

Fig. S2 Sequence alignment of CoMA and related enzymes. The sequence of CoMA without the signal peptide was used. The protein sequence alignments were generated via the MUSCLE alignment in MEGA 7.0. Numbering of amino acid residues from the N-terminus of mature enzymes is shown on the right. The black boxes represent the seven conserved regions of α -amylases (Region I-VII) and two conserved sequence regions which found only in the CD-/pullulan-degrading enzymes. The asterisks represent the invariant catalytic amino acids including Asp-328, Glu-357, and Asp-424 (ThMA numbering). The enzymes are: ThMA (PDB no. 1SMA), NPase (PDB no. 1J0K), BSMA (accession no. Q45490), CDase (PDB no. 1EA9), HOAMYA (PDB no. 1WZA), TAA (PDB no. 2TAA).

ThMA MRKEAIHRSIDNFAYAYDSEFLHLRLQTKKNDVDHVELLFGDPYEWHDGAWQFOTMPMRKTGSDGLFDYWLAEVKPPYR 80
 NPase MRKEAIYHRPADNFAYAYDSEFLHLRLRQTKKDDIDRVELLHGDPYDQNGAWQFOMMPMRKTGSDGLFDYWFAEVKPPYR 80
 BSMA MFKEAIYHRPKDNFAYAYDSEFLHLRLRQTKKNDVHVRLLYGDYFVWENGHWQVSYQSMQKSGTDELFDYWIAEIVPPYR 80
 CDase MFEAVYHRPRKNSFYAYNGTIVHLRLRQTKKDDMTAVYALAGDKYVWDH---TMEYVPMTKLAIDELFDYWECEVTPPYR 77
 HOAMYA -----
 CoMA -----
 TAA -----
 ruler 1.....10.....20.....30.....40.....50.....60.....70.....80

ThMA RLRVGFVLRAGGEEKLVYTEKGFYHEAPSDDTAYYFCFPFLHRVDFLQAPDVKVDTVMYQIFPERFANGNPALSPKGRPW 160
 NPase RLRVGFVLRAGGEEKLVYTEKGFYFVPTDDTAYYFCFPFLHRVDFLQAPDVKVDTVMYQIFPERFANGNPALSPKGRPW 160
 BSMA RLRVGFELTSENEQIVYTEKGFYRQAPMDTAYYFCFPFLNKIDVFOAPEWVKDTIYQIFPERFANGNEALNPAGTLPLW 160
 CDase RVKYGFLQLQCEKRWMTPEYDFLEPPAN-PRDLRFYFPFINEVDVFOPPAWVKDAIFYQIFPERFANGDTRNDPEGTPLW 156
 HOAMYA -----BEKHCITYEIVVRSFYDSG---DGIGDL 26
 CoMA -----PTESAPGNITLAAEAGDAWYRGAVFYEIVVRSFQDSNG---DGVGDL 70
 TAA -----APPADWRSQSIIVFLITDRFARTDG---STH 27
 ruler90.....100.....110.....120.....130.....140.....150.....160

ThMA GSEDPPTPSFFGGDLQGIIDHLDYLADLITGIYLLHIFRSP-----SNHKYDTADYFEIDPHFGDKEIKLTLVKRC 232
 NPase GSEDPPTPSFFGGDLQGIIDHLDYLVDLGITGIYLLHIFRSP-----SNHKYDTADYFEVDPHFGDKEIKLTLIDRC 232
 BSMA GSADPTPSFFGGDFEGIIQKLDHLVDLGVNGIYFPIFKAS-----SNHKYDTADYFEIDPQFGDKQIFKRLVELC 232
 CDase GSADPTPSFFGGDLQGIIDHLDLHSLKLVNAVYFPIFKAT-----SNHKYDTADYFEIDPQFGDKQIFKRLVELC 228
 HOAMYA KGLHEKLDYLNDDGPE---TADLGVNGLWLMPIFKSP-----SYHGVDVLDYKINPDYTDLEDHKLVEAA 91
 CoMA QGLISRLDYLNDGNEPA---TDDLGVDAIWLWVEFASP-----SYHGVDVSDYERIQAYGSLDELQRLCDEA 135
 TAA ATCNTADQKCGGAWOGLIDKLDYIQGMEETAIWIFVIAOLPQDCAYGDYHGYWQTDIYSLINENYGTADDLKLSSAL 107
 ruler170.....180.....190.....200.....210.....220.....230.....240

Region VI

ThMA HEKGIRVMLDAVFNHSCGYEFAPFQDVLKNGAASRYKDWFHIREFPLOTEP-RPNYDT-----FAFVPHMPKL 298
 NPase HEKGIRVMLDAVFNHSCGYEFAPFQDVKNGESSKYKDWFHIREFPLOTEP-RPNYDT-----FRFVPMQKL 298
 BSMA HOKAIRVMLDAVFNHSCGYEFPFQDVLKNGENSKYKDWFHIREFPLOTEP-RPNYDT-----FAFTPMMPKL 298
 CDase HERGIRVLLDAVFNHSGRTFPFQDVLKNGENSKYKDWFHIREFPLOTEP-RPNYDT-----FAFELMPKL 295
 HOAMYA HORGIKVIIIDLPINHTSERHPWFLKASRD-KNSEYRDYVWAG--PDITKETKIDGGRVWHYSPTGMYYGVFWSGMPDL 168
 CoMA HRRGMRVIIIDLVINHTSERHPWFLSKSS-ESAKRDWYQWRANPAWAQFDLYSQTNTHCQDTGMYGVFWSGMPDL 214
 TAA HERGMVMDVVAVNHMGYDAGSSVDYVSKPSSQDYFHPFCFIQNYEDQTOVEDCWLG---DNTVSTPDL 176
 ruler250.....260.....270.....280.....290.....300.....310.....320

Region I

Region V

ThMA NTAHPVVKRYLLDVAIYWIREFDIDGWRLLDVAN-----EIDHCFWREFRQAVKALKPDVYIIGEIWHDAMP 364
 NPase NTAHPVVKRYLLDVAIYWIREFDIDGWRLLDVAN-----EIDHCFWREFRQAVKALKPDVYIIGEIWHDAMP 364
 BSMA NTEHQEVKNYLLDVAIYWIREFDIDGWRLLDVAN-----EVDHCFWREFRQAVKTIKPDAYIIGEIWHDAMP 364
 CDase NTEHQEVKNYLLDVAIYWIREFDIDGWRLLDVAN-----EVDHCFWREFRQAVKTIKPDAYIIGEIWHDAMP 364
 HOAMYA NYNPPVQEKVIGIAKYWLKCG-VDGFRLDGAHIFPPP-----AVYDKMSTWVEKFRCEIEVKP-VYLVGEVHWSIET 240
 CoMA NITQIAVRKNEVRLALNLQRC-VDGFRLDAAEYLLIETGGGAGQADPEEFAFWKKEFAAHVRSVKPDVLYGEAWSETPS 293
 TAA DITKDVVKNEDYDNGSVSNYSIDGLRDTVK-----HVCDFWEGYKKAAG---VYCIGEVLDGPA 237
 ruler330.....340.....350.....360.....370.....380.....390.....400

Region II

Region III

ThMA WLRGDC--FDAVMN-----YPTADAALRFFAKEDMSASEFADRLMHVLHSPKQVN---EAAFNLLGSHDTPRLLIV 431
 NPase WLRGDC--FDAVMN-----YPTDGLVLRFFAKEETSAARQFANQMMHVLHSPNNVN---EAAFNLLGSHDTSRILIV 431
 BSMA WLRGDC--FDAVMN-----YPTINGLRFRAQEFIRASQVFGMMTHVLHSPTNVN---EAFNLLGSHDTPRLLIL 431
 CDase WLRGDC--FDAVMN-----YPTINAVLDFFIHQADAEKFSMLGKQLAGYPRQNS---EVMFNLLGSHDTPRLLIQ 428
 HOAMYA VAPYFK--VGFDS-----TFNFKLAEAVIATAKAGFPFGFNKKAHXYGV-DREVGFNGYIDAPPLTNDHONRILLQ 310
 CoMA VAKYKQ--SATVFGGDELPLNPFMSARVIEGYNAGNSGGVASKLLEMKNYPAGV---ADAPPLTNDHMRRLAQ 366
 TAA YTCFYQNVMDGVLN-----YPLIYPIILNARKSKSSGSDDYNNMINTVKSDQDPSLLG---TFVENHNDPRFASY 304
 ruler410.....420.....430.....440.....450.....460.....470.....480

Region IV

ThMA CGGDVRKVKLLFLFQLTFTGSPCIYYGDEIGMTGGND---PECRKCMVWD-----PEK 481
 NPase CGGDVRKVKLLFLFQLTFTGSPCIYYGDEIGMTGGND---PECRKCMVWD-----PMQ 481
 BSMA CREEDVRKAKLSFLFQLSFTGSPCIYYGDEIGMTGGND---PGCRKMIWD-----EHQ 481
 CDase ADGDKRKMKLAFLFQFTYFTGSPCIYYGDEIVGLDGGHD---PGCRKMEWD-----ETK 478
 HOAMYA LGQDRNKARVAASLYLTLPGNPFIIYGEIGMRGQCP---HEVIREPEPQWVNGSGEGETIYWEPMYNDG---FLSVEQEEK 385
 CoMA FSNDAKALGLAAAVLLTLFGAPFLIYGEVGLGNGANNDESKRTMPWSAAAGGGFTTGSWPWYAFSGGRELVANVESQR 446
 TAA LN-DIAAKNVAAFIILNDGFLPIIYAGQOHHVAGGND---PANREAWLS-----GYF 353
 ruler490.....500.....510.....520.....530.....540.....550.....560

Region VII

ThMA QNKELHYHVQKLIARLKOYRSLR-RGDVAFLTADDEVN-HLVYAKIDGNETVMIIINRSNEAAEIP-MPLDARGKWLVLN 558
 NPase QNKELHQHVQKLIARLKOYRSLR-RGEISFLHADDEMN-HLYYKKTIDGDETVLVIINRSDQKADIP-IPLDARGTWLVLN 558
 BSMA QNRQLFRHVQKLIARLKAAYKAFNRGNLHFIDANDET-NHLIYKKTIDGDEEAILVLVNNNEQEIET-LPLSLKGLLTLN 559
 CDase HDKDLFAFYQTVIRLRQAHAALR-TGTQKFLTAEKNSR-QIAYTREDDDQTLVVMN--NDKAGET-LTLPVRFHQVTHL 553
 HOAMYA NQDSLNNHYRRLIHFERNENPVFY-TGKTEIING--GLN-VVAFRRYNDKRDLYVHNLVNRVVKIK---VASGNWTLF 457
 CoMA NPSLLSRYRNLIFARQSEALR-NGGLRLFTATTGMSRTLAFVRLGDQOVLVIHNESTAQESVGPDDVEATLAEPLFL 525
 TAA TDSLEYKLIASANAIRN-YAISKDTGCFVYKPYKDDTTIARRKTGTDGQIVLILSNKQASGDSYTLSSLSGASTAGCQ 432
 ruler570.....580.....590.....600.....610.....620.....630.....640

ThMA LTG---ERFAAEAE---TLCVSLPPYGFVLYAVESW----- 588
 NPase LTG---ERFAAEAE---TLCTSLPPYGFVLYAIEHW----- 588
 BSMA WTN---EQFSAEAD---TLKSTLPPYGFVLYKIEDWL----- 590
 CDase WOD---DVLTAAGC---QLTVKLPAYGFVLYKASSD----- 583
 HOAMYA NSGKEIITPVEDNN---KLMYIIPAYTIVLEKE----- 488
 CoMA DSG---VAPLGGTC---AKKTSIPARSIGIWRLR----- 554
 TAA LTEVIGCTTVVVGSDGNVPPVPMAGCLPRVLYPTEKLAGSKICSDSS 478
 ruler650.....660.....670.....680.....

Fig. S3 Inhibition of CoMA amylase activity by acarbose. Action of CoMA on acarbose, soluble starch and a mixture of acarbose and soluble starch in 20 mM Tris-HCl (pH 7.0) at 30°C for up to 12 h. Acb, acarbose; M, maltooligosaccharide standards; G1 to G3, glucose to maltotriose.

Fig. S3

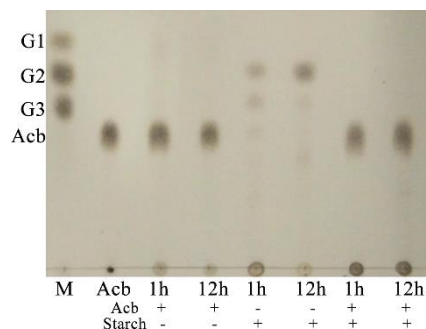


Table S1 The activity location of the expressed CoMA in *E. coli*.

Location	Resting cell	Crude lysate	Culture supernatant
Activity ^a (U.mL ⁻¹)	76.3±7.1	80.8±10.3	ND ^b

^a The recombinant *E. coli* BL21(DE3) cells harboring the pET29a-coMA (500 mL) after induced were harvested by centrifugation and resuspended in an equilibration buffer (20 mL). This cells were cut into two parts. One part was set as resting cell; and the other was sonicated and set as crude lysate. The culture supernatant of recombinant *E. coli* BL21(DE3) was concentrated to 20 mL using ultrafiltration columns. The activity of these were measured for 10 min under standard conditions and recombinant *E. coli* BL21 with pET29a(+) was used as control.

^b ND, no activity detected.