). There also exist nylon-6 cyclic oligomers.

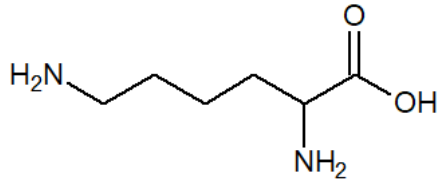
Because this paper may be of interest to a fairly wide range of scientists, we wish to clarify that when we are specifying a nylonase gene we use italicized letters, beginning with lower case (i.e., *nylB*), but when we are specifying nylonase proteins we use non-italicized letters, beginning with upper 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 oligomers<sup>2</sup> 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.<sup>3</sup> 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).<sup>4</sup> Most “nylonases” can only digest waste product nylons that have already been degraded into short oligomers.

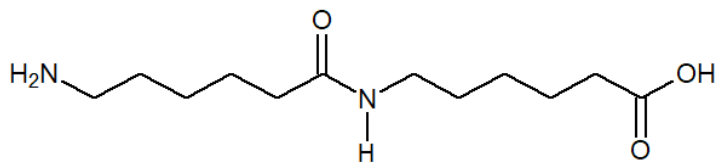


6-aminohexanoic acid

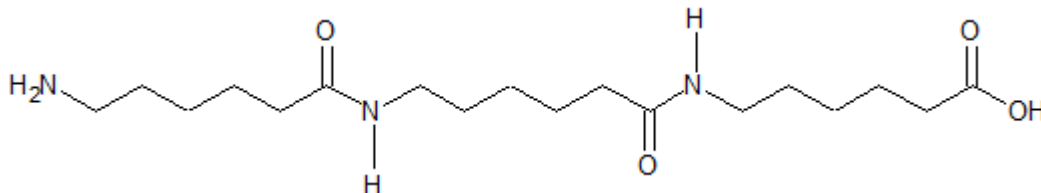


lysine

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.<sup>5,6</sup> 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).<sup>7</sup> 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*.<sup>8</sup> 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.<sup>9</sup>

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).<sup>10</sup> This bacterium was extracted from soil samples and then tested for ability to grow on nylon waste by-products.<sup>11</sup> KI72's nylon-eating ability was reported by Kinoshita in 1973 and 1975.<sup>12</sup> Even though Kinoshita acknowledged Fukumura's earlier 1996 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.<sup>13</sup> It appears that Kinoshita believed his nylonase gene was a newly evolved gene that arose due to the introduction of man-made nylon.<sup>14,15</sup> In his 1977 paper, Kinoshita described a particular nylonase<sup>16</sup> (eventually named NylA in a later paper by Okada<sup>17</sup>), 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.”<sup>18</sup>

Some popular literature has claimed that KI72 was extracted from the nylon-rich environment of the waste water of a nylon factory.<sup>19,20,21</sup> 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.<sup>22</sup> 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.<sup>23</sup> 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.<sup>24,25</sup>

After Kinoshita's initial discovery of *nylA*, three other nylonase genes were discovered in the very same KI72 strain, namely *nylB*, *nylB'*, and *nylC*.<sup>26</sup> 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.<sup>27</sup> 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.<sup>28</sup>

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.<sup>29</sup> 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.<sup>30</sup>

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.<sup>31,32</sup> 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.<sup>33</sup> 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*.<sup>34</sup> 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*.<sup>35</sup>

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.”<sup>36</sup> 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.<sup>37</sup>

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 transformation<sup>38</sup> (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.<sup>39</sup> The popular narrative also implied that the hypothetical frame shift mutation was directly observed under reproducible laboratory conditions.<sup>40</sup>

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.<sup>41</sup> 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.<sup>42</sup>

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.<sup>43</sup> 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.<sup>44,45</sup>

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.<sup>46</sup>

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<sub>A</sub> 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.<sup>47</sup> 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*,<sup>48</sup> 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).<sup>49</sup> 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.<sup>50</sup>

A diagram in S. Negoro's 2000 paper<sup>51</sup> 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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- <sup>2</sup> Negoro, S. Biodegradation of Nylon Oligomers. *Appl Microbiol Biotechnol* (2000) 54:461-466. Springer-Verlag 2000.
- <sup>3</sup> Kinoshita, S. Purification and Characterization of 6-Aminohexanoic-Acid-Oligomer Hydrolase of *Flavobacterium* sp. KI72. *Eur. J. Biochem.* 116, 547-551 (1981).
- <sup>4</sup> 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."
- <sup>5</sup> Ebata, M. Morita, K. Hydrolysis of  $\epsilon$ -Aminocaproic Compounds by Trypsin. *The Journal of Biochemistry*, Vol 46, No. 4, 1959.
- <sup>6</sup> 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.
- <sup>7</sup> 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.
- <sup>8</sup> 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.
- <sup>9</sup> 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
- <sup>10</sup> 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 Alkaliphilic Bacteria, *Agromyces* sp. KYR5. *Journal of Bioscience and Bioengineering*. Vol. 104, No. 6, 521-524, 2007. DOI: 10.1263/jbb.104.521."
- <sup>11</sup> Kinoshita, S. Kageyama, S. Iba, K. Yamada, Y. Okada, H. "Utilization of a Cyclic Dimer and Linear Oligomers of  $\epsilon$ -Aminocaproic Acid by *Achromobacter guttatus* KI 72." *Agr. Biol. Chem.*, 39 (6), 1219-1223, 1975.
- <sup>12</sup> The reference for 1973 discovery was in the end note of Kinoshita's 1975 "Utilization of Cyclic Dimer and Linear Oligomers of  $\epsilon$ -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.
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- <sup>14</sup> 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).
- <sup>15</sup> Kinoshita, S. Terada, T. Taniguchi, T. Takene, Y. Masuda, S. "Purification and Characterization of 6-Aminohexanoic-Acid-Oligomer Hydrolase of *Flavobacterium* sp. KI72.
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- <sup>17</sup> Okada, H. Negoro, S. Kimura, H. Nakamura, S. "Evolutionary adaptation of plasmid-encoded enzymes for degrading nylon oligomers." *Nature* Vol. 306 November 1983.
- <sup>18</sup> Kinoshita, S. Purification and Characterization of 6-Aminohexanoic-Acid-Oligomer Hydrolase of *Flavobacterium* sp. KI72. *Eur. J. Biochem.* 116, 547-551 (1981).
- <sup>19</sup> <http://biologos.org/blogs/dennis-venema-letters-to-the-duchess/intelligent-design-and-nylon-eating-bacteria/>
- <sup>20</sup> <https://ncse.com/cej/5/2/new-proteins-without-gods-help>
- <sup>21</sup> Miller, K. Only a Theory: Evolution and the Battle for America's Soul. Penguin June 12, 2008.
- <sup>22</sup> 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.
- <sup>23</sup> 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.
- <sup>24</sup> The UNIPROT number for the 6-aminohexanoate hydrolase in *Cryobacteria arcticum*, an organism found in Arctic is A0A1B1BHD0.

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- <sup>25</sup> 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.
- <sup>26</sup> 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.
- <sup>27</sup> 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.
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- <sup>31</sup> Rost, B. “Twilight zone of protein sequence alignments.” *Protein Engineering* vol. 12 no.2 pp85-94, 1999.
- <sup>32</sup> Rost, B. “Protein structures sustain evolutionary drift.” *Fold Des*. 1997;2(3):219-24.
- <sup>33</sup> 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*
- <sup>34</sup> The UNIPROT entry for *nylB\_1* for *Bacillus cereus* is A0A0K6IYF0
- <sup>35</sup> The UNIPROT entry for *nylB* for *Streptococcus pneumoniae* is A0A0u0EFW2.
- <sup>36</sup> 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).
- <sup>37</sup> Okada, H. Negoro, S. Kimura, H. Nakamura, S. “Evolutionary adaptation of plasmid-encoded enzymes for degrading nylon oligomers.” *Nature* Vol. 306 November 1983.
- <sup>38</sup> 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.
- <sup>39</sup> <http://biologos.org/blogs/dennis-venema-letters-to-the-duchess/intelligent-design-and-nylon-eating-bacteria/>
- <sup>40</sup> Miller, K. Only a Theory: Evolution and the Battle for America’s Soul. Penguin June 12, 2008.
- <sup>41</sup> <http://biologos.org/blogs/dennis-venema-letters-to-the-duchess/intelligent-design-and-nylon-eating-bacteria/>
- <sup>42</sup> <https://ncse.com/cej/5/2/new-proteins-without-gods-help>
- <sup>43</sup> 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.
- <sup>44</sup> Marks, R. Dembski, W. Sanford, J. Behe, M. Gordon, M. *Biological Information: New Perspectives*. <http://www.biologicalinformationnewperspectives.org/>
- <sup>45</sup> Axe, D. “Estimating the Prevalence of Protein Sequences Adopting Functional Enzyme Folds” (*Axe, J Mol Biol* 341, 1295-1315, 2004).
- <sup>46</sup> <https://ncse.com/cej/5/2/new-proteins-without-gods-help>
- <sup>47</sup> Okada, H. Negoro, S. Kimura, H. Nakamura, S. “Evolutionary adaptation of plasmid-encoded enzymes for degrading nylon oligomers.” *Nature* Vol. 306 November 1983.
- <sup>48</sup> 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.
- <sup>49</sup> Okada, H. Negoro, S. Kimura, H. Nakamura, S. “Evolutionary adaptation of plasmid-encoded enzymes for degrading nylon oligomers.” *Nature* Vol. 306 November 1983.
- <sup>50</sup> 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.
- <sup>51</sup> 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](http://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 tanacetii  
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](http://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)  
Paeniglutamibacter 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 gelatinovor (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:

The screenshot shows the UniProtKB entry for A0A0K6IYF0 (A0A0K6IYF0\_BACCE). The page is viewed in a browser window with the address bar showing the URL. The UniProt logo and search bar are at the top. The main content area includes a navigation menu on the left and a central panel with the following information:

- Protein:** Submitted name: 6-aminohexanoate-dimer hydrolase
- Gene:** nyIB\_1
- Organism:** Bacillus cereus
- Status:** Unreviewed - Annotation score: ●○○○○ - Protein predicted<sup>i</sup>
- Function:**
  - GO - Molecular function<sup>i</sup>**
    - 6-aminohexanoate-dimer hydrolase activity Source: UniProtKB-EC
  - Keywords<sup>i</sup>**
    - Molecular function: Hydrolase Imported
- Names & Taxonomy<sup>i</sup>**

Protein names <sup>1</sup>	Submitted name: 6-aminohexanoate-dimer hydrolase Imported (EC:3.5.1.46 Imported)
Gene names <sup>1</sup>	Name: nyIB_1 Imported ORF Names: BN2127_JRS1_00149 Imported
Organism <sup>1</sup>	Bacillus cereus Imported
Taxonomic identifier <sup>1</sup>	1396 [NCBI]
Taxonomic lineage <sup>1</sup>	Bacteria > Firmicutes > Bacilli > Bacillales > Bacillaceae > Bacillus > Bacillus cereus group
Proteomes <sup>1</sup>	UP000182087 Component <sup>1</sup> : Unassembled WGS sequence
- Family & Domains<sup>i</sup>**

The left sidebar contains a 'Display' section with various filters, including 'Function', 'Names & Taxonomy', 'Subcellular location', 'Pathology & Biotech', 'PTM / Processing', 'Expression', 'Interaction', 'Structure', 'Family & Domains', 'Sequence', 'Similar proteins', 'Cross-references', 'Entry information', and 'Miscellaneous'. The bottom of the screenshot shows the Windows taskbar with the date and time as 5:38 PM on 7/13/2017.

- 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
- [Top](#)

**Protein** Submitted name: **6-aminohexanoate-dimer hydrolase**

**Gene** **ny1B**

**Organism** *Streptococcus pneumoniae*

**Status** Unreviewed - Annotation score: ●○○○○ - Protein predicted<sup>1</sup>

### Function<sup>1</sup>

**GO - Molecular function<sup>1</sup>**

- 6-aminohexanoate-dimer hydrolase activity Source: UniProtKB-EC

[Complete GO annotation...](#)

**Keywords<sup>1</sup>**

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

### Names & Taxonomy<sup>1</sup>

Protein names <sup>1</sup>	Submitted name: 6-aminohexanoate-dimer hydrolase Imported (EC:3.5.1.46 Imported)
Gene names <sup>1</sup>	Name: <b>ny1B</b> Imported ORF Names: ERS020515_01316 Imported
Organism <sup>1</sup>	<i>Streptococcus pneumoniae</i> Imported
Taxonomic identifier <sup>1</sup>	1313 [NCBI]
Taxonomic lineage <sup>1</sup>	Bacteria > Firmicutes > Bacilli > Lactobacillales > Streptococcaceae > Streptococcus
Proteomes <sup>1</sup>	UP000039704 Component <sup>1</sup> : Unassembled WGS sequence

### Family & Domains<sup>1</sup>



## 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](http://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](http://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](http://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  
Coproccoccus comes  
Corallocooccus 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 chromiirens*  
*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  
Methylobrevis 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 burhodogranaria 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  
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. AD87  
Paenibacillus sp. E194  
Paenibacillus sp. FJAT-22460  
Paenibacillus sp. FSL A5-0031  
Paenibacillus sp. FSL H7-0357  
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 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\_FULLL\_69\_31  
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)  
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  
Providencia burhodogranaria 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 stibiirens 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'*]

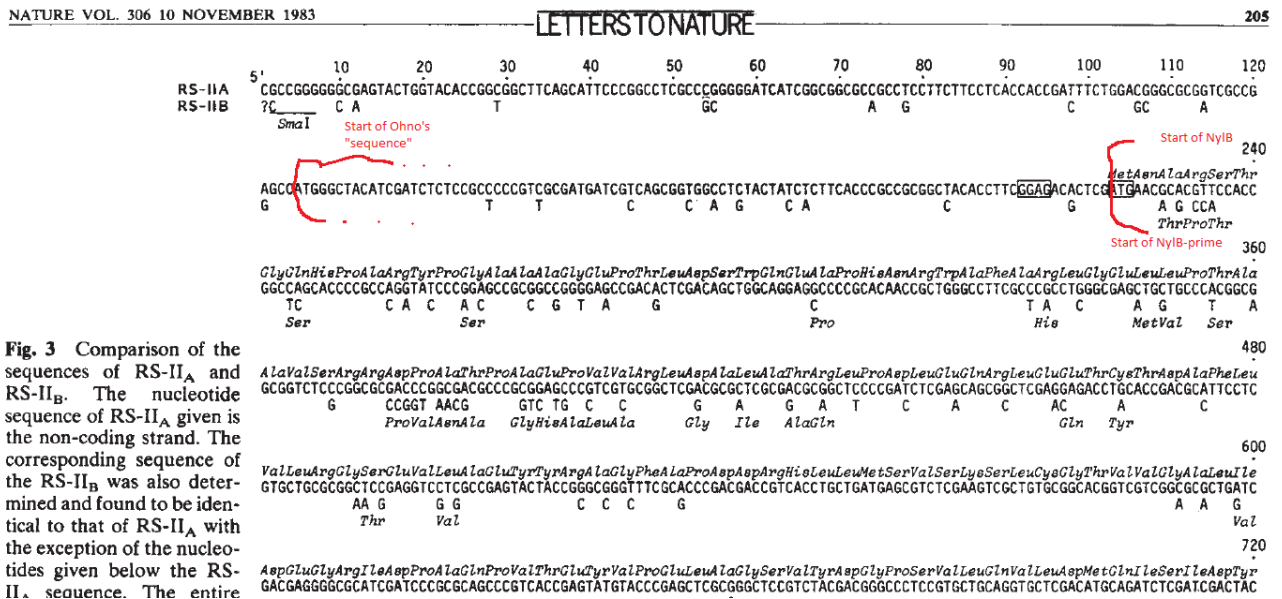
WP\_012476894.1 6-aminohexanoate-dimer hydrolase [Flavobacterium sp. KI723T1]

# 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<sub>A</sub> 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.



**Fig. 3** Comparison of the sequences of RS-II<sub>A</sub> and RS-II<sub>B</sub>. The nucleotide sequence of RS-II<sub>A</sub> given is the non-coding strand. The corresponding sequence of the RS-II<sub>B</sub> was also determined and found to be identical to that of RS-II<sub>A</sub> with the exception of the nucleotides given below the RS-II<sub>A</sub> sequence. The entire

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

3ACGCGGTGTAG**GCGGCGTGACC**GCGGCTCGGCACCCGGCCGGGATCCTCTA

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<sub>A</sub> (of *nylB*) and RS-II<sub>B</sub> (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<sub>B</sub>. 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
GGTGGCCTCTACTATCTCTTCACCCGCCGCGGCTACACCTTCGGAGACACT
CG agaacgcacgtccacc
ggccagcaccgccaggtatcccggagccgcggccggggagccgacactcgacagctgg
caggaggccccgcacaaccgctgggcttcgcccgctggcgagctgctgcccacggcg
gcggttccccggcgacgcccggcagcggcgagcccgtctgctgggctcgacgcgctc
gcgacgcggctccccgatctcgagcagcggctcgaggagacctgcaccgacgattctc
gtgctgcgcggctccgaggtctcgcgagtagtaccggggcggttcgacccgacgac
cgtaacctgctgatgagcgtctgaagtcgctgtgcggcacggctgctggcgcgctgatc
gacgagggggcgcacgatcccgcgcagcccgtcaccgagtagtaccgagctcgcgggc
tccgtctacgacgggcccctcgtgctgcaggtgctcgacatgcagatctcgatcgactac
aacgaggactacgctgatccggcctcggaggtgcagaccacgatcgtccgccggctgg
cgcacgcggcgagacggggaccgccgacacctacgagttctcaccacctccgcggc
gacggcgaccggcgagttccagtactgctcggcgaacaccgacgtgctcgctggatc
gtcgagcgggtcaccggtctgcgctacgtcgaagcgtctccacgtacctgtggcggaag
ctcgacgccgatcgggatcgcaccatcacggctgaccagaccggcttcggcttcgcaac
gggggctctcctgcaccgcgggatctcgcacgcgtgggcccgatgatgctcgacggc
ggcgtcgtccccggcgacgggtcgtatgcagggctgggtgaaagcgtgctggccggc
ggctcccgcgaagccatgaccgacgaggtttcacctccgattccccgagggcagctac
acgcgccagtgggtgctgcacgggcaacgagcgcggcaacgtgagcggcatcggcatccac
ggccagaacctctggctcgatccgcgcaccgactcggtagctgaagctctcgtcgtgg
cccgateccgacaccggcactggcagggctgcagagcgggatctgctcgacgtcagc
cgtgccctcgacgcggtgtag GCGGCTGA

```

## 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  
PVVVHVGQRARQRERHRHPRPEPLARSAHRLGDRQALVVARSRHPALARAAERDPARRQPC  
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 BLAST 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 four red bars, indicating high alignment scores across the query length.

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

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

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

This is a zoomed-in view of the "Sequences producing significant alignments:" table from the screenshot above. It shows the same four entries with their respective scores and accession numbers.

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



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:

ATGGGCTACATCGATCTCTCCGCCCCGTCGCGATGATCGTCAGC  
 GGTGGCCTTACTATCTCTTACCCGCCGCGGCTACACCTTCGGAGAACT  
 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 | 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"/> <a href="#">Flavobacterium sp. K1723T1 plasmid pOAD2 DNA, complete sequence</a>	318	318	100%	2e-83	99%	<a href="#">D26094.1</a>
<input type="checkbox"/> <a href="#">Flavobacterium sp. K172 E11 (ny1B) gene coding for an enzyme degrading nylon oligomers</a>	318	318	100%	2e-83	99%	<a href="#">X00046.1</a>
<input type="checkbox"/> <a href="#">Agromyces sp. KY5R ftsX, smpB, ny1B, ny1C, oppF, oppD, oppC, oppB, oppA, penDE, ny1C', ny1B', smpB, genes, complete cds</a>	302	532	100%	2e-78	98%	<a href="#">AB264778.2</a>

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

**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 ATGGGCTACATCGATCTCTCCGCCCGTCGCGATGATCGTCAGCGGTGGCCTCTACTAT 60  
 Sbjct 4087 ATGGGCTACATCGATCTCTCCGCCCGTCGCGATGATCGTCAGCGGTGGCCTCTACTAT 4028

Query 61 CTCTTCACCCGCCGCGGTACACCTTCGGAGACACTCGA-GAACGCACGTTCCACGGCC 119  
 Sbjct 4027 CTCTTCACCCGCCGCGGTACACCTTCGGAGACACTCGATGAACGCACGTTCCACGGCC 3968

Query 120 AGCACCCCGCCAGGTATCCCGGAGCCGGCCGGGGAGCCGACACTCGACAGCTGG 175  
 Sbjct 3967 AGCACCCCGCCAGGTATCCCGGAGCCGGCCGGGGAGCCGACACTCGACAGCTGG 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.

ATGGGCTACATCGATCTCTCCGCCCGTCGCGATGATCGTCAGC  
 GGTGGCCTCTACTATCTCTTCACCCGCCGCGGTACACCTTCGGAGACACT  
 CG **ag**aacgcacgttccacc

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 `ATGAACGCACGTCCAC`. 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 as follows:

<b>RID</b>	TAT0DJNV015 (Expires on 08-18 17:38 pm)	<b>Database Name</b>	nr
<b>Query ID</b>	lcl Query_44336	<b>Description</b>	All non-redundant GenBank CDS translations+PDB+SwissProt+PIR+PRF excluding environmental samples from WGS projects
<b>Description</b>	None	<b>Program</b>	BLASTP 2.6.1+ <a href="#">Citation</a>
<b>Molecule type</b>	amino acid		
<b>Query Length</b>	427		

Below the query details, a message states: "No significant similarity found. For reasons why, click here". Other reports are listed as "Search Summary". The footer includes the NCBI logo, contact information, and logos for NIH and USA.gov. The system tray 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.

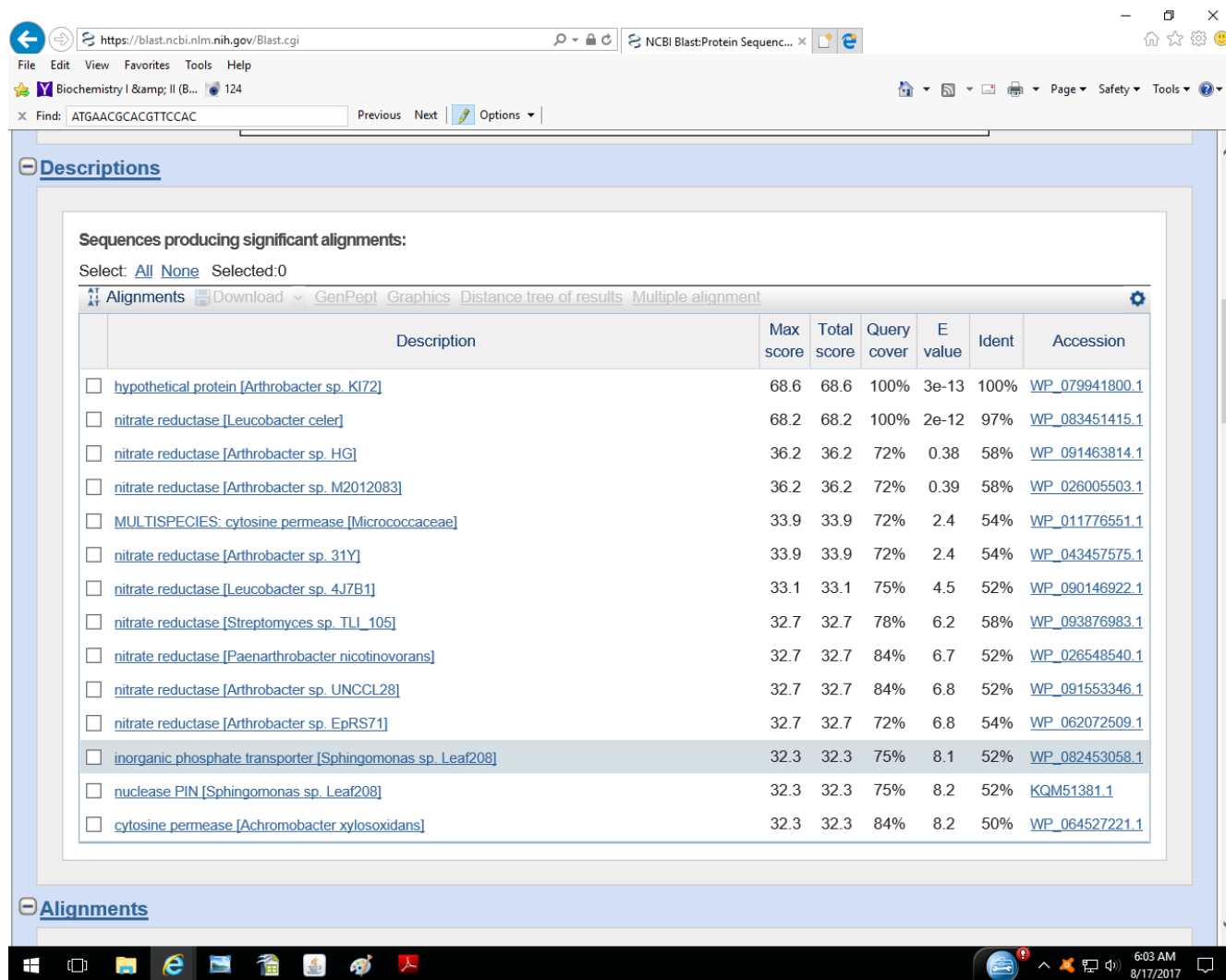
The sequence is:

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 table includes columns for Description, Max score, Total score, Query cover, E value, Ident, and Accession. The top result 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 notable results include nitrate reductase from Leucobacter celer (E value 2e-12) and inorganic phosphate transporter from Sphingomonas sp. Leaf208 (E value 8.1).

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