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## Functional and Structural Characterization of a Novel Isoamylase From *Ostreococcus tauri* and Role of the N-Terminal Domain

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### Article History

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Ostreococcus_tauri      1 MATRAIASARSRAPRAREGTSLARARRWTRSPHGRVSGRRSRATRWTLDAVSTEDAEGWRRETSKAKET
Chlamydomonas_reinhardtii 1 .....MLEQAPGLAPGSARRDAACSVAREATNVR.....IVTAFALTPGRAGVSGRRIL
Sulfolobus_solfataricus 1 .....MALFRTDRDPLR.....LDDPVTLESMWEDD...DEVLPFLS
Arabidopsis_thaliana    1 .....MDALKCSS.SFLHHTKLTNLFNSHTFPKIS.....APNFKPLFRPISIS...
Helianthus_annuus      1 .....MEKLRTSYSLFHTPKLTSTHTFQTFNPNF.....HINFOTQSRNTAA..V
Jatropha_curcas        1 .....MDLLQPSSTSRILFTPLMLLPFKKNSDN.....FLKPRTRTSCNKS
Klebsoridium_nitens    1 .....MTMVQISSDGFKKPAKNPVLDDTAGPRR.....YSTLQOS.....
Oryza_sativa_Indica     1 .....MASLPHCLSARPLVAAAPGRPGPGPGWLRGGARRRNAAFSAGNAGRVRGLRRV
Pisum_sativum          1 .....MYSLSPPHTTTTSHSTHALINKTK.....LLELNHNHS.RTISIT
Ricinus_communis       1 .....MELLQFATSSRSLGVTLMSCNKNKKKE.....CGEFVMTSTSRIRIRI
Solanum_tuberosum     1 .....MELLHCPISITYK.PKLSPHNHLFSRRSSN.....GVDFESIWKRSSS..V
Spinacia_oleracea      1 .....MTPMELAHCTLFSLSSSPKLCINPQ.KIIRSLN.....INHISPHSTSTIGSL
Theobroma_cacao        1 .....WOLTCCTFLQYAKLMLALRQTTPKRLNLTYSNNHATTSTTRM
Triticum_aestivum      1 .....MTMMALAKAP.CLCARPSLAARAR.RPGPGPAPRLR...RWR...PNATAGKGVG...EV
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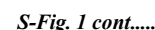
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Ostreococcus_tauri      71 RPTRTIVADAGRGVDDVHARTVAREAKRRTPAPRGVPGVCDTFRCDABALNTRVVGCPDDTVVFVVT
Chlamydomonas_reinhardtii 71 PPSRVSVLEAPTLSSSPATVSTKKLFCEPSSGGOPASTAYGALCPAPALLNSIDADT..GAIYFVVS
Sulfolobus_solfataricus 71 ENAEKVELLEQLTNRQYKFTETISVK.....LDDPVTLESMWEDD...DEVLPFLS
Arabidopsis_thaliana    44 .....AKDRSNEAEN.....IAVVEKPLKSDRFISDLPSPFPPTVRDD...DEVLPFLS
Helianthus_annuus      47 AVNAGDAG.....ELDTAVV.....EKEKPPSPRR.FOLLGKNPTFPFATVLQD...GVFIYS
Jatropha_curcas        47 SHPLIQASRRSSGGGGEVET.....WYVADLKLKRRYQSEHAF.....GVFIYS
Klebsoridium_nitens    37 .....DGRDFGNGLN.....VLYDTF.....ORCASTLISLAQGR..GVFIIT
Oryza_sativa_Indica     57 ASAVEVGVEDEEGVEVEEEEE...VEAVVMPEYALGGACRVLASMPAPLLATALDG...GVFIYS
Pisum_sativum          44 SSKSNKNTFAIGNRVGVET.....EIAVVEIKHQ.FEVRSPFPFPFATAQED...GVFIYS
Ricinus_communis       48 RRCYYSVVTAKARDEVT.....AVVVKRPFRR.YOFSKMPAF..TADG...GVFIYS
Solanum_tuberosum     45 VNAAVDSGRGGVVKTAATAV.....VVEKPTTERC.FEVLSSKPLFPFATADG...GVFVPS
Spinacia_oleracea      50 NSRTPRLMANARETSABIE.....TSVTERQAQTTREIFFGNMPPFVARDDG...GVFIYS
Theobroma_cacao        47 VVKARGGGGGARGCAEAD.....TAVVEKPKIR.FOISDPHGFPLLDG...GVFIYS
Triticum_aestivum      50 CAAVEAATKAEDDDDEE.....AAEDRYALGACRVLASMPAPLLATALDG...GVFIYS
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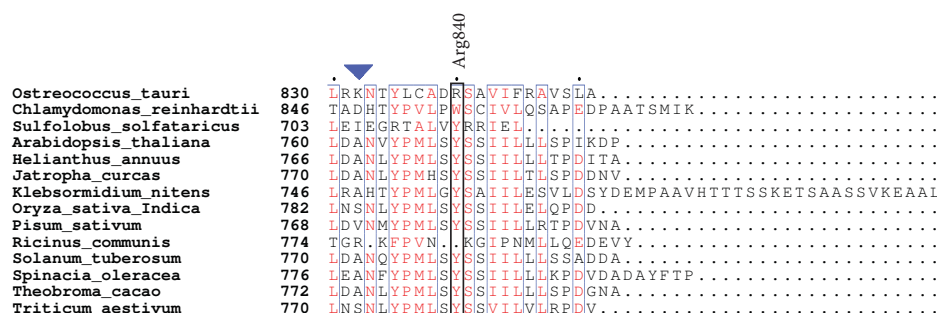
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Ostreococcus_tauri      141 SAATAVSLVAPTEGLARGETAGETE.LDETTNCGSVHIALPRAEVL..FYRVDPYFEEA...R
Chlamydomonas_reinhardtii 118 SRAESVSLVTEADLNAGNATFEPLDPYV..EGDVHMLPDLRODLLFYRVDPHGEEDKDPY...R
Sulfolobus_solfataricus 99 ENAEKVELLEQLTNRQYKFTETISVK.....LDDPVTLESMWEDD...DEVLPFLS
Arabidopsis_thaliana    93 TNSVSTATLCISLSDLRONKVTETIQLDPSR..NCGVHMLFLRGDFDKMLFYRFDKFPFEE...FY
Helianthus_annuus      99 HNAITSATLCMTPSDLQPKRITETIPLDQLTLNCGDVHMLFLKGDFTDMLFYKFDKFPFEE...FY
Jatropha_curcas        102 TNVAVSASLCISLSDLRONKVTETIQLDPLTV..EGDVHMLFLKGDFTDMLFYKFDKFPFEE...FY
Klebsoridium_nitens    78 DGRDVTGCTGEGDQNGTVTAVPLDPLQLP..NCGVHMLFLKGDFTDMLFYKFDKFPFEE...FY
Oryza_sativa_Indica     120 AGASAASLCISLSDLEADVEVEEVLDPDLV..NCGVHMLFYIECELHMLFYRFDKFPFEE...FY
Pisum_sativum          101 LNARSATLCISLSDLFKNKVTETIYALDPLV..NCGVHMLFLKGDFTDMLFYKFDKFPFEE...FY
Ricinus_communis       101 SDVSRASATLCISLSDLRONKVTETIQLDPLTV..EGDVHMLFLKGDFTDMLFYKFDKFPFEE...FY
Solanum_tuberosum     102 RNATATATLCISLSDLPKRVETQIFLDPLA..NCGDVHMLFLKGDFTDMLFYKFDKFPFEE...FY
Spinacia_oleracea      108 GNSVSTATLCISLSDLRONKVTETIQLDPLV..NCGDVHMLFLKGDFTDMLFYKFDKFPFEE...FY
Theobroma_cacao        104 ANAVSATLCISLSDLRONKVTETIQLDPLTV..EGDVHMLFLKGDFTDMLFYKFDKFPFEE...FY
Triticum_aestivum      108 GGLTAAALCISLSDLPKRVETIQLDPLV..NCGDVHMLFYIECELHMLFYRFDKFPFEE...FY
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Ostreococcus_tauri      207 FDKSKILLDPYARFVS.....RPEYQASKKED.....GTEDCWPOYAGGVKKLRSDGKED...G
Chlamydomonas_reinhardtii 188 HDKRRVVLDPYAVAVL.....RRRWQMGPNLPYGEGLVGMPTWPOAAALPA...ARGSA...G
Sulfolobus_solfataricus 99 FNPKKVLDPYARAINGSVINWDVFGKIGQDN.....QDLTYDERDSGEVYKS..VVNIP...D
Arabidopsis_thaliana    159 YDSNILLDPYARATIS.....RGEFGVLGDD.....NCWPQACMGV...TREEE...G
Helianthus_annuus      165 YDSQILLDPYARAVVS.....RGEFGVLGDD.....DCWSQACMGV...SV.DE...G
Jatropha_curcas        168 FESSEVILLDPYARAVIS.....RGEFGVLGDD.....NCWPQACMGV...AARDK...G
Klebsoridium_nitens    144 FDETKIMVDPYARAVS.....RGEFGVLGDD.....NCWPQACMGV...DQED...G
Oryza_sativa_Indica     186 FDVSNVVLDPYARAVIS.....RGEFGVLGDD.....NCWPQACMGV...LPYST...G
Pisum_sativum          167 YDSERVILLDPYARAVIS.....RGEFGVLGDD.....NCWPQACMGV...FDPEE...G
Ricinus_communis       171 FDSQVILLDPYARAVIS.....RGEFGVLGDD.....NCWPQACMGV...ASQAE...G
Solanum_tuberosum     168 FDSQVILLDPYARAVIS.....RGEFGVLGDD.....NCWPQACMGV...SASDG...G
Spinacia_oleracea      174 YDSERVILLDPYARAVIS.....RGEFGVLGDD.....NCWPQACMGV...SPNDE...G
Theobroma_cacao        170 YDSKILLDPYARAVIS.....RGEFGVLGDD.....NCWPQACMGV...TSEDE...G
Triticum_aestivum      174 LDSENVILLDPYARAVIS.....RGEFGVLGDD.....NCWPQACMGV...LPYST...G
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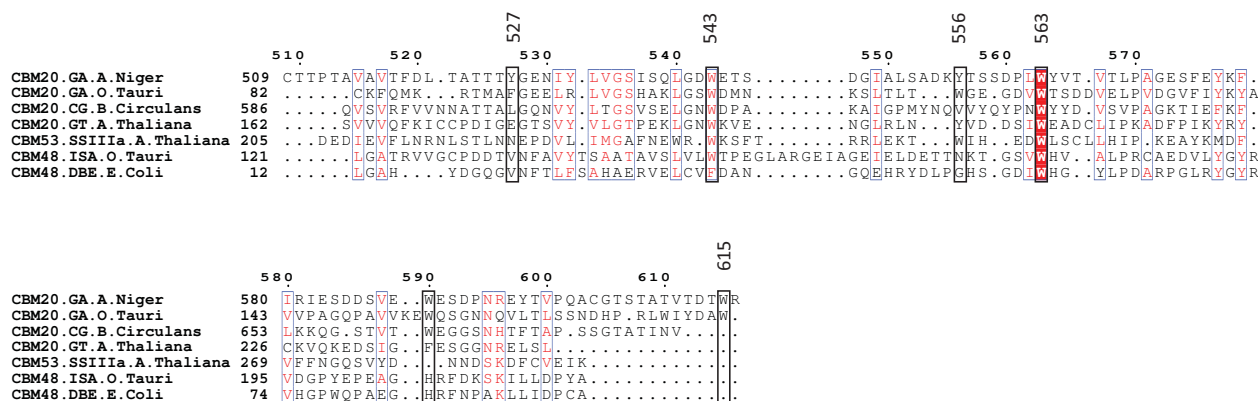
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Ostreococcus_tauri      265 VTSPK...RFMRDLVVYEARGCHAD.LETKAK.FSTAAIEEA..LPY..OLGVAIS...HEHEM
Chlamydomonas_reinhardtii 249 DPLN...LPMESLVIVEVRGTAH.ASSGVAAPGTAGMVER..LDY..LSGVAIS...VFEDEL
Sulfolobus_solfataricus 161 SDFTRGKKVPLKDTVIVEVRGTAH.ASSGVAAPGTAGMVER..LDY..LSGVAIS...VFEDEL
Arabidopsis_thaliana    213 DPLA...FAQRDLVIVEVRGTAH.ESSRTECP..LGVVVK..LDH..E.GNCIO...HEDEL
Helianthus_annuus      217 DPLK...YQKDLVIVEVRGTAH.ESSRTECP..LGVVVK..LDH..E.GNCIO...HEDEL
Jatropha_curcas        193 DIAPN...IAQKDLVIVEVRGTAH.ESSRTECP..LGVVVK..LDH..E.GNCIO...HEDEL
Klebsoridium_nitens    217 DPLK...YQKDLVIVEVRGTAH.ESSRTECP..LGVVVK..LDH..E.GNCIO...HEDEL
Oryza_sativa_Indica     221 DPLK...YQKDLVIVEVRGTAH.ESSRTECP..LGVVVK..LDH..E.GNCIO...HEDEL
Pisum_sativum          221 DPLK...YQKDLVIVEVRGTAH.ESSRTECP..LGVVVK..LDH..E.GNCIO...HEDEL
Ricinus_communis       217 DPLK...YQKDLVIVEVRGTAH.ESSRTECP..LGVVVK..LDH..E.GNCIO...HEDEL
Solanum_tuberosum     223 DPLK...YQKDLVIVEVRGTAH.ESSRTECP..LGVVVK..LDH..E.GNCIO...HEDEL
Spinacia_oleracea      219 DPLK...YQKDLVIVEVRGTAH.ESSRTECP..LGVVVK..LDH..E.GNCIO...HEDEL
Theobroma_cacao        223 DPLK...YQKDLVIVEVRGTAH.ESSRTECP..LGVVVK..LDH..E.GNCIO...HEDEL
Triticum_aestivum      223 DPLK...YQKDLVIVEVRGTAH.ESSRTECP..LGVVVK..LDH..E.GNCIO...HEDEL
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S-Fig. 1 cont....





**SUPPLEMENTARY FIG. 1.** Alignment using ESPrnt V 3.0 program with default parameters. The protein sequence belongs to OstalISA1 (Ostreococcus tauri Isoamylase 1); Isoamylase from Chlamydomonas reinhardtii (AAP85534.1); Trex from Sulfolobus solfataricus (WP\_009989784); Isoamylase 1 from Arabidopsis thaliana (NP\_181522); Isoamylase 1 from Helianthus annuus (XP\_022037404); Isoamylase 1 from Jatropha curcas (XP\_012075534); Isoamylase from Klebsormidium nitens (GAQ88556); Isoamylase 1 from Oryza sativa (AII21931); Isoamylase 1 from Pisum sativum (AAZ81835); Putative Isoamylase from Ricinus communis (EEF32503); Isoamylase 1 from Solanum tuberosum (NP\_001274937); Isoamylase 1 from Spinacia oleracea (XP\_021857581); Isoamylase 1 isoform 2 from Theobroma cacao (EOY32085); Isoamylase D1 from Triticum aestivum (AAP44580). Catalytic aminoacids are black boxed and tagged according to Ostreococcus tauri numbering. Numbers above sequence refers to O. tauri. The color code is as follows: Red box, white character means "Strict identity"; Red character or black bold means "Similarity in a group"; Blue frame means "Similarity across groups". Blue arrowheads mark the amino acids involved in dimerization (in ChlreISA1).



**SUPPLEMENTARY FIG. 2.** CBM amino acid sequence alignment. Numbering refers to 1ACZ. Y527, W543, Y556, W590 y W563: Aminoacids involved in carbohydrate binding. W615: Aminoacid with important role on CBM structure. CBM20GA.A.Niger is CBM20 (glucoamylase - 1ACZ) from A. niger; CBM20AG. O.Tauri is CBM20 (glucan 1,4- $\alpha$ -glycosidase) from O. tauri; CBM20CG.B.Circulans is CBM20 (cyclomaltodextrin glucanotransferase) from B. circulans; CBM20GT.A. Thaliana is CBM20 (4- $\alpha$ -glucanotransferase) from A. thaliana; CBM53SSIII.A.Thaliana is CBM53 (starch synthase III) from A. thaliana; CBM48ISA.O.Tauri is CBM48 (isoamilase) from O. tauri, CBM48.DBE.E.Coli is CBM48 (glycogen debranching enzyme) from E. coli.