

**Figure S1 Carbon utilization profiles of *R. opacus* strains.** Turbidity data measure bacterial growth, indicative of substrate utilization. Washed cells grown on LB plates for 3 days were suspended in 28 ml IF-0a GN/GP Base Inoculating Fluid (Biolog) and 0.3 ml Redox Dye Mix D (Biolog) and adjusted to an OD<sub>660</sub> of 0.01. Aliquots of 50 μl the mixture were dispensed into each well of the BIOLOG PM microplates. Data are based on the absorbance following incubation of the plates for 5 days. The well color development for each sample was calculated as the mean value of the OD<sub>595</sub> in the three replicates of each substrate, subtracted of the average OD<sub>595</sub> in the background wells. The order of the carbon sources is the rank of the growth on 190 substrates for PD630. Error bars show the standard deviations.

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Hyg-XI  MNYQPTPEDRFTFGLWTVGWQGRDPFGDATRRALDPVETVQRLAELGAHGVTFHDDDLIPFGSSDTERESHIKRFRQALD
Ave-XI  MNYQPTPEDRFTFGLWTVGWQGRDPFGDATRRALDPVETVQRLAELGAHGVTFHDDDLIPFGSSDTERESHIKRFRQALD
Cha-XI  MSYQPTPEDRFTFGLWTVGWQGRDPFGDATRRALDPVETVQRLAELGAHGVTFHDDDLIPFGSSDTEREAHIKRFRQALD
Coe-XI  MNYQPTPEDRFTFGLWTVGWQGRDPFGDATRQALDPAESVRRLELGAHGVTFHDDDLIPFGSSDTERESHIKRFRQALD
Sp8-O1  MSFQPTPEDRFTFGLWTVGWQGRDPFGDATRPALDPVETVQRLAELGAYGVTFHDDDLIPFGSSDTERESHIKRFRQALD  80
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Hyg-XI  ATGMTVPMATTNLFTHPVFKDGAFTANDRDVRRYALRKTIRNIDLAAELGAKTYVAWGGREGAESGAAKDVRVALDRMKE
Ave-XI  ATGMAVPMATTNLFTHPVFKDGAFTANDRDVRRYALRKTIRNIDLAAELGAKTYVAWGGREGAESGAAKDVRVALDRMKE
Cha-XI  ATGMKVPMATTNLFTHPVFKDGAFTANDRDVRRYALRKTIRNIDLLEVELGAQVYVAWGGREGAESGAAKDVRVALDRMKE
Coe-XI  ATGMKVPMATTNLFTHPVFKDGAFTANDRDVRRYALRKTIRNIDLLEVELGASVYVAWGGREGAESGAAKDVRDALDRMKE
Sp8-O1  ATGMTVPMATTNLFTHPVFKDGGFTANDRDVRRYALRKTIRNIDLAAELGAKTYVAWGGREGAESGGAKDVRDALDRMKE  160
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Hyg-XI  AFDLLGEYVTSQGYDLRFAIEPKPNEPRGDILLPTVGHALAFIERLERPELYGVNPEVGHEQMAGLNFPHGIAQALWAGK
Ave-XI  AFDLLGEYVTAQGYDLRFAIEPKPNEPRGDILLPTVGHALAFIERLERPELYGVNPEVGHEQMAGLNFPHGIAQALWAGK
Cha-XI  AFDLLGEYVTSQGYDLRFAIEPKPNEPRGDILLPTVGHALAFIERLERPELYGVNPEVGHEQMAGLNFPHGIAQALWAGK
Coe-XI  AFDLLGEYVTEQGYDLKFAIEPKPNEPRGDILLPTVGHALAFIERLERPELYGVNPEVGHEQMAGLNFPHGIAQALWAGK
Sp8-O1  AFDLLGEYVIAQGYDLRFAIEPKPNEPRGDILLPTVGHALAFIERLERPELYGVNPEVGHEQMAGLNFPHGIAQALWAGK  240
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Hyg-XI  LFHIDLNGQSGIKYDQDLRFGAGDLRAAFWLVDLLESAGYEGPRHFDFKPPRTEDLDGVWASAAGCMRNYLILKERAALF
Ave-XI  LFHIDLNGQSGIKYDQDLRFGAGDLRAAFWLVDLLESAGYEGPKHFDKPPRTEDLDGVWASAAGCMRNYLILKERTAAF
Cha-XI  LFHIDLNGQSGIKYDQDLRFGAGDLRAAFWLVDLLESAGYTGPRHFDFKPPRTEDLDGVWASAAGCMRNYLILKERAALF
Coe-XI  LFHIDLNGQSGIKYDQDLRFGAGDLRAAFWLVDLLERAGYAGPRHFDFKPPRTEDFDGVWASAAGCMRNYLILKDRAAAF
Sp8-O1  LFHIDLNGQSGIKYDQDLRFGAGDLRAAFWLVDLLETAGYEGPRHFDFKPPRTEDFDGVWASAAGCMRNYLILKDRAAAF  320
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Hyg-XI  RADPEVQEALRASRLDQLAQPTAADGLEDLLADRAAFEDFDVEAAAARGMAFERLDQLAMDHLLGARG
Ave-XI  RADPEVQEALRAARLDELAQPTAGDGLTALLADRATAFEDFDVEAAAARGMAFEQLDQLAMDHLLGARG
Cha-XI  RADPEVQEALRAARLDELAQPTAADGLQALLADRSASFESFDVEAAAARGMAFEHLDQLAMDHLLGARG
Coe-XI  RADPQVQEALAAARLDELARPTAEDGLAALLADRSAYDTFDVDAARARGMAFEHLDQLAMDHLLGAR-
Sp8-O1  RADPEVQEALRAARLDQLAQATAADGLDALLADRSASFEDFDVDAARARGMAFEHLDQLAMDHLLGARG  388
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**Figure S2 Comparison of ORF-1 in pXsp8 insert with several xylose isomerases at the deduced amino acid sequence level using the CLUSTALW program.** Hyg-XI, xylose isomerase of *S. hygroscopicus* accession no. AEY87893; Ave-XI, xylose isomerase of *S. avermitilis* accession no. NP\_828358; Cha-XI, xylose isomerase of *S. chartreusis* accession no. ZP\_09957234; Coe-XI, xylose isomerase of *S. coelicolor* accession no. NP\_625460; Sp8-O1, ORF-1 of the 3603-bp insert. Numbering of the amino acids starts at the N-terminus of all proteins. The introduction of gaps is indicated by dashes. Asterisks, colons and periods indicate identical, strongly similar and weakly similar conserved residues, respectively, among all of these enzymes.

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Cha-XK MSAAEGLVVGVDSTQSTKALVVDVSTGRVVASGQAPHTVSSGSGRES DPRQWWDALCEALRQCGDAAHEAAA VSVGGQQHG
Coe-XK MSAAEGLVVGVDSTQSTKALVVDAAATGRVVASGQAPHTVSSGTGRES DPRQWWDALGEALSQCGEAAREAAA VSVGGQQHG
Ave-XK MSAAEGLVVGVDSTQSTKALVVDASTGRVVASGQAPHTVSAGAGRES DPRQWWDALCEALHQCGDAAHEAAA I SVGGQQHG
Hyg-XK MSAAEGLVVGVDSTQSTKALVVDAAATGQVVASGQAPHTVSSGAGRES DPRQWWDALCEALRQCGDAAHEAAA VSI GGGQQHG
Sp8-O2 MSAAEGLVVGVDSTQSTKALVVDAAATGQVVASGQAPHTVTTGGGRES DPRQWWDALCEALRQCGDAAHEAAA I SVGGQQHG 83

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Cha-XK LVTLDDRGE PVRPALLWNDVRSAPQARRL TEELGGAKFWAERTGSVPAASFVTVKWAWLAEHEPEAARATKAVRLPHDY LTER
Coe-XK LVTLDARGE PVRPALLWNDVRSAPQARRL IDELGGAKAWAERTGSVPSASFVTVKWAWLTEHEPEAARAVKAVRLPHDY LTER
Ave-XK LVTLDAHGDPVRPALLWNDVRSAPQARRL IEELGGPKAWAERTGSVPGPSFTVTWKAWLTEHEPEAVRATAAVRLPHDY LTER
Hyg-XK LVTLDERGE PVRPALLWNDVRSAPQAGRLVEELGGPKAWAERTGSVPGASFVTVKWAWLAEHEPEAVRATRAVRLPHDY LTER
Sp8-O2 LVTLDERGE PVRPALLWNDVRSAPQARRLVEELGGPKAWAERTGSVPGASFTASKWAWLAEHEPEAARATRAVRLPHDY LTER 166

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Cha-XK LTGQGTDRGDASGTGWWASGTERYDEEILAQVGLDLPALLPRVVRPGEIAGTVRDSHDL PFSKGTLVAPGTGDNAAAALGLGV
Coe-XK LTGEGTDRGDVSGTGWWASGTEAYDEEILARVALDLPALLPRVVRPGEVAGTVRDGHGLPFSKGTLVAAAGTDNAAAALGLGL
Ave-XK LTGQGTDRGDVSGTGWWASATESYDEEVLTHVGLDLPALLPRVVRPGEVVGTVRDSHDL PFSKGTLVAPGTGDNAAAALGLGL
Hyg-XK LTGQGTDRGDASGTGWWASGTEAYDEETLAHVGLDLPALLPRVVRPGEVAGTVRDSHDL PFSKGTLVAPGTGDNAAAALGLGL
Sp8-O2 LTGQAVTDRGDASGTGWWASGTERYDEDTLAHLGLDLPALLPRVVRPGEVAGTVRDGHGLPFSKGTLVASGTGDNAAAALGLGL 249

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Cha-XK RPGVPVMSLGTSGTVYAVSQRRPADPSGTVAGFADARGDWLPLACTLNCTLAVDRVATLLGLDREAVEPGTAVTLLPYLDGER
Coe-XK RPGVPVMSLGTSGTAYAVSQRRPADPTGTVAGFADARGDWLPLACTLNCTLAVDRVASLLGLDREAVEPGTDVTL PFLDGER
Ave-XK RPGTPVLSLGTSGTVYAVSTRRPADPTGTVAGFADARGDWLPLACTLNCTLAVDRVAALLGLDREAVEPGTGVTL PLYDGER
Hyg-XK RPGTPVLSLGTSGTVYAVSRRRPTDPTGTVAGFADARGDWLPLACTLNCTLAVDRVAALLGLDREAVESGGSVTL PLYDGER
Sp8-O2 LPGTPVLSLGTSGTVYAVSRHRPADPTGTVAGFADARGDWLPLACTLNCTLAVDRVATLLGLDREAVAPGGSVTL PFLDGER 332

**:*:***:***** *****:***** ** .:***** *****:
Cha-XK TPNLPHASGLLHGLRHDTTAGQLLQAAYDGAVHSLLGALDLVLDDEDADPSAPLLLIGGGARGTAWQQTVRRLS GRPVQVPEAK
Coe-XK TPNLPHSGLLHGLRHDTTAGQLLQAAYDGAVHSLLGALDLVLDADADPSAPLLLIGGGARGTAWQQTVRRLS GRPVQIPEAR
Ave-XK TPDLPHASGLLHGLRHDTTAGQLLQAAYDGAVHALLGALDLVLDADADRSAPLLLIGGGARGTAWQQTVRRLS GRPVQVPEAR
Hyg-XK TPNLPHASGVLHGLRHDTTAGQLLQAAYDGAVHALLGALDLVLDDEDADRDTPLLLIGGGARGTAWQQTVRRLS GRPVQVPEAR
Sp8-O2 TPDLPNASGLLHGLRHDTTPGQLLQAAYDGAVHSLLGALDLVLDDEDADRDSPLLLIGGGARGHAWQQTVRRLS GRPVQ ILRLA 415
Hyg-CB MSDTSPYGFELVRRGYDRAQVDERISKLVSDRDSALARITALEKRIEELHLETQNAQAQITDAEPSYAGLGARVEKILRLA 81
Ave-CB MSDTSPYGFELVRRGYDRAQVDERISKLVSDRDSALARITALEKRIEELHLETQNAQAQVTDAEPSYAGLGARVEKILRLA 81
Cha-CB MSDTSPYGFELVRRGYDRAQVDERISKLVSDRDSALARITALEKRIEELHLETQNAQAQISDAEPSYAGLGARVEKILRLA 81
Coe-CB MSDTSPYGFELVRRGYDRAQVDERISKLVSDRDSALARITALEKRIEELHLETQNAQAQVNDAEPSYAGLGARVEKILRLA 81
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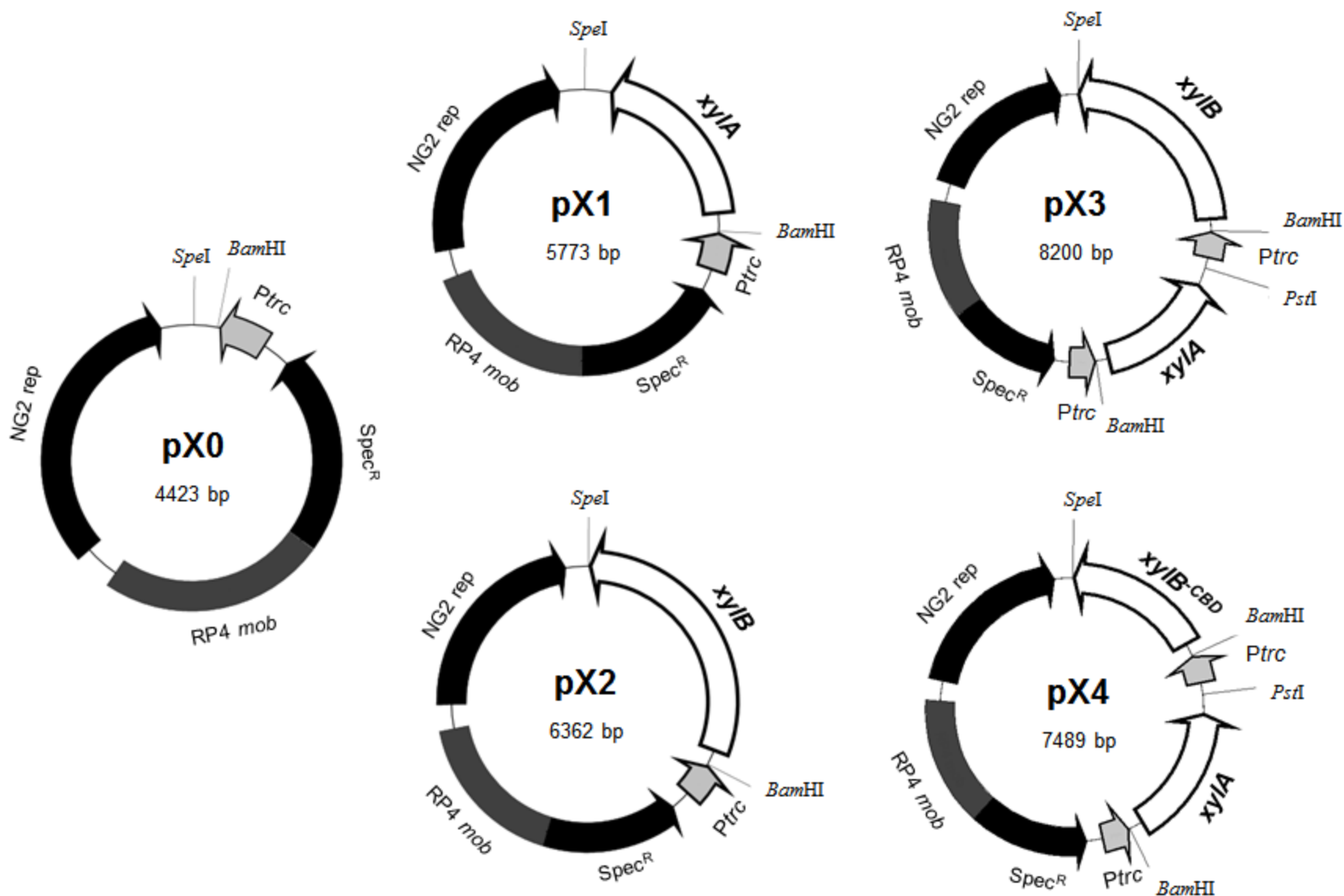
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Coe-XK ELVALGAAAQAAGLLTGEDAAAVARRWNTAAGPVLDAVERDEATLNRIITGVLSDAAPLLERDAASR 481
Ave-XK ELVALGAAAQAAGVLTGEDPAAVARRWNTAHGPVLDAVERDEATLARISGVLSDAAPLLEREPDRR 481
Hyg-XK ELVALGAAAQAAGLLTGEDPAAVARRWNTAAGPVLEAVERDRITLDRITGVLSDAAPLLERGTDTTE 481
Sp8-O2 EEEAKELREEARRAAEQHRELAESAAQQVRNDAESYATERKAKAEDEGSRIVEKAKGEASQLRADAQKDAQSKREEADALFEE 498
Hyg-CB EEEAKELREEARRAAEQHRDLAESAAQQVRNDAESYAAERKAKAEDEGVRIVEKAKSDAAQLRAEAQKDAQSKREEADALFEE 164
Ave-CB EEEAKDLREEARRASEQHRELAESAAQQVRNDAESFAADRKSKAEDEGVRIVEKAKSDASQLRQEAQKDAQSKREEADALFEE 164
Cha-CB EEEAKDLREEARRAAEQHRELAESAAQQVRNDAESYSAERKAKAEDEGLRIVEKAKSDAAQLRAEAQKDAQSKREEADALFEE 164
Coe-CB EEEAKDLREEARRAAEQHRELAESSAQQVRNDAESYAAERKAKAEDEGVRIVEKAKGDASQLRSEAQKDAQSKRDEADALFEE 164
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Sp8-O2 TRAKAAQAADDFETNLAKRREQSERDLASRQQAQKAEKRLAEIEHRAEQRLRLEAEKLRRTDAERRARQTVETAQRQAEDIVADANA 581
Hyg-CB TRAKAAQAADDFETNLAKRREQSERDLASRQQAQKAEKRLAEIEHRAEQRLRLEAEKLRRTDAERRARQTVETAQRQAEDIVADANA 247
Ave-CB TRAKAAQAADDFETNLAKRREQSERDLASRQQAQKAEKRLAEIEHRAEQRLRLEAEKLRRTDAERRARQTVETAQRQAEDIVADANA 247
Cha-CB TRAKAAQAADDFETNLAKRREQSERDLASRQQAQKAEKRLAEIEHRAEQRLRLEAEKLRRTDAERRARQTVETAQRQAEDIVADANA 247
Coe-CB TRAKAAQAADDFETNLAKRREQSERDLASRQQAQKAEKRLAEIEHRAEQRLRLEAEKLRRTDAERRARQTVETAQRQSEDIVADANA 247
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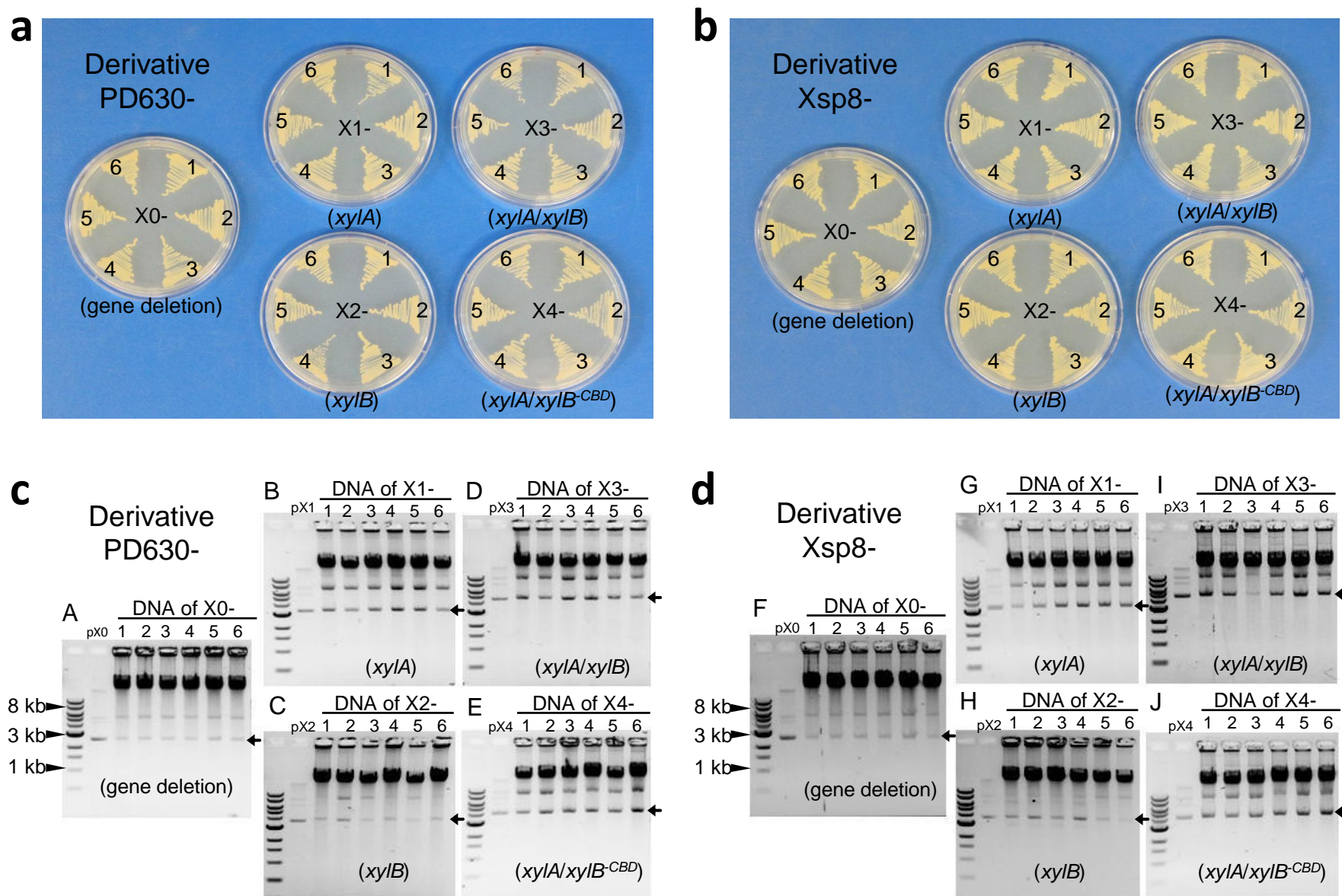
Sp8-O2 KADRIRSESERELAALTNRRDSINAQLTNVREMLATLTGAAVAAAGT-TGEDEPVSIRGVPAQQTR 645
Hyg-CB KADRIRSESERELAALTNRRDSINAQLTNVREMLATLTGAAVAAAGT-STDDEPISIRGVPAQQSR 311
Ave-CB KADRIRSESERELAALTNRRDSINAQLTNVREMLATLTGAAVAAAGT-PSTDDEPISIRGVPAQQSR 312
Cha-CB KADRIRSESERELAALTNRRDSINAQLTNVREMLATLTGAAVAAAGT-PAEDEPISIRGVPAQQSR 311
Coe-CB KADRIRSESERELAALTNRRDSINAQLTNVREMLASLTGAAVAAAPS--VEDESVSIRGVPAQQSR 310
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**Figure S3 Comparison of ORF-2 in pXs8 insert with several xylulose kinases and cellulose-binding proteins at the deduced amino acid sequence level using the CLUSTALW program.** Cha-XK, xylulose kinase of *S. chartreusis* accession no. ZP\_09957235; Coe-XK, xylulose kinase of *S. coelicolor* accession no. NP\_733525; Ave-XK, xylulose kinase of *S. avermitilis* accession no. NP\_828357; Hyg-XK, xylulose kinase of *S. hygrosopicus* accession no. AEY87894; Sp8-O2, ORF-2 of the 3603-bp insert; Hyg-CB, cellulose-binding protein of *S. hygrosopicus* accession no. AEY91743; Ave-CB, cellulose-binding protein of *S. avermitilis* accession no. NP\_824035; Cha-CB, cellulose-binding protein of *S. chartreusis* accession no. ZP\_09956458; Coe-CB, cellulose-binding protein of *S. coelicolor* accession no. NP\_629535. Numbering of the amino acids starts at the N-terminus of all proteins. Sequences of xylulose kinases and cellulose-binding proteins are depicted in black and blue letters, respectively. The introduction of gaps is indicated by dashes. Asterisks, colons and periods indicate identical, strongly similar and weakly similar conserved residues, respectively, among all of these enzymes.



**Figure S4 Construction of plasmid pX0, pX1, pX2, pX3 and pX4.** These plasmids contain the NG2 origin of replication from pEP2, the RP4 *mob* element from pSUP301, a spectinomycin resistance marker derived from the omega interposon and the *trc* promoter from pTrc99A<sup>47</sup>. See for methods for details.



**Figure S5 Elucidation of the molecular targets involved in improvement of xylose metabolism in *R. opacus* PD630.** (a,b) Growth of *Rhodococcus* isolates on LB plates. Six colonies each plate in Figure 6a, b were randomly streaked onto LB plate and incubated for 3 days. The isolates were designated PD630X0-1 to -6, PD630X1-1 to -6, PD630X2-1 to -6, PD630X3-1 to -6 and PD630X4-1 to -6, respectively, from pX0, pX1, pX2, pX3 and pX4 plates in Figure 6a, and Xsp8X0-1 to -6, Xsp8X1-1 to -6, Xsp8X2-1 to -6, Xsp8X3-1 to -6 and Xsp8X4-1 to -6, respectively, from pX0, pX1, pX2, pX3 and pX4 plates in Figure 6b. (c,d) Detection of the plasmid DNA in *R. opacus* derivatives. Each strain was grown in LB medium supplemented with spectinomycin for 24 h in a flask. The total genomic DNA of each strain was prepared and subjected to 0.7% agarose gel electrophoresis. Arrows in panels A and F, pX0; arrows in panels B and G, pX1; arrows in panels C and H, pX2; arrows in panels D and I, pX3; arrows in panels E and J, pX4. Filled arrowheads indicate size of relevant markers (1 kb ladder).