

Supplementary material

Increasing the diversity of of nylonases for poly(ester amide) degradation

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Table S1. Sequences of NylC₁₋₆.

Protein ID	Genbank Accession	Donor organism	Sequence
NylC ₁	PP841629	<i>Leucobacter chromiirestiens</i> JG 31	MNPSSHEPHALAEIDSGAVIDPAPRLAGAPVFGGPGNGD FDLVPQRSDGRERLRFDFPGVRIGSAHYAEGPTGATVIH LPAGARTAVDARGGAVGLAGGYDFNHAICLAGGSVYG LEAGAGVSDALLERFENRTGFADLQLVSSAIYDFSARST SVYPDRELGRAAFDFAVEGEFAQGRAGAGMSASSGKVD WERTEITGQGAAFRQIGDVRILAVVVPNPVGVILDRAGR VVRGNYDAATGERRHPVFDYAEFAEHRAAETQSGNTT ISALITNVKMSTVELNQFAKQVHSSMHRGIQPFHTDMDG DTLFAVTTDEIDLPISPGSSRGRLSVNATALGAIASEVMW DALLEAAK
NylC ₂	PP841630	<i>Microbacterium oxydans</i>	MTDIHRLAPVLPAGPRRSNGDYALAPVHGTDGRQVEYD FPGVLLGTAEYAEGPTGATVMSIPAGARTFTDRRGAV GASGLYGYNHAICLAGGSVYGLSAVAGVSEALFERAEH RSGFDQLQLVSGAIYDYSVRENSVFPDTALGKAALQAA RQGVIEVGRVGGASASSGKVDPARVEFTGQGAAFRQV GDVKLVVTVLNPYGVIVDRAGRIIRGNFDATTGERRHP ALDYEEAIGESRLVESMSGNTTITAVITNVQLSDVELKQF GLQVHSSMHRGIQPFHTPLDGDTLFALTTDEVALPEDPG TSRGRLSLNSTAVATLAGETAWDAILCAAG
NylC ₃	PP841631	<i>Streptomyces</i> 63005	sp.MTVTDRAGTPARAIPAPSPFGPARTNDDFELTPQSPGQG TVEFDFPGVEVGTAEYAEGPTGTTVVHVPAGARMFIDE RGGAIGLSYGDKQFAHAICLSGGSLYGLGACAGVADEL RSRVDNRVGWNDLKC VSGAIYDFATRDNVVPDAALG RAALRTAEPGRFPVGRAGAGISASVGKIDWTRCEFSGQG AAFRQVGDVKILAAATVVNAVGVVVDRTGTVVRGNHDP ATGTRRLPHLDYEAFAFGDGPPTMQGNTTISVLTNVR LDDRALEQFGRQVHGSMNRGIQPFHTSLDGDTLFLLTTD EVDLPTTPSRIGAHALNAVGLGSIAAEVMWDAILSSAR
NylC ₄	PP841632	<i>Variovorax boronicumulans</i>	MKNDVKAQDLVPRLWQGPSDGRALSDFPQLKIGIAE YEEGPTGCTVLHVEGGADCEVDVRRGGAPLLGGYTRV DAISLAGGSLYGLEAATGVTSRMLAERDGA VGWGKIAL VSGAIYDFGGRTNSIYDPKALGAAAYRSAQVGTFLPGA RGAGRSAAVGKFAAYPDYEREAGGQGA AFAMVGGTRV FVATVVNALGVIVDRQGRVVRGLRHRRTGVRHHPREVL VPTASATVEAPVTPGNTVATTENTTLTVLVTQPMPLF LRQLGRQVHSSMARAIQPFHTPHDGDILFALSTGTGTAS VDPFVLAEAASDLAWDAVLQAVDF
NylC ₅	PP841633	<i>Gordonia</i> sp.	MTFLARAGNRITDVAGIAVGHHRIDDEVVATGTEPG VGWATGTTVVTVDAGATS AVDVRGGGPGTRETDLLEP GNSVRGANAI VLTGGSAYGLSAADGVMRGLEARGIGLP MDEQGHVPIVPAAVIFDLPVGEWARRPDEEFGALALD AADADFAVGSVGAGAGARAGALKGGVGTASITLADGP ARGLTVGALVVANPVGQVIDPATGLPWGGDDLAAYGL GAPAA GELTRLRELEAKGTVLNTTIGVVATDATLSVPSV RRLAMSGHDGLARAVRPAHSPLDGDTLFAVATGRRTAD PEAAVDIPPGMEPEIAVVAALAEASATVVQRAIVSAVVH ATSVAGIPSYRDVVASAFGWDSF
NylC ₆	PP841634	<i>Rhodococcus</i> sp.	MIVPGPTDSDLVDVAGLSVGHWQRLDPEVTVGTPEIPGTG AATGCTVVLADPSAVASVDVRGGGPGTRETDLDP SHS VQRVDAVLLTGSAYGLAAADGVMRYLEKNGKIPMG APGAVVPIVPGAVIFDLPVGGQWSARPTADSGCTAAATAC STHLERGSAGAGTGARAGALKGGIGSASVRITDGPAAAG VTVAALMVANPVGSVFDVPTGLPWGVDAARAEELGLS SPSASDLAAARALGDKGTVLNTTIGVVATDAPLSKAAC RRVAVTGHDLGRAIRPAHSPLDGDITIFALSTGTADVTD ALRDSIPSIPAFAAELPILAAVCEAAA VVERAIVDAILSA TSVADIPAYRDVLPVSVFGR

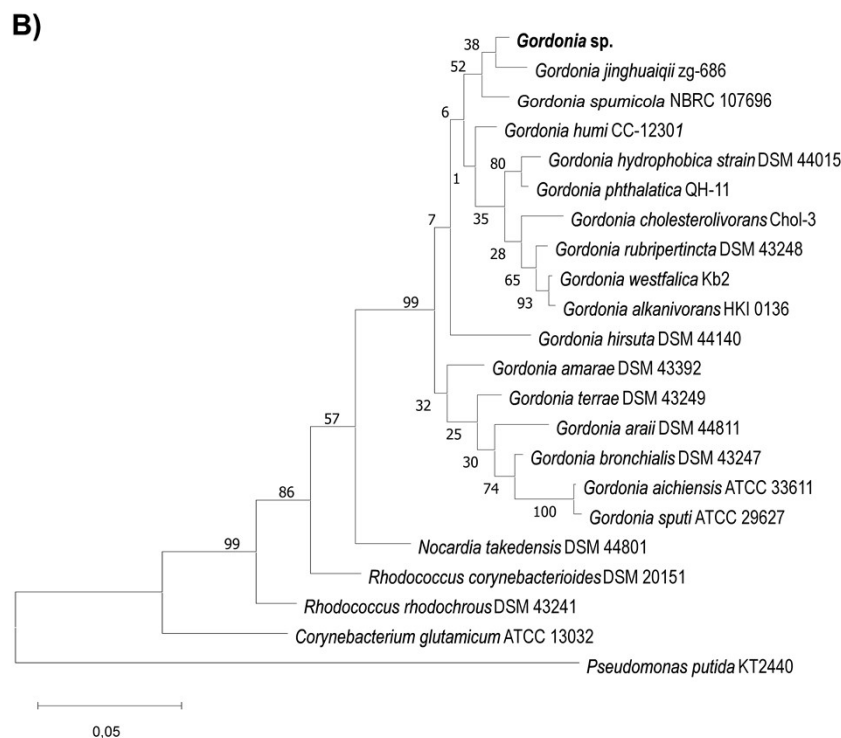
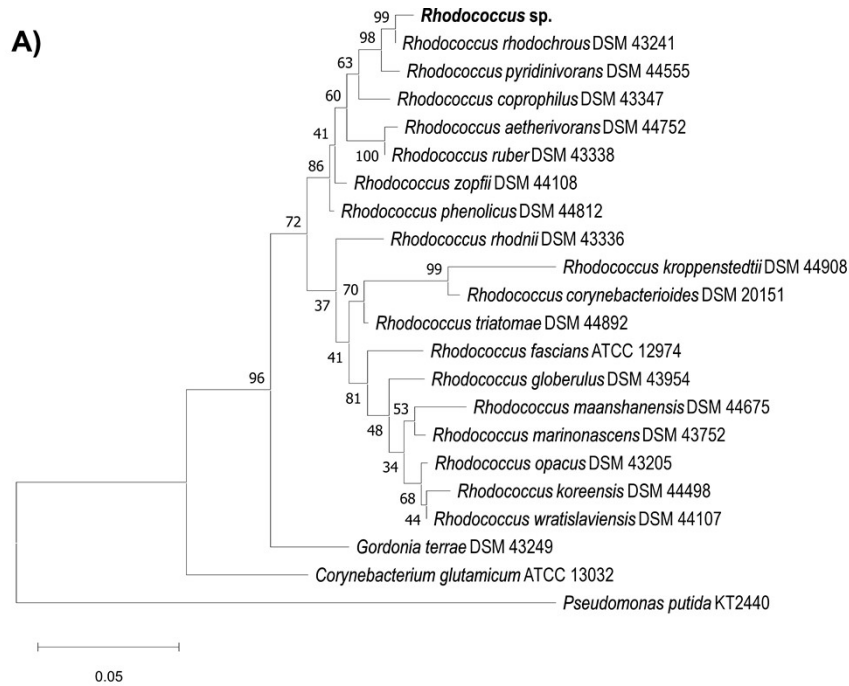


Figure S1. Position of the novel isolates in the phylogenetic tree based on 16S rRNA, inferred by using the Maximum Likelihood method and Tamura-Nei model.¹ A) The tree with the highest log likelihood (-6943.31) for the *Rhodococcus* sp. isolate, source of NylC₆ B) The tree with the highest log likelihood (-5975.33) for *Gordonia* sp., source of NylC₅. The novel strains are printed in bold. The percentage of trees in which the associated taxa clustered together are shown below the branches. The trees are drawn to scale, with branch lengths measured in the number of substitutions per site. Evolutionary analyses were conducted in MEGA11.²

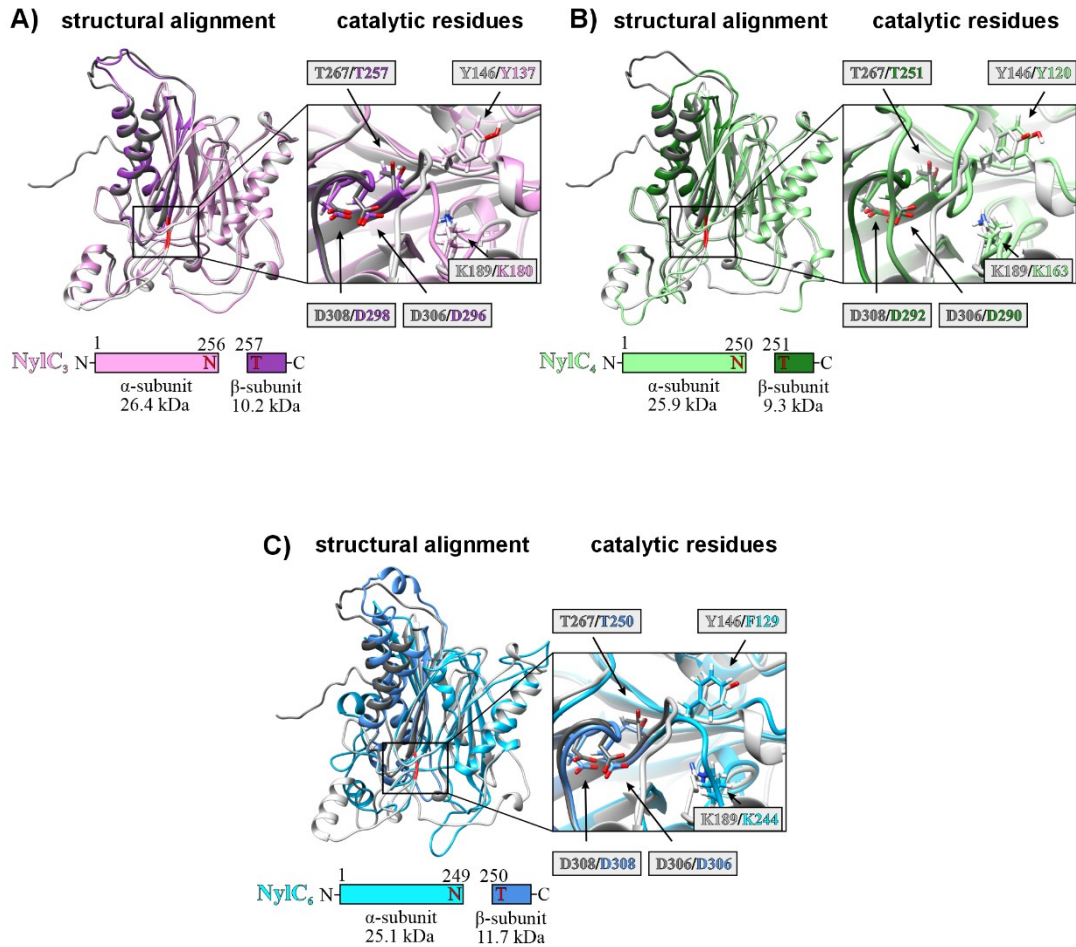


Figure S2. Structural alignments of NylC_{p2} and NylC_{3,4,6}. Protein structures of NylC₁₋₆ were predicted using ColabFold³ and were aligned to NylC_{p2}. The holistic structural alignments are shown as well as the alignments of the catalytic residues (box). The N/T-autocleavage site is highlighted in red.

Table S2 RMSD of structural alignments of NylC_{p2} and NylC₁₋₆, provided by Chimera MatchMaker

Enzyme	Overall (number of atom pairs)	Pruned (number of atom pairs)
NylC ₁	2.998 Å (353)	0.525 Å (328)
NylC ₂	9.723 Å (352)	0.531 Å (299)
NylC ₃	6.64 Å (349)	0.562 Å (292)
NylC ₄	11.107 Å (333)	0.687 Å (260)
NylC ₅	29.458 Å (337)	0.93 Å (160)
NylC ₆	23.941 Å (341)	0.875 Å (171)

References

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3. M. Mirdita, K. Schütze, Y. Moriwaki, L. Heo, S. Ovchinnikov and M. Steinegger, *bioRxiv*, 2021, DOI: 10.1101/2021.08.15.456425, 2021.2008.2015.456425.