Purification and characterization of the acetone carboxylase of *Cupriavidus metallidurans* strain CH34

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1. Abstract

The acetone carboxylase (Acx) is a key enzyme involved in the biodegradation of acetone by bacteria. Excepted for the *Helicobacteriaceae* family, genome analyses revealed that bacterial that possess Acx are associated to the soil such as *Cupriavidus metallidurans* strain CH34. Acx of CH34 forms a heterohexameric complex $\alpha_2\beta_2\gamma_2$ and can only carboxylate acetone and 2-butanone in the ATP dependent pathway to acetoacetate and 3-keto-2-methylbutyrate, respectively.

Acetone is a toxic compound found in air, water and soil, both naturally , as a pollutant (6, 18) and also produced by mammals and bacteria (9, 12, 13, 25). Acetone can be also degraded by various bacteria using a CO_2-dependent pathway including acetone carboxylase as key enzyme, a property of increasing interest for bioremediation purposes (1-3, 7-8, 10-11, 17, 19-23). Acetone carboxylase is a member of a protein family that also contains acetophenone carboxylase and ATP-dependent hydantoinases/oxoprolinases. While the members of this family share several similar characteristics, they differ with respect to the substrates, the products of ATP hydrolysis and structural properties (19).

As shown in figure 1, genome analyses revealed a lot of bacterial species that possess the acetone carboxylase and thus which are potentially able to detoxify acetone. Most of these bacteria were found in soil such as *Cupriavidus metallidurans* or in contact with soil (*e.g.* by plant symbiosis), and belong to Proteobacteria and especially β-Proteobacteria. In the α-Proteobacteria class, the Rhizobiales and the Rhodobacterales orders were found to contain Acx. In the δ-Proteobacteria class, only one species (*Geobacter uraniireducens* Rf4), up to now, was discovered to contain an Acx, with around 30 % aa sequence identity with the CH34 Acx depending on the subunit. The only pathogenic species that possess the enzyme are those belonging to the *Helicobacteriaceae* family (□-Proteobacteria), which are found in the mammalian stomach.

In general, similar gene organization for the Acx operon was found, with three genes *acx* A, B and C encoding the three acetone carboxylase subunits (β, α and γ subunits, respectively) and one regulator *acx*R, which was identified as a σ^{54} or σ^{70} specific transcriptional regulator and can be divergently transcribed (21). The only known paralogous enzyme with similar biochemical function is acetophenone carboxylase from *Aromatoleum aromaticum*. This

68 enzyme consist of a heterooctamer of four subunits, whose the corresponding genes
69 *apc*ABCDE are clustered as an operon. Acetone carboxylase does not contain a paralogue of
70 ApcE.

71 **Acetone carboxylase induction in *Cupriavidus metallidurans***

72 A recent study focused on acetone metabolism in *C. metallidurans* CH34, a
73 Betaproteobacterium found in industrial biotopes highly contaminated in metals (15), showed
74 an overexpression of the acetone carboxylase when grown in spaceflight conditions (14),. As
75 observed in *Rhodobacter capsulatus* and *Xanthobacter autotrophicus, C. metallidurans* Acx
76 subunits were induced at a high level (19.28 ± 4.54 % of the total proteins) when acetone was
77 present in the culture (Fig. 2) (21-22). An *acxR*$^-$ knock-out mutant was constructed in this
78 study. This mutant, in which no acetone carboxylase was produced (Fig. 2), was unable to
79 growth at the expense of acetone or isopropanol. A large expression of this enzyme could
80 compensate a low turnover number for catalysis allowing a reasonable rate of acetone
81 carboxylation to support growth with a relatively low doubling time (4 to 20 hours for *X.*
82 *autotrophicus* , *R. capsulatus and C. metallidurans* CH34) (21).

83

84 ***Acetone carboxylase purification and characterization***

85 The partial characterization of acetone carboxylase were conducted in *X autotrophicus* strain
86 Py2, *Rhodococcus rhodochrous* strain B276, *R. capsulatus* strain B10, in *Alicycliphilus*
87 *denitrificans* strain K601, two species of *Paracoccus* and very recently in *A. aromaticum* and
88 showed high structural similarities (1, 7-8, 17, 19-22).

89 In this study, the Acx of CH34 was purified according to a two-step procedure consisting of
90 anion DEAE-Sepharose chromatography followed by Sephacryl S300 molecular filtration
91 (24, 25). The native molecular weight of the acetone carboxylase complex was determined by
92 gel filtration and was estimated to be 388 +/- 15kDa, corresponding to an $\alpha_2\beta_2\gamma_2$ configuration

93 (86, 76 and 19 kDa for the α, β and γ subunits), as described previously in other bacteria (7, 8,
94 19-21). The absorption spectrum of acetone carboxylase between 250 and 350 nm exhibited a
95 maximal peak at 287.2 nm, which is close to the value obtained in *X. autotrophicus* (281 nm)
96 (20).
97 ***Enzymatic activity***
98 Depending on the species, the properties of Acx differ with regard to the substrates and
99 cofactors required to support the carboxylation reaction (1, 7, 19-22).
100 The *C. metallidurans* enzyme showed a poor stability and a maximum activity in a pH range
101 of 6.5 - 8.0.
102 Of all the tested high-energy compounds (ATP, ITP, UTP or GTP), only Mg-ATP supported
103 acetone carboxylation in CH34 resulting in acetoacetate formation (Fig. 3). Similar results
104 were obtained in *X. autotrophicus*, A. aromaticum and *R. capsulatus*, while in *R.*
105 *rhodochrous*, no activity was observed with ATP (7, 19, 21). As observed in these bacteria,
106 acetone carboxylase reaction in CH34 showed that ATP is hydrolyzed into AMP and 2
107 inorganic phosphates.
108
109 NH_4^+ ions have also been shown to increase acetone carboxylase activity (21). Yet
110 tests performed in the presence of NH_4Cl (100 mM) showed no significant increase in
111 acetoacetate production by Acx of CH34. In contrast, as observed in *X. autotrophicus*,
112 potassium (40 mM) and CO_2 source ($KHCO_3$) stimulated the CH34 acetone carboxylase
113 activity.
114 The specific activities obtained with the purified enzyme of CH34 were 0.4 to 0.6U/mg
115 compared to 0.08-0.240 U/mg for the other Acx. Nevertheless, the comparison in terms of
116 activity has to be taken cautiously due to the differences observed with the stability of the
117 purified enzymes.

Among all the tested substrates, only acetone and 2-butanol were identified as substrates of the *C. metallidurans* Acx (Fig. 3). Interestingly, we showed that *C. metallidurans* CH34 was also able to growth in presence of 2-butanone as sole carbon source. Studies in *X. autotrophicus and A. aromaticum* also revealed that 2-butanone was the only alternative substrate of acetone carboxylase (19, 20). In *R. rhodochrous*, acetone carboxylase was found to utilize a wider range of substrates, including 2-butanone, which was consumed at rate to identical acetone, and also 2-pentanone, 3-pentanone and 2-hexanone, which were degraded at 70, 40 and 42 % the rate of acetone, respectively (7). NMR analyses revealed that carboxylation of 2-butanone by Acx of CH34 produces 3-keto-2-methylbutyrate (Fig. 4). Recently, Schühle and Heider suggested that Acx of *A. aromaticum* carboxylates only methyl groups adjacent to carbonyl and proposed that butanone was transformed to 3-oxopentanoic acid. Nevertheless, no experimental characterization of the carboxylated product was realized from butanone by Acx of *A. aromaticum* (19).

We propose for *C. metallidurans*, that 3-keto-2-methylbutyrate obtained by carboxylation of butanone was then activated to coenzyme A (CoA) thioester and thiotically cleaved to Propionyl-CoA and Acetyl-CoA as observed in the leucine catabolism pathway.

In conclusion, *C.metallidurans* CH34 is able to degrade acetone and, besides acetone, only 2-butanone using an ATP dependent pathway including the Acx enzyme. The corresponding *acx* genes are located on the second chromosome or chromid

Figures

Figure 1. Synteny of the acetone carboxylase operon. Amino acid sequence comparisons were performed using the NCBI Blast2 tool on the Expasy Proteomic Server and the complete database Swissprot/TrEMBL without any restriction. Underlined bacteria names represent

143 those in which enzyme was already formally identified in the literature. **(A)** Kingdom of
144 Bacteria; **(B)** Phylum of Proteobacteria; **(C)** Class of ε-Proteobacteria.
145
146 **Figure 2.** Acetone carboxylase expression. SDS-PAGE of protein extracts (10 µg) from
147 CH34 grown in the presence of (1) 9 mM gluconate, (2) 25 mM acetone, (3) 25 mM
148 isopropanol and (4) 25 mM *n*-propanol. (5) Protein extract (10 µg) from *AcxR*$^-$ mutant
149 cultivated in presence of acetone 25mM. (6) Purified acetone carboxylase (5 µg).
150
151 **Figure 3.** Comparison of the acetone and acetophenone carboxylases from various species.
152 [1] (19) [2] (20, 21) [3] (21) [4] (7) [5] (11).
153 a : The products of the enzymatic reaction were not identified in *X. autotrophicus and* and *R.*
154 *capsulatus*. For *A. aromaticum*, the nature of the product was suggested by the authors but not
155 experimentally identified.
156 b : The product of the enzymatic reaction was not identified.
157 ND : Not Determined.
158
159 **Figure 4.** Determination by NMR analyses of acetone carboxylase reaction products with 2-
160 butanone as substrate. (A, B) Enzymatic reactions realized without substrates; (C, D, E, F)
161 Enzymatic reactions realized with 8 mM, 12 mM, 16 mM and 24 mM ATP respectively; (G)
162 Enzymatic reaction realized with 16 mM ATP in presence of 2 concentrated Acx.
163
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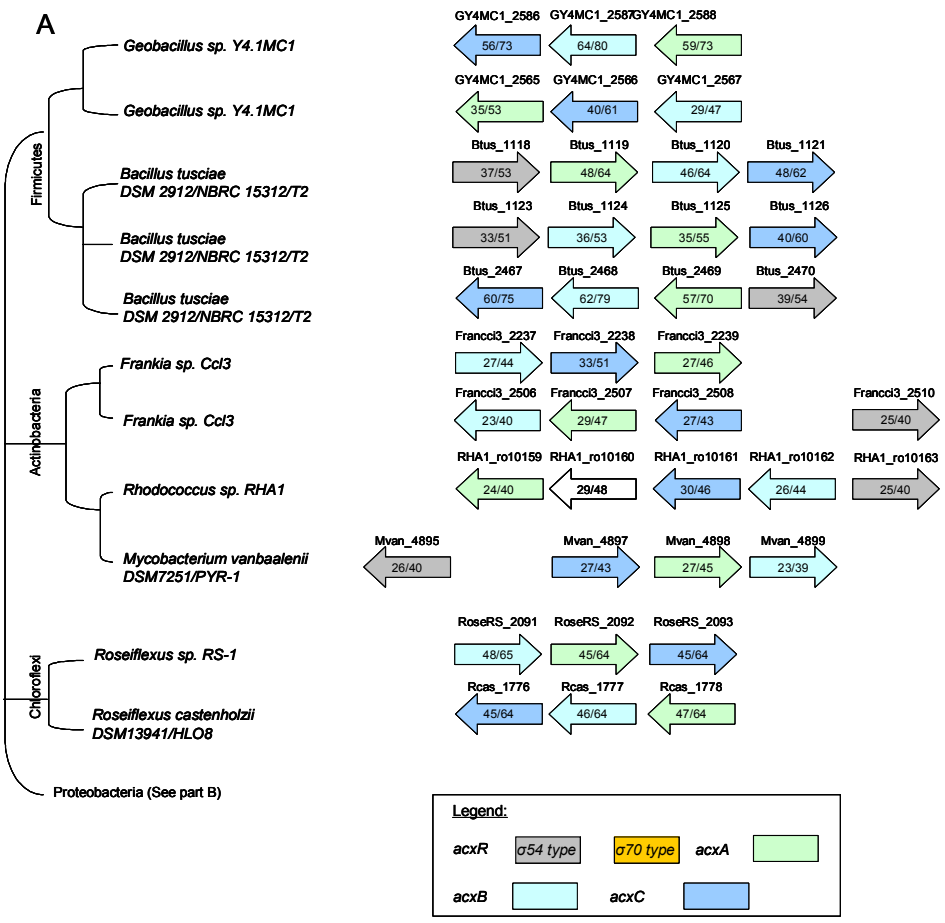
Figure 1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Geobacillus sp. Y4.1MC1
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Bacillus tusciae DSM 2912/NBRC 15312/T2
Firmicutes
Frankia sp. CcI3
Frankia sp. CcI3
Rhodococcus sp. RHA1
Mycobacterium vanbaalenii DSM7251/PYR-1
Actinobacteria
Roseiflexus sp. RS-1
Roseiflexus castenholzii DSM13941/HLO8
Chloroflexi
Proteobacteria (See part B)
GY4MC1_2586 56/73
GY4MC1_2587 64/80
GY4MC1_2588 59/73
GY4MC1_2565 35/53
GY4MC1_2566 40/61
GY4MC1_2567 29/47
Btus_1118 37/53
Btus_1119 48/64
Btus_1120 46/64
Btus_1121 48/62
Btus_1123 33/51
Btus_1124 36/53
Btus_1125 35/55
Btus_1126 40/60
Btus_2467 60/75
Btus_2468 62/79
Btus_2469 57/70
Btus_2470 39/54
Francci3_2237 27/44
Francci3_2238 33/51
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Francci3_2506 23/40
Francci3_2507 29/47
Francci3_2508 27/43
Francci3_2510 25/40
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RHA1_ro10163 25/40
Mvan_4895 26/40
Mvan_4897 27/43
Mvan_4898 27/45
Mvan_4899 23/39
RoseRS_2091 48/65
RoseRS_2092 45/64
RoseRS_2093 45/64
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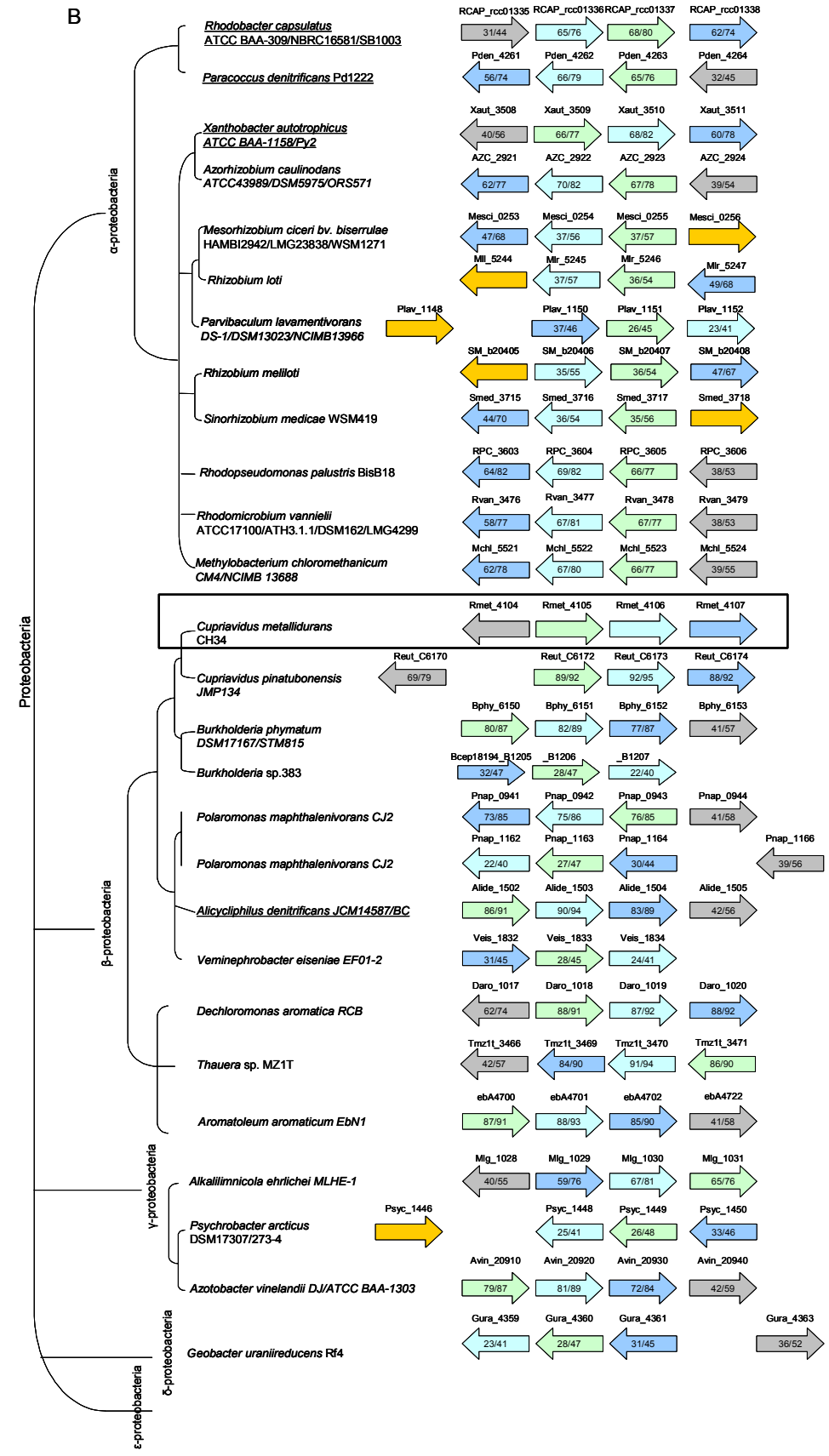
σ54 type
σ70 type
acxA
acxB
acxC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Proteobacteria
α-proteobacteria
β-proteobacteria
γ-proteobacteria
δ-proteobacteria
ε-proteobacteria
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Paracoccus denitrificans Pd1222
Xanthobacter autotrophicus ATCC BAA-1158/Py2
Azorhizobium caulinodans ATCC43989/DSM5975/ORS571
Mesorhizobium ciceri bv. biserrulae HAMBI2942/LMG23838/WSM1271
Rhizobium loti
Parvibaculum lavamentivorans DS-1/DSM13023/NCIMB13966
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Sinorhizobium medicae WSM419
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Methylobacterium chloromethanicum CM4/NCIMB 13688
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Burkholderia phymatum DSM17167/STM815
Burkholderia sp.383
Polaromonas maphthalenivorans CJ2
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Alicycliphilus denitrificans JCM14587/BC
Verninephrobacter eiseniae EF01-2
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Thauera sp. MZ1T
Aromatoleum aromaticum EbN1
Alkalilimnicola ehrlichei MLHE-1
Psychrobacter arcticus DSM17307/273-4
Azotobacter vinelandii DJ/ATCC BAA-1303
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Xaut_3508 Xaut_3509 Xaut_3510 Xaut_3511
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Mesci_0253 Mesci_0254 Mesci_0255 Mesci_0256
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62/78 67/80 66/77 39/55
Rmet_4104 Rmet_4105 Rmet_4106 Rmet_4107
Reut_C6170 Reut_C6172 Reut_C6173 Reut_C6174
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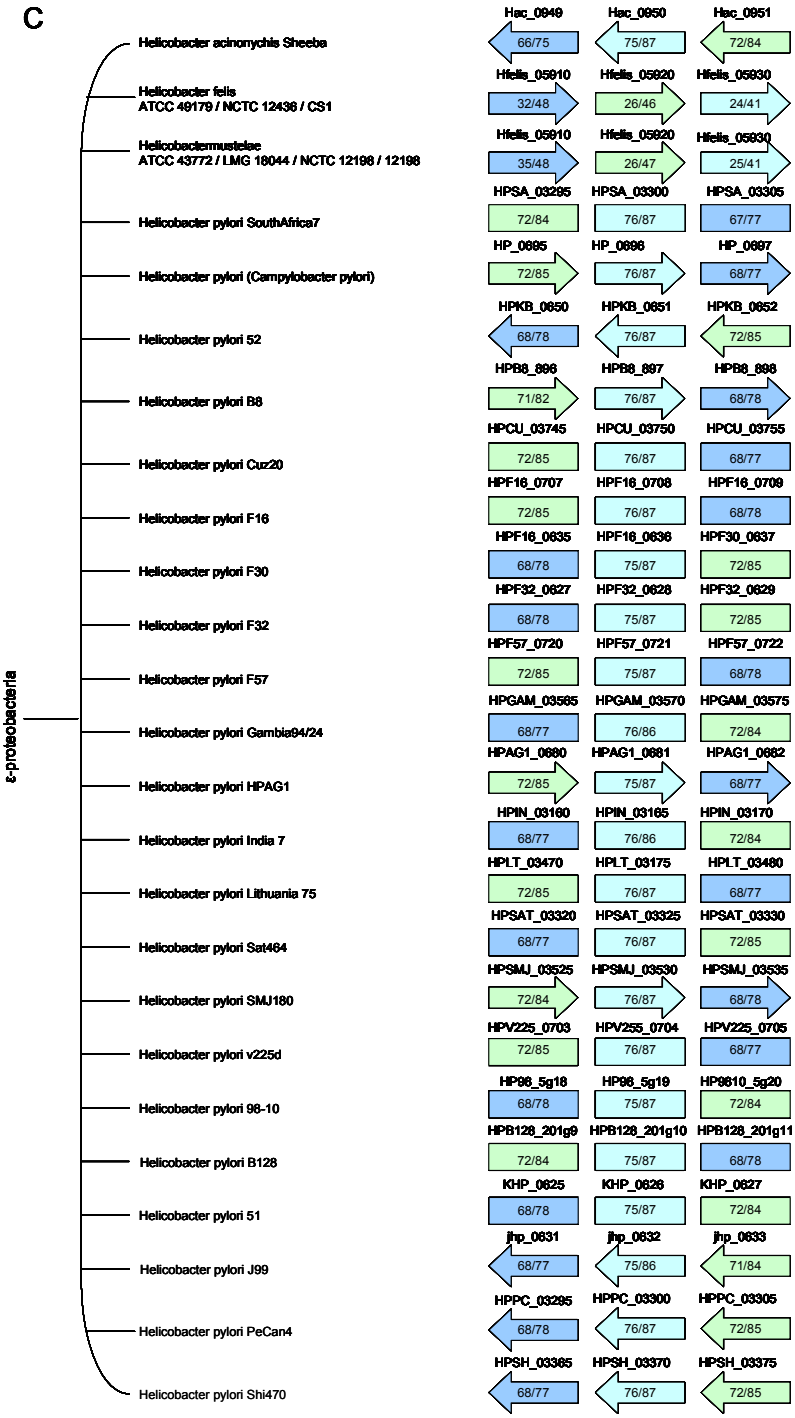
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Helicobacter pylori HPAG1
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HPPC_03295 68/78
HPPC_03300 76/87
HPPC_03305 72/85
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HPSH_03375 72/85

Figure 2

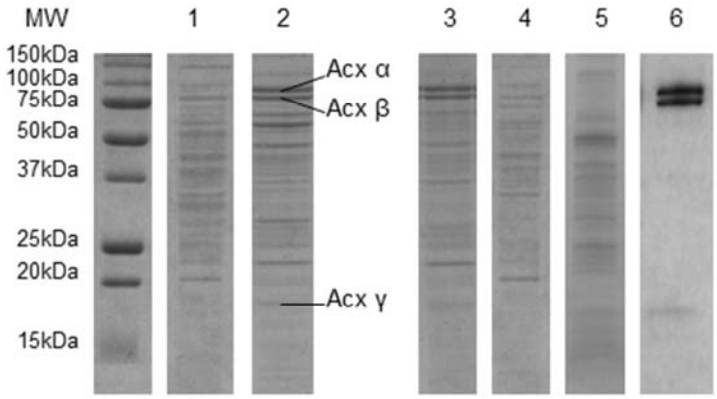
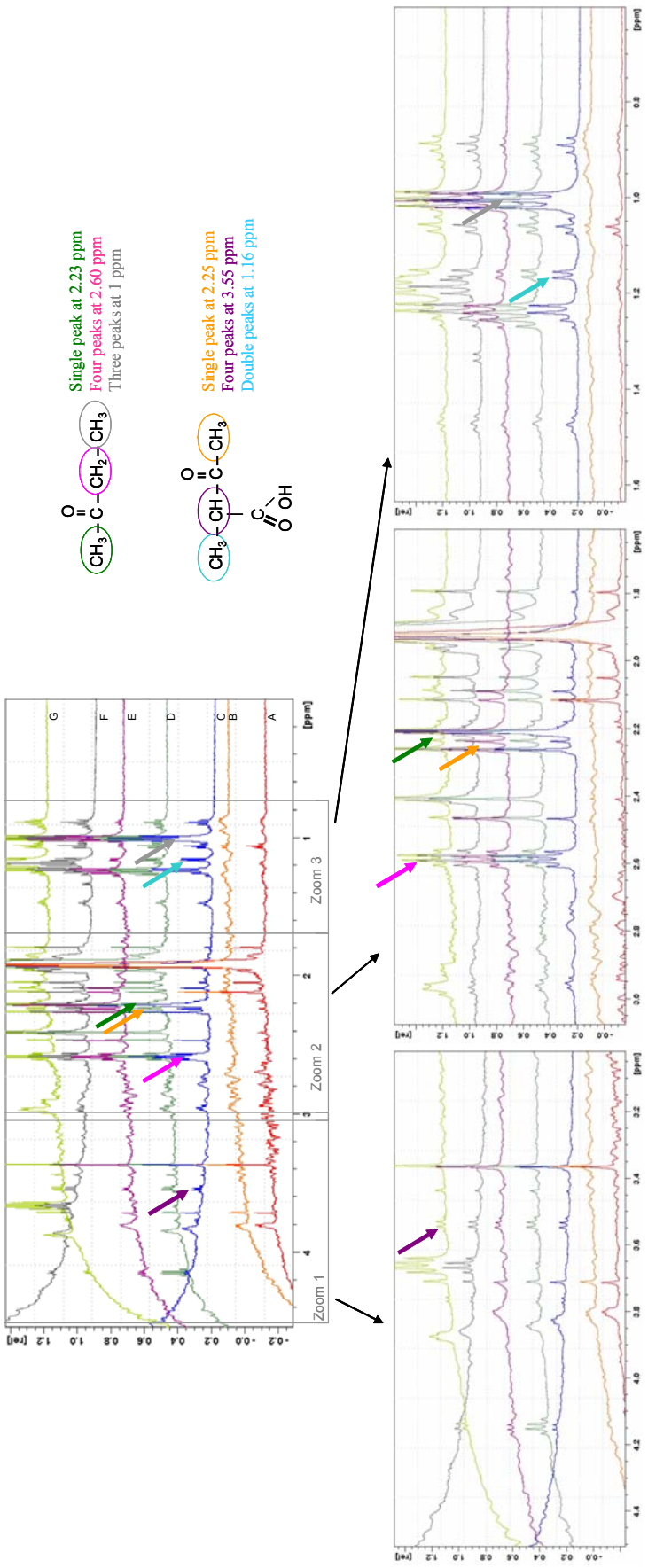
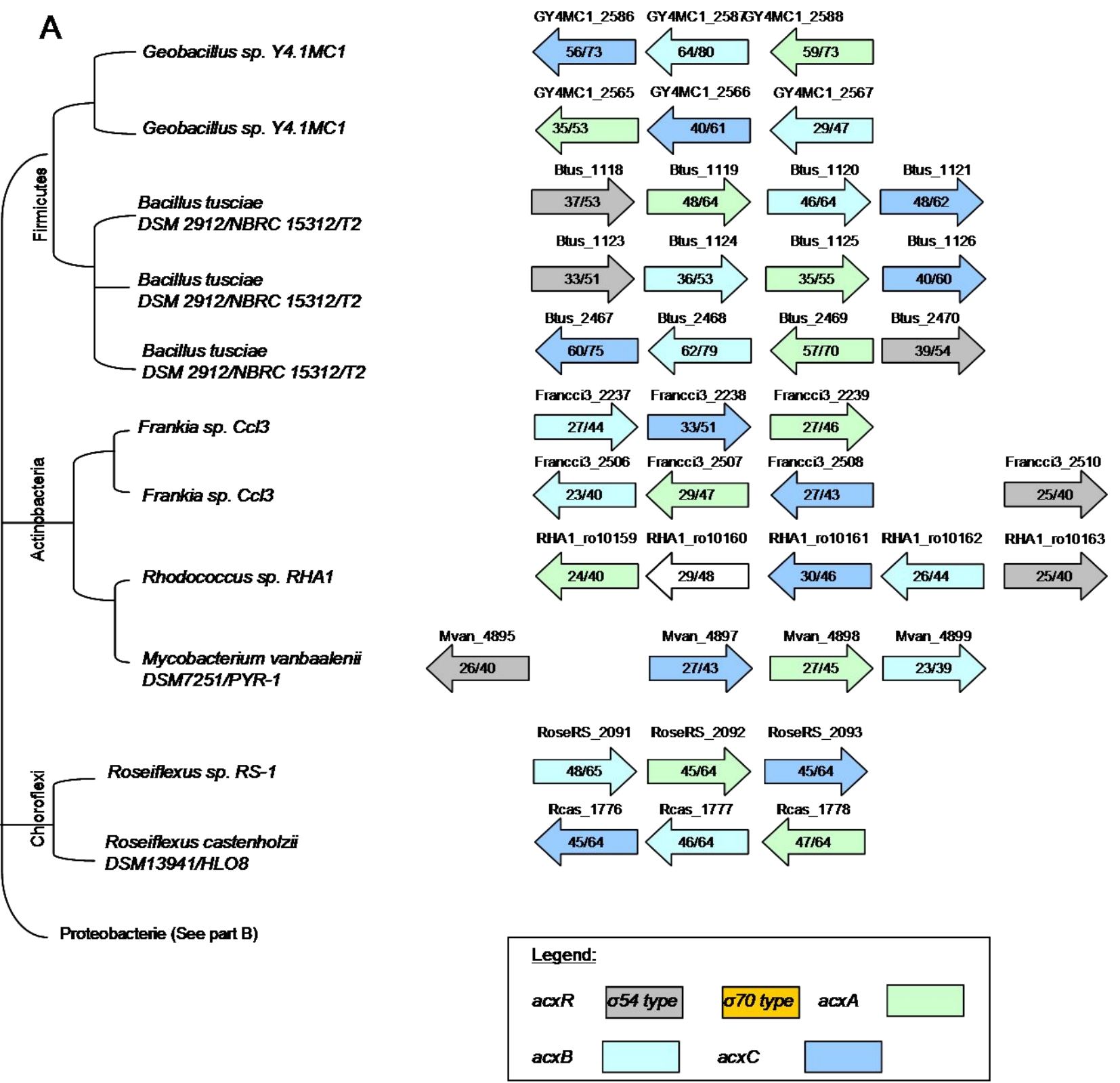


Figure 3

	Acetone carboxylase			Acetophenone carboxylase
	A. aromaticum [1] *X. autotrophicus* [2] *R. capsulatus* [3]	*C. metallidurans CH34*	*R. rhodochrous* [4]	*A. aromaticum* [5]
Subunit composition.	$\alpha_2\beta_2\gamma_2$ (85/75/20 kDa in *A. aromaticum*) (85/78/20 kDa in *X. autotrophicus*) (85/78/20 kDa in *R. capsulatus*)	$\alpha_2\beta_2\gamma_2$ (88/76/19 kDa)	$\alpha_2\beta_2\gamma_2$ (85/74/16 kDa)	$(\alpha\beta\beta'\gamma)_2 + \varepsilon_2$ (85/75/70/15 + 34 kDa)
Cofactors. ATP	++++	++++	-	++++
GTP	-	-	++++	-
ITP	-	-	++++	-
UTP	-	-	+++	-
CTP	-	ND	++	-
XTP	ND	ND	+	ND
Substrates:	acetone →acetoacetate 2 butanone → 2 ketovalerate [a]	acetone →acetoacetate 2 butanone →3 keto 2 methylbutyrate	acetone →acetoacetate 2 butanone [b] 2-pentanone [b] 3-pentanone [b] 2-hexanone [b]	acetophenone → benzoylacetate propiophenone [b] 4-acetyl pyridine [b]
No-substrates:	2-pentanone 3-pentanone 2-hexanone Chloroacetone	2-pentanone 3-pentanone 2-hexanone *n*-propanol isopropanol acetaldehyde dimethylsulfoxyde		ring substituted acetophenone derivatives aliphatic ketones

Figure 4


Single peak at 2.23 ppm
Four peaks at 2.60 ppm
Three peaks at 1 ppm
Single peak at 2.25 ppm
Four peaks at 3.55 ppm
Double peaks at 1.16 ppm
Zoom 1
Zoom 2
Zoom 3
[ppm]
[rel]


A
Geobacillus sp. Y4.1MC1
Geobacillus sp. Y4.1MC1
Bacillus tusciae DSM 2912/NBRC 15312/T2
Bacillus tusciae DSM 2912/NBRC 15312/T2
Bacillus tusciae DSM 2912/NBRC 15312/T2
Firmicutes
Frankia sp. Ccl3
Frankia sp. Ccl3
Rhodococcus sp. RHA1
Mycobacterium vanbaalenii DSM7251/PYR-1
Actinobacteria
Roseiflexus sp. RS-1
Roseiflexus castenholzii DSM13941/HLO8
Chloroflexi
Proteobacterie (See part B)
GY4MC1_2586 56/73
GY4MC1_2587 64/80
GY4MC1_2588 59/73
GY4MC1_2565 35/53
GY4MC1_2566 40/61
GY4MC1_2567 29/47
Btus_1118 37/53
Btus_1119 48/64
Btus_1120 46/64
Btus_1121 48/62
Btus_1123 33/51
Btus_1124 36/53
Btus_1125 35/55
Btus_1126 40/60
Btus_2467 60/75
Btus_2468 62/79
Btus_2469 57/70
Btus_2470 39/54
Francci3_2237 27/44
Francci3_2238 33/51
Francci3_2239 27/46
Francci3_2506 23/40
Francci3_2507 29/47
Francci3_2508 27/43
Francci3_2510 25/40
RHA1_ro10159 24/40
RHA1_ro10160 29/48
RHA1_ro10161 30/46
RHA1_ro10162 26/44
RHA1_ro10163 25/40
Mvan_4895 26/40
Mvan_4897 27/43
Mvan_4898 27/45
Mvan_4899 23/39
RoseRS_2091 48/65
RoseRS_2092 45/64
RoseRS_2093 45/64
Rcas_1776 45/64
Rcas_1777 46/64
Rcas_1778 47/64
Legend:
acxR
σ54 type
σ70 type
acxA
acxB
acxC

B

Proteobacteria

α-proteobacteria

Rhodobacter capsulatus ATCC BAA-309/NBRC16581/SB1003

Paracoccus denitrificans Pd1222

Xanthobacter autotrophicus ATCC BAA-1158/Py2

Azorhizobium caulinodans ATCC43989/DSM5975/ORS571

Mesorhizobium ciceri bv. biserrulae HAMBI2942/LMG23838/WSM1271

Rhizobium loti

Parvibaculum lavamentivorans DS-1/DSM13023/NCIMB13966

Rhizobium meliloti

Sinorhizobium medicae WSM419

Rhodopseudomonas palustris BisB18

Rhodomicrobium vannielii ATCC17100/ATH3.1.1/DSM162/LMG4299

Methylobacterium chloromethanicum CM4/NCIMB 13688

Cupriavidus metallidurans CH34

Cupriavidus pinatubonensis JMP134

β-proteobacteria

Burkholderia phymatum DSM17167/STM815

Burkholderia sp.383

Polaromonas maphthalenivorans CJ2

Polaromonas maphthalenivorans CJ2

Alicycliphilus denitrificans JCM14587/BC

Verminephrobacter eiseniae EF01-2

Dechloromonas aromatica RCB

Thauera sp. MZ1T

Aromatoleum aromaticum EbN1

γ-proteobacteria

Alkalilimnicola ehrlichei MLHE-1

Psychrobacter arcticus DSM17307/273-4

Azotobacter vinelandii DJ/ATCC BAA-1303

δ-proteobacteria

Geobacter uraniireducens Rf4

ε-proteobacteria

RCAP_rcc01335 31/44 | RCAP_rcc01336 65/76 | RCAP_rcc01337 68/80 | RCAP_rcc01338 62/74

Pden_4261 56/74 | Pden_4262 66/79 | Pden_4263 65/76 | Pden_4264 32/45

Xaut_3508 40/56 | Xaut_3509 66/77 | Xaut_3510 68/82 | Xaut_3511 60/78

AZC_2921 62/77 | AZC_2922 70/82 | AZC_2923 67/78 | AZC_2924 39/54

Mesci_0253 47/68 | Mesci_0254 37/56 | Mesci_0255 37/57 | Mesci_0256

Mll_5244 | Mlr_5245 37/57 | Mlr_5246 36/54 | Mlr_5247 49/68

Plav_1148 | Plav_1150 37/46 | Plav_1151 26/45 | Plav_1152 23/41

SM_b20405 | SM_b20406 35/55 | SM_b20407 36/54 | SM_b20408 47/67

Smed_3715 44/70 | Smed_3716 36/54 | Smed_3717 35/56 | Smed_3718

RPC_3603 64/82 | RPC_3604 69/82 | RPC_3605 66/77 | RPC_3606 38/53

Rvan_3476 58/77 | Rvan_3477 67/81 | Rvan_3478 67/77 | Rvan_3479 38/53

Mchl_5521 62/78 | Mchl_5522 67/80 | Mchl_5523 66/77 | Mchl_5524 39/55

Rmet_4104 | Rmet_4105 | Rmet_4106 | Rmet_4107

Reut_C6170 69/79 | Reut_C6172 89/92 | Reut_C6173 92/95 | Reut_C6174 88/92

Bphy_6150 80/87 | Bphy_6151 82/89 | Bphy_6152 77/87 | Bphy_6153 41/57

Bcep18194_B1205 32/47 | _B1206 28/47 | _B1207 22/40

Pnap_0941 73/85 | Pnap_0942 75/86 | Pnap_0943 76/85 | Pnap_0944 41/58

Pnap_1162 22/40 | Pnap_1163 27/47 | Pnap_1164 30/44 | Pnap_1166 39/56

Alide_1502 86/91 | Alide_1503 90/94 | Alide_1504 83/89 | Alide_1505 42/56

Veis_1832 31/45 | Veis_1833 28/45 | Veis_1834 24/41

Daro_1017 62/74 | Daro_1018 88/91 | Daro_1019 87/92 | Daro_1020 88/92

Tmz1t_3466 42/57 | Tmz1t_3469 84/90 | Tmz1t_3470 91/94 | Tmz1t_3471 86/90

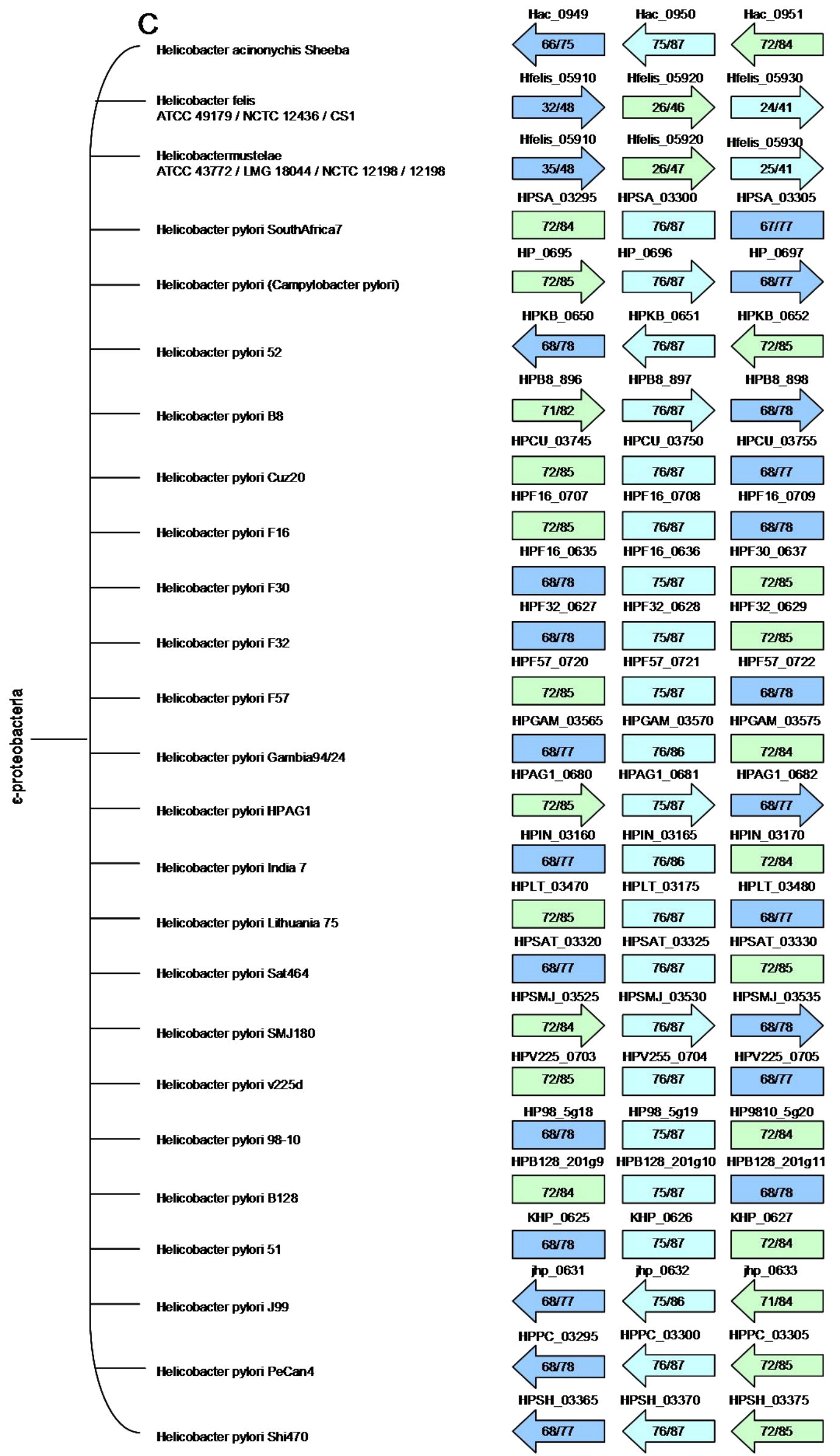
ebA4700 87/91 | ebA4701 88/93 | ebA4702 85/90 | ebA4722 41/58

Mlg_1028 40/55 | Mlg_1029 59/76 | Mlg_1030 67/81 | Mlg_1031 65/76

Psyc_1446 | Psyc_1448 25/41 | Psyc_1449 26/48 | Psyc_1450 33/46

Avin_20910 79/87 | Avin_20920 81/89 | Avin_20930 72/84 | Avin_20940 42/59

Gura_4359 23/41 | Gura_4360 28/47 | Gura_4361 31/45 | Gura_4363 36/52


C
ε-proteobacteria
Helicobacter acinonychis Sheeba
Helicobacter felis
ATCC 49179 / NCTC 12436 / CS1
Helicobactermustelae
ATCC 43772 / LMG 18044 / NCTC 12198 / 12198
Helicobacter pylori SouthAfrica7
Helicobacter pylori (Campylobacter pylori)
Helicobacter pylori 52
Helicobacter pylori B8
Helicobacter pylori Cuz20
Helicobacter pylori F16
Helicobacter pylori F30
Helicobacter pylori F32
Helicobacter pylori F57
Helicobacter pylori Gambia94/24
Helicobacter pylori HPAG1
Helicobacter pylori India 7
Helicobacter pylori Lithuania 75
Helicobacter pylori Sat464
Helicobacter pylori SMJ180
Helicobacter pylori v225d
Helicobacter pylori 98-10
Helicobacter pylori B128
Helicobacter pylori 51
Helicobacter pylori J99
Helicobacter pylori PeCan4
Helicobacter pylori Shi470
Hac_0949 66/75
Hac_0950 75/87
Hac_0951 72/84
Hfelis_05910 32/48
Hfelis_05920 26/46
Hfelis_05930 24/41
Hfelis_05910 35/48
Hfelis_05920 26/47
Hfelis_05930 25/41
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HPSA_03305 67/77
HP_0695 72/85
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HPKB_0651 76/87
HPKB_0652 72/85
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HPCU_03750 76/87
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HPF16_0635 68/78
HPF16_0636 75/87
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HPF32_0627 68/78
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HPF57_0722 68/78
HPGAM_03565 68/77
HPGAM_03570 76/86
HPGAM_03575 72/84
HPAG1_0680 72/85
HPAG1_0681 75/87
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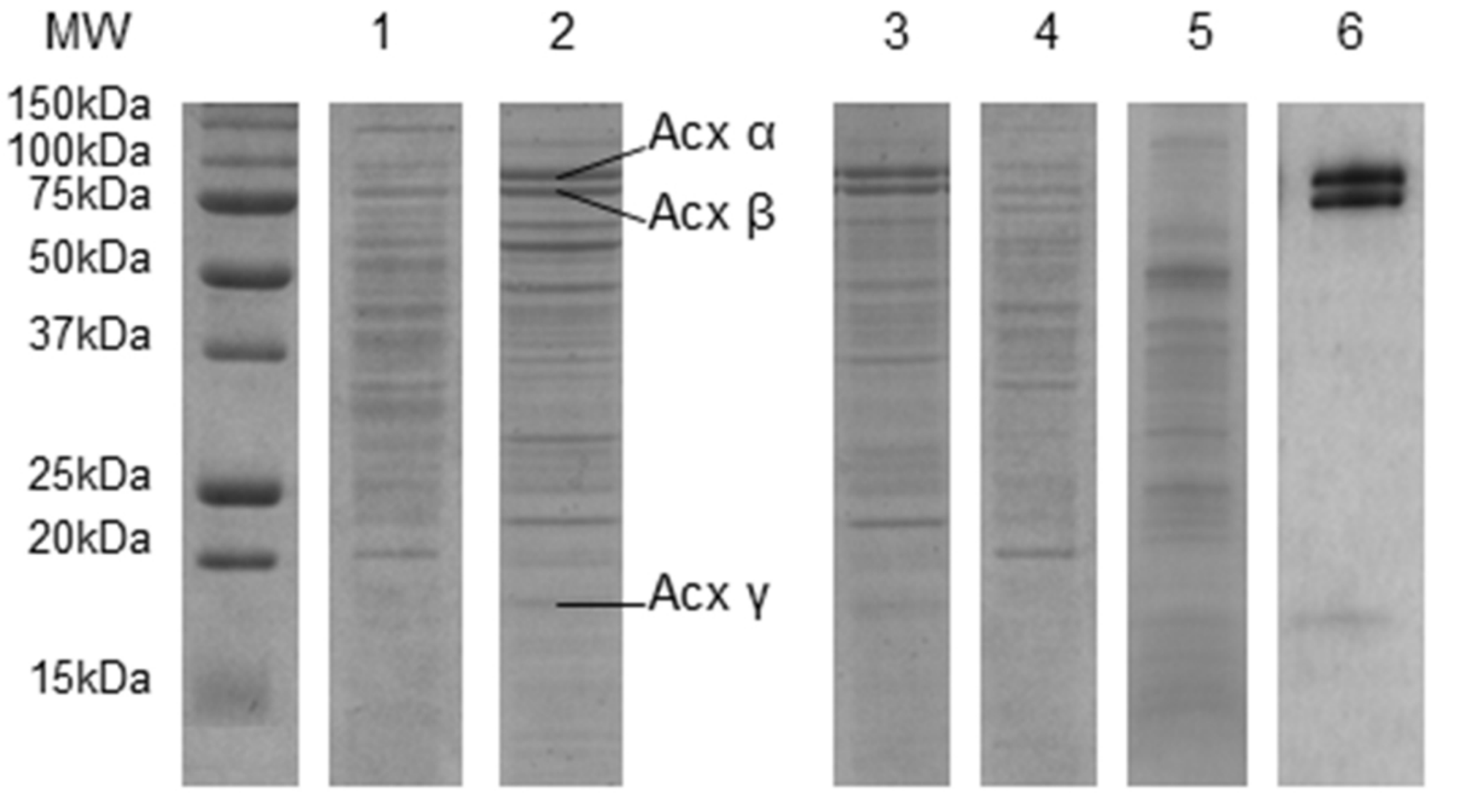
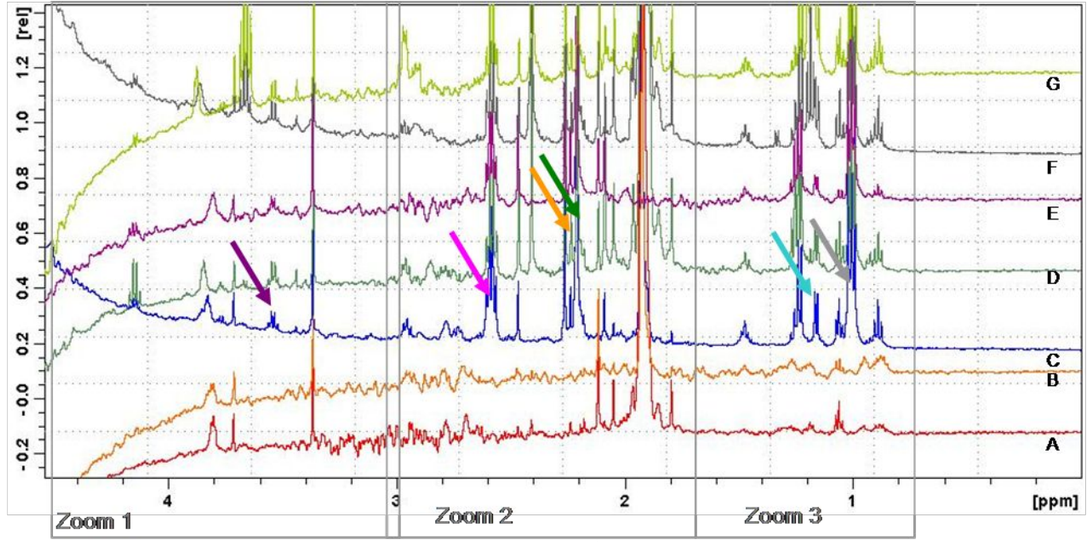
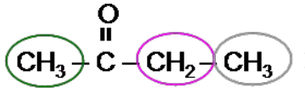
HPV225_0705 68/77
HP98_5g18 68/78
HP98_5g19 75/87
HP9810_5g20 72/84
HPB128_201g9 72/84
HPB128_201g10 75/87
HPB128_201g11 68/78
KHP_0625 68/78
KHP_0626 75/87
KHP_0627 72/84
jhp_0631 68/77
jhp_0632 75/86
jhp_0633 71/84
HPPC_03295 68/78
HPPC_03300 76/87
HPPC_03305 72/85
HPSH_03365 68/77
HPSH_03370 76/87
HPSH_03375 72/85

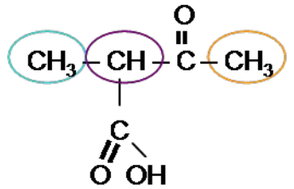

MW
1
2
3
4
5
6
150kDa
100kDa
75kDa
50kDa
37kDa
25kDa
20kDa
15kDa
Acx α
Acx β
Acx γ

	Acetone carboxylase			Acetophenone carboxylase
	A. aromaticum [1] *X. autotrophicus* [2] *R. capsulatus* [3]	*C. metallidurans CH34*	*R. rhodochrous* [4]	*A. aromaticum* [5]
Subunit composition:	$\alpha_2\beta_2\gamma_2$ (85/75/20 kDa in *A. aromaticum*) (85/78/20 kDa in *X. autotrophicus*) (85/78/20 kDa in *R. capsulatus*)	$\alpha_2\beta_2\gamma_2$ (86/76/19 kDa)	$\alpha_2\beta_2\gamma_2$ (85/74/16 kDa)	$(\alpha\beta\beta'\gamma)_2 + \varepsilon_2$ (85/75/70/15 + 34 kDa)
Cofactors: ATP	++++	++++	-	++++
GTP	-	-	++++	-
ITP	-	-	++++	-
UTP	-	-	+++	-
CTP	-	ND	++	-
XTP	ND	ND	+	ND
Substrates:	acetone → acetoacetate 2-butanone → 2-ketovalerate [a]	acetone → acetoacetate 2-butanone → 3-keto-2-methylbutyrate	acetone → acetoacetate 2-butanone [b] 2-pentanone [b] 3-pentanone [b] 2-hexanone [b]	acetophenone → benzoylacetate propiophenone [b] 4-acetyl pyridine [b]
No-substrates:	2-pentanone 3-pentanone 2-hexanone Chloroacetone	2-pentanone 3-pentanone 2-hexanone *n*-propanol isopropanol acetaldehyde dimethylsulfoxyde		ring substituted acetophenone derivatives aliphatic ketones





Single peack at 2.23 ppm
Four peaks at 2.60 ppm
Three peaks at 1 ppm



Single peak at 2.25 ppm
Four peaks at 3.55 ppm
Double peacks at 1.16 ppm