Additional file 2. Alignment of *Streptomyces spp.* ChiJ sequences, where a dot indicate an identical amino acid residue as in S. clavuligerus ChiJ and a dash indicate a gap.

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                               I                              II
                              10        20        30        40        50        60        70
                                P      ◊   ◊                  P  P      P
                      ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ChiJ S. clavuligerus  AETCAVKSKPAGKVLQGYWENWDGAANGVHPPLGWIPITDPRIRAHGYNVLNAAFPVIRSDGTVLWENGM
ChiJ S. griseus       V.........T.............................S..TQ.....I................D..
ChiJ S. sp. C         PD.....P.............................V..G.........V................D.F
ChiJ S. sp. ACTE      .D.....PR.Q.....................F..TS.A..L.P......V................D..
ChiJ S. sp. Mg1       .D..P..PR.T...............A.......F..T....S...D.....I.......L....A..QD..
ChiJ S. coelicolor    .D................................F..T.....Q.G..............L....A...D..
ChiJ S. sp. e14       .D................................F..T.....Q.G..............L....A...D..
ChiJ S. lividans      .D................................F..T.V...Q.G..............L....A...D..
ChiJ S. ghanaensis    .D...L.P..............SS......F..T....S..AQ.....I............A......
ChiJ S. avermitilis   .D......R.S.............SS......F..T....S..A......I............A...D..
ChiJ S. sp. AA4       SD.....PR.S.P............S.....GM..V..N..S.A......I.......L.....Q..D..

                        III             IV
                              80        90       100       110       120       130       140
                                                      ◊◊               C                ◊ ◊
                                             P                 C C       P              * * *   P
                      ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ChiJ S. clavuligerus  DTTVKVPTPAEMCQAKADGQTILMSIGGAAAGIDLSSRAVADRFIATIVPILKRFNFDGIDIDIETGLVG
ChiJ S. griseus       .N....A..........S.L.T...............S......VQ.......KY...............
ChiJ S. sp. C         .S..............Q..L.V..................N.T......V.....L..KY...............
ChiJ S. sp. ACTE      .A..R..........S.L.V.L.....T.....N.T......VE.V.....AN.............A.
ChiJ S. sp. Mg1       .A..E.A..........A.A...............N.A......V..L.....KY...............
ChiJ S. coelicolor    .RG...A......A...S...L.L.....T.....N.T......VD.......EY.............T.
ChiJ S. sp. e14       .RG...A......A...S...L.L.....T.....N.T......VD.......EY.............T.
ChiJ S. lividans      .RG...A......A...S...L.L.....T.....N.T......VD.......EY.............T.
ChiJ S. ghanaensis    ..G...A......A...A.....L.....T.....N.T.......E.......TY.............I.
ChiJ S. avermitilis   ..G...A..........S.....L.............S......V.....L..KY...............
ChiJ S. sp. AA4       .S....S......A..DV.A.................SS...K.VS.......KY.............SS

                           V                   VI
                             150       160       170       180       190       200       210
                                 C
                                 P          CC  CP     ◊    ◊      P
                      ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ChiJ S. clavuligerus  TGNINQLSASQANLIHIIDGVLAQMPPNFGLTMAPETAYVTGGSVVYGSIWGSYLPIIKKYADNGRLWWL
ChiJ S. griseus       S..........S...R.......A..............................................
ChiJ S. sp. C         S.S.TR..P......R..........AG.................A......A...VV............
ChiJ S. sp. ACTE      S.D.G...P..S...R.......A...G.........................A...V............
ChiJ S. sp. Mg1       S.T.TR..P..T...R....I.....AG.................T......A...V..R..........
ChiJ S. coelicolor    S..........S...R.......R..S...................I.......A.........E.......
ChiJ S. sp. e14       S..........S...R.......R..S...................I.......A.........E.......
ChiJ S. lividans      S..........S...R.......R..S...................I.......A.M...............
ChiJ S. ghanaensis    ........P..T...R......KR..DG..........................A.................
ChiJ S. avermitilis   S...G..........R......G...S.......................A......A....V............
ChiJ S. sp. AA4       S.D..S........ER......S...SG.................T......A............Q....

                                                                                     VII
                             220       230       240       250       260       270       280
                          ◊
                        ◊ *◊   ◊                                                     C
                      ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ChiJ S. clavuligerus  NMQYYNGSMYGCSGDSYAAGTVAGFRAQTDCLNRGLVIQGTTIRVPYDKQAPGLPAQPGAGGGHMAPGLV
ChiJ S. griseus       .................S.......T.......K.........K......V..............ST...
ChiJ S. sp. C         .................S.......K......D....V....V.......V............Y......
ChiJ S. sp. ACTE      .................E....Q..T......DK...V....VK......V..............S.AQ.
ChiJ S. sp. Mg1       ......................Q..T.......Q................V..............S.A..
ChiJ S. coelicolor    ............A....S....Q..T......DK.........V......V............Y.S.SP.
ChiJ S. sp. e14       ............A....S....Q..T......DK.........V......V............Y.S.SP.
ChiJ S. lividans      ............A....S....Q..T......DK.........V......V............Y.S.S..
ChiJ S. ghanaensis    .................P....Q..TV.......K................V................SQ.
ChiJ S. avermitilis   .................S....Q..T.......K...V.....V......V......S......Y.S.S..
ChiJ S. sp. AA4       .................E....Q..TV..Q..DK..TV........V...V............Y.D..S.

                          VII
                             290       300       310
                              C
                           P PPP      ◊◊
                      ....|....|....|....|....|....|....|....
ChiJ S. clavuligerus  TQAWNGYGNALKGLMTWSLNWDGSRNWTFGDNVRALQGR
ChiJ S. griseus       S....H.KGS...............G.......KS....
ChiJ S. sp. C         A....P..GR.............................
ChiJ S. sp. ACTE      A....T.SG-........I......D.......KN....
ChiJ S. sp. Mg1       A....R.AGG........V....A......N..KT....
ChiJ S. coelicolor    S....H.NG...............K.....T..KS....
ChiJ S. sp. e14       S....H.NG...............K.....T..KS....
ChiJ S. lividans      S....H.NG...............K.....T..KS....
ChiJ S. ghanaensis    A....H..TS........I.....K........K.....
ChiJ S. avermitilis   S...RH..SG........I.....K........K.....
ChiJ S. sp. AA4       S..YQSVPG-.....D..I......G.......KS....
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As outlined in Fig. 3, asterisks (*) indicate positions of predicted catalytic residues, diamonds (◊) indicate predicted substrate-interacting residues, P indicate residues under positive selection, C indicate co-evolving residues. Positions of highly variable regions are indicated (I through VII).