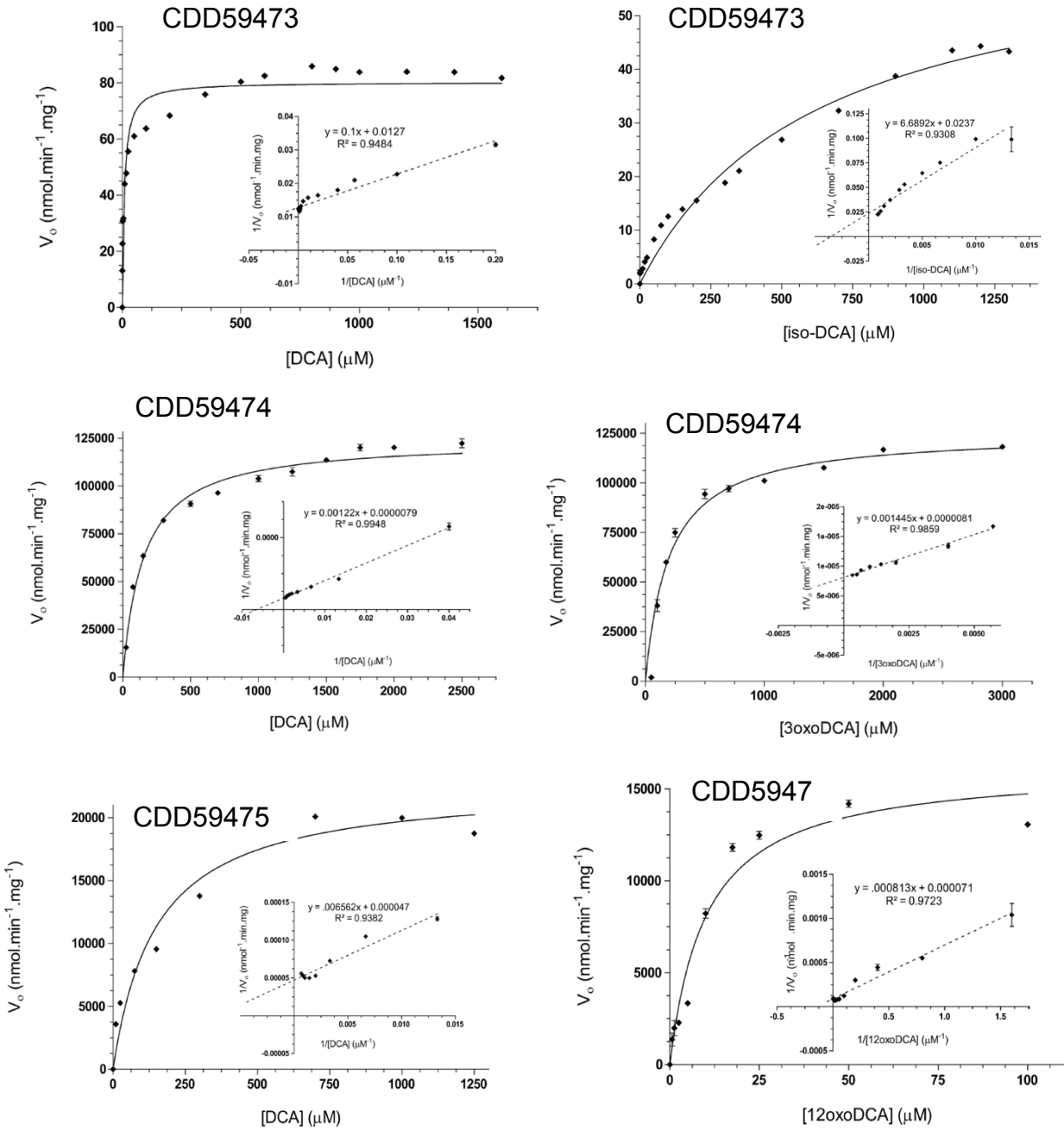


# Supplementary Material

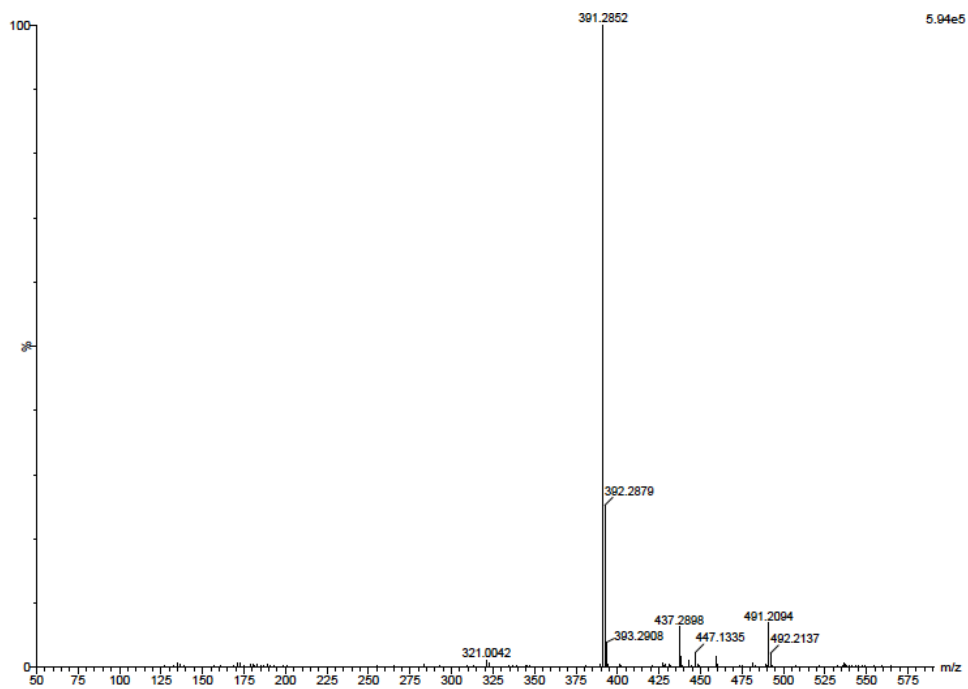
## Figure S1.



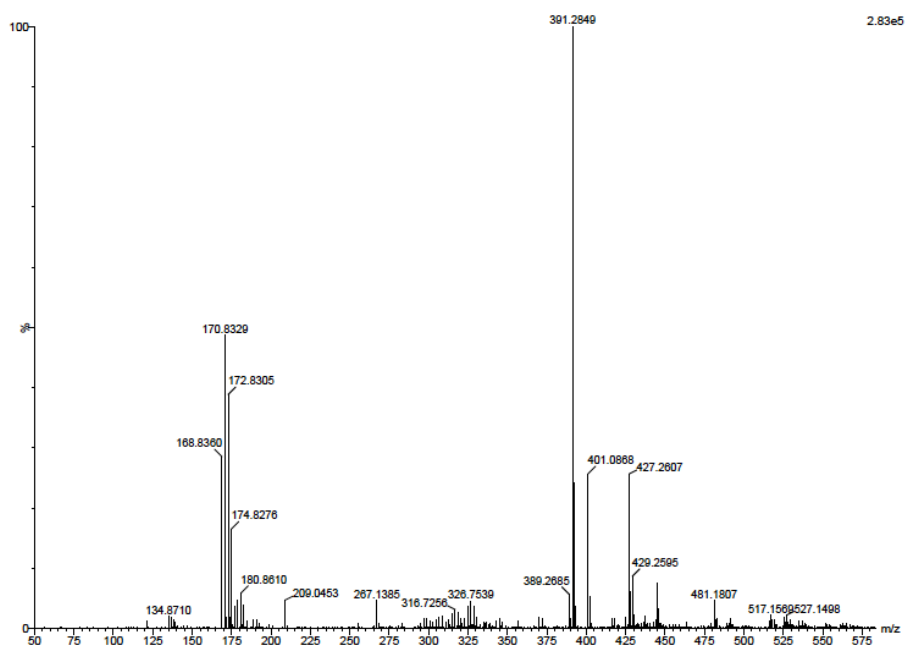
**Figure S1. Kinetic analysis of recombinant CDD59473, CDD59474, and CDD59475 from metagenomic sequence of *Eggerthella* CAG:298** Michaelis-Menten and Lineweaver-Burk plot of initial velocity with varying concentrations of substrate in the oxidative (left panel) and reductive (right panel) directions. Purified recombinant enzyme at a final concentration of 0.05 nM was added to 50  $\mu$ M DCA/12-oxoDCA and 150  $\mu$ M NAD<sup>+</sup>/NADH in 500  $\mu$ l buffer. The oxidation/reduction of NAD(H) was measured by continuous spectrophotometry at 340 nm for 5 min and the initial velocities were used calculated to calculate kinetic constants (Table 1). Each data-point represents  $\pm$  S.E.M from four independent experiments.

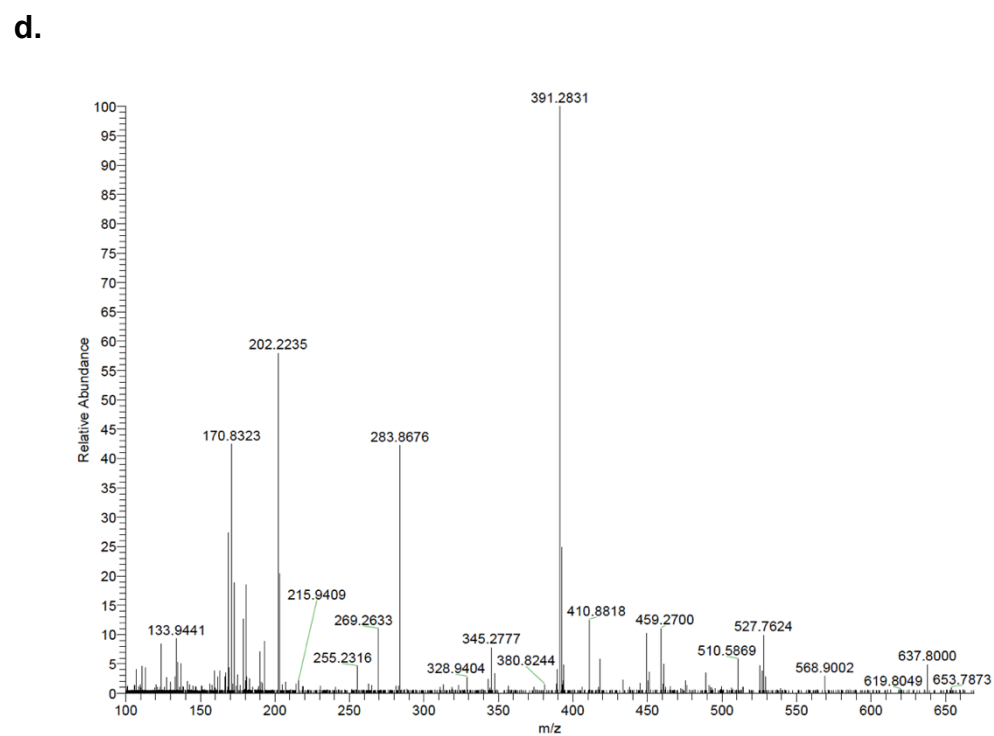
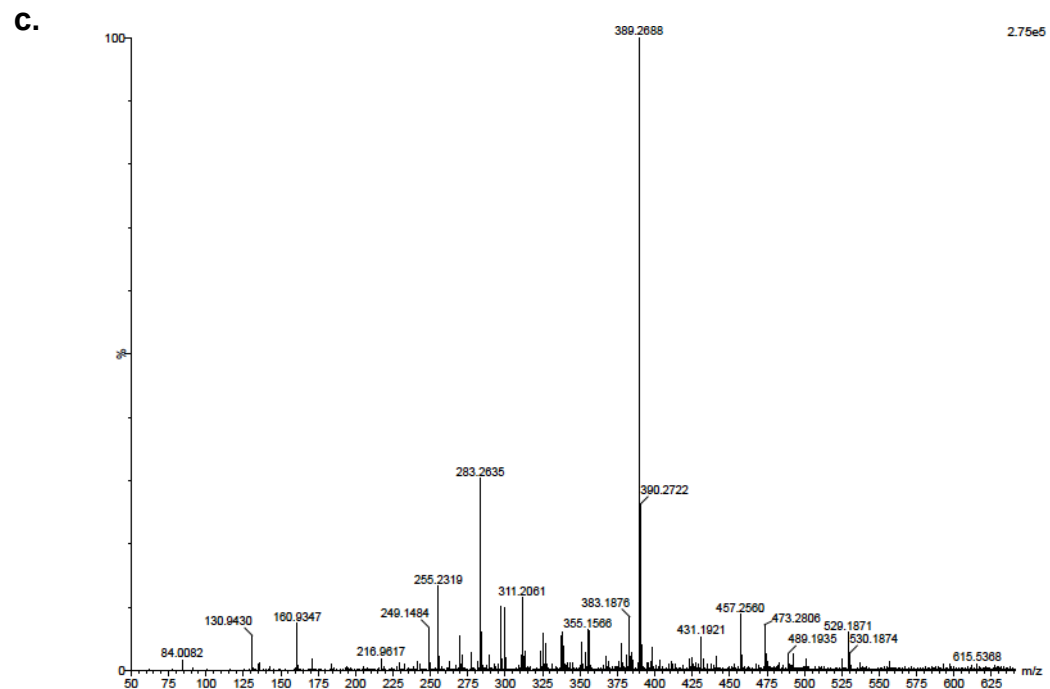
Figure S2.

a.



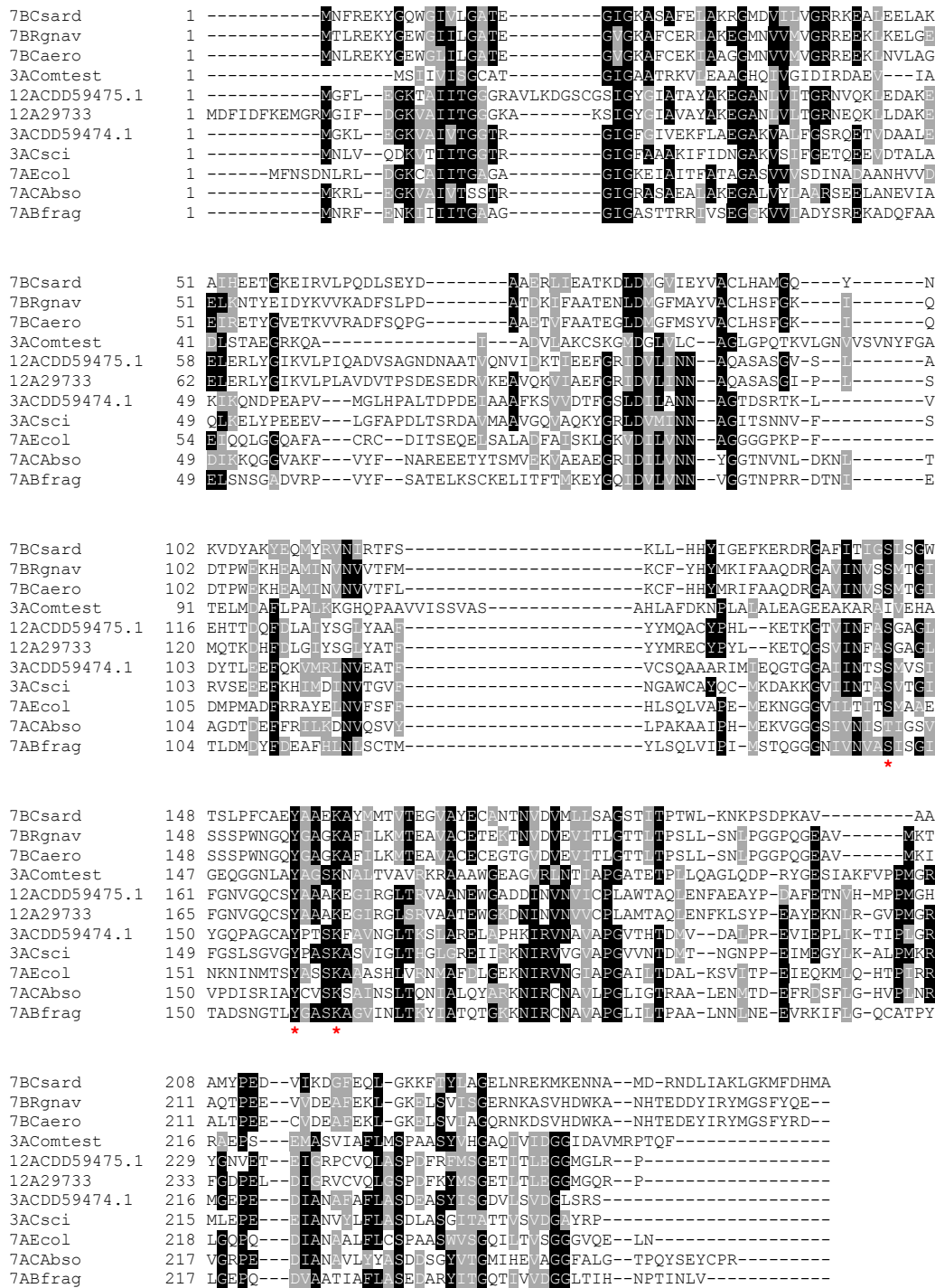
b.





**Figure S2. Mass spectrometry of enzymatic reaction products. a.) DCA standard, scraped from TLC plate after resolution in solvent system 75:20:2**

toluene:dioxane:glacial acetic acid (v/v/v). **b.**) MS analysis of reaction product co-migrating with iso-DCA, catalyzed by rCDD59473 in the presence of NADPH and 3-oxoDCA (see Fig. 2A) **c.**) MS analysis of reaction product co-migrating with 3-oxoDCA, catalyzed by rCDD59474 in the presence of NADP<sup>+</sup> and DCA (TLC not shown). **d.**) MS analysis of reaction product co-migrating with 12-oxoDCA, catalyzed by rCDD59475 in the presence of NADPH (see Fig. 2C).



**Figure S3. CLUSTAL alignment of biochemically characterized gut bacterial hydroxysteroid dehydrogenases. Circles represent conserved pyridine nucleotide**

binding sites; asterisks (\*) represent active-site residues conserved in SDR-family.

“7BCsard” AET80684.1 7-beta-hydroxysteroid dehydrogenase *Clostridium sardiniense*;

“7BRgnav” AGN52919.1 7-beta-hydroxysteroid dehydrogenase *Ruminococcus gnavus*;

“7BCaero” CZQ36308.1 unnamed protein product *Collinsella aerofaciens*; “3AComtest”

WP\_003078312.1 3-alpha-hydroxysteroid dehydrogenase/carbonyl reductase

*Comamonas testosteroni*; “12A29733” ERJ00208.1 oxidoreductase, short chain

dehydrogenase/reductase family protein *Clostridium* sp. ATCC 29733; “3ACSci”

AAC45414.1 3-alpha hydroxysteroid dehydrogenase *Clostridium scindens*; “7AEcoi”

>AKF67908.1 7-alpha-hydroxysteroid dehydrogenase, NAD-dependent *Escherichia coli*;

“7ABfrag” AAD49430.2 7-alpha-hydroxysteroid dehydrogenase *Bacteroides fragilis*;

“7ACADSO” 5EPO\_A Chain A, The Three-dimensional Structure Of *Clostridium*

*absonum* 7alpha-hydroxysteroid dehydrogenase.

**Table S1: Sequences used in phylogenetic analysis**

Phylogenetic tree identifier	Protein Locus Tag	Function	Organism	Ref
3A_CDD59473	CDD59473	3 $\alpha$ -HSDH	<i>Eggerthella</i> sp. CAG:298	TS
3A_CDD59474	CDD59474	3 $\alpha$ -HSDH	<i>Eggerthella</i> sp. CAG:298	TS
12A_CDD59475	CDD59475	12 $\alpha$ -HSDH	<i>Eggerthella</i> sp. CAG:298	TS
3A_Elen_0690	Elen_0690	3 $\alpha$ -HSDH	<i>Eggerthella lenta</i> DSM 2243	13
3B_Elen_1325	Elen_1325	3b-HSDH	<i>Eggerthella lenta</i> DSM 2243	13
3B_Elen_0198	Elen_0198	3b-HSDH	<i>Eggerthella lenta</i> DSM 2243	13
3A_Rumgna_02133	Rumgna_02133	3 $\alpha$ -HSDH	<i>Ruminococcus gnavus</i> ATCC 29149	13
3B_Rumgna_00694	3B_Rumgna_00694	3b-HSDH	<i>Ruminococcus gnavus</i> ATCC 29149	13
12A_COLAER_RS06615	COLAER_RS06615	putative 12 $\alpha$ -HSDH	<i>Collinsella aerofaciens</i> ATCC 25986	30
12A_COPCOM_01375	COPCOM_01375	putative 12 $\alpha$ -HSDH	<i>Coproccoccus comes</i> ATCC 27758	30
7A_Ecoli	BAA01384	7 $\alpha$ -HSDH	<i>Escherichia coli</i>	53
3A_BAIA2Csi	BAIA2_CLOSV	3 $\alpha$ -HSDH	<i>Clostridium scindens</i> ATCC 35704	38, 39
3A_BAIAChyl	ACF20977	3 $\alpha$ -HSDH	<i>Clostridium hylemonae</i> DSM 15053	54
3A_BAIAChir	EEA86309	3 $\alpha$ -HSDH	<i>Clostridium hiranonis</i> DSM 13275	55
3A_RUMHYD_02185	RUMHYD_02185	putative 3 $\alpha$ -HSDH	<i>Blautia hydrogenotropica</i> DSM 10507	30
12A_Csp29733	ERJ00208.1	12 $\alpha$ -HSDH	<i>Clostridium</i> sp. strain ATCC 29733	15, 29



3A_DORFOR_00173	DORFOR_00173	putative 3 $\alpha$ -HSDH	<i>Dorea formicigenerans</i> ATCC 27755	30
3A_RUMTOR_01195	RUMTOR_01195	putative 3 $\alpha$ -HSDH	<i>Ruminococcus torques</i> ATCC 27756	30
3A_RUMOBE_03494	RUMOBE_03494	putative 3 $\alpha$ -HSDH	<i>Ruminococcus obeum</i> ATCC 29174	30
7A_NP_810824Beth	NP_810824	putative 7 $\alpha$ -HSDH	<i>Bacteroides thetaiotaomicron</i> VPI-5482	10, 30, 61
3A_EDT24590.1Cperf	EDT24590.1	putative 3 $\alpha$ -HSDH	<i>Clostridium perfringens</i> B str. ATCC 3626	27, 56
7A_AAA53556.1Csord	AAA53556.1	7 $\alpha$ -HSDH	<i>Clostridium sordellii</i> ATCC 9714	57
7A_CsciAAB61151.1	AAB61151.1	7 $\alpha$ -HSDH	<i>Clostridium scindens</i> VPI 12708	58
7A_CLOHIR_RS06190	CLOHIR_RS06190	putative 7 $\alpha$ -HSDH	<i>Clostridium hiranonis</i> DSM 13275	30
7A_BACOVA_01473	BACOVA_01473	putative 7 $\alpha$ -HSDH	<i>Bacteroides ovatus</i> ATCC 8483	30
7A_BF9343_RS16010	7A_BF9343_RS16010	putative 7 $\alpha$ -HSDH	<i>Bacteroides fragilis</i> NCTC 9343	30
7A_BACFIN_RS13120	BACFIN_RS13120	putative 7 $\alpha$ -HSDH	<i>Bacteroides fingoldii</i> DSM 17565	30
7A_ANACAC_03661	7A_ANACAC_03661	putative 7 $\alpha$ -HSDH	<i>Anaerostipes caccae</i> DSM 14662	30
7A_CD630_00650	CD630_00650	putative 7 $\alpha$ -HSDH	<i>Clostridium difficile</i> 630	30
7B_COLAER_RS09325	COLAER_RS09325	7b-HSDH	<i>Collinsella aerofaciens</i> ATCC 25986	59, 60
7B_RUMGNA_RS11340	RUMGNA_RS11340	7b-HSDH	<i>Ruminococcus gnavus</i> ATCC 29149	41
12A_CLOHYLEM_04236	12A_CLOHYLEM_04236	12 $\alpha$ -HSDH	<i>Clostridium hylemonae</i> DSM 15053	30, unpublished data

12A_CLOSCI_02455	CLOSCI_02455	putative 12 $\alpha$ -HSDH	<i>Clostridium scindens</i> ATCC 35704	30, unpublished data
12A_COLAER_01483	COLAER_01483	putative 12 $\alpha$ -HSDH	<i>Collinsella aerofaciens</i> ATCC 25986	30
20B_Bdes	WP_051643274.1	20b-HSDH	<i>Butyricoccus demolans</i> ATCC 43058	40
20B_Ccad1	WP_051196374.1	20b-HSDH	<i>Clostridium cadaveris</i> AGR2141	40
20B_Ccad2"	WP_027640050	20b-HSDH	<i>Clostridium cadaveris</i> AGR2141	40
20B_BScar	BAQ31198.1	20b-HSDH	<i>Bifidobacterium scardovii</i> DSM 13734	40
20B_Plym	WP_024111275.1	20b-HSDH	<i>Propionimicrobium</i> sp. BV2F7	40
WP_025022266.1	WP_025022266.1	aldo/keto reductase	<i>Lactobacillus hayakitensis</i>	TS
KRM19149.1	KRM19149.1	oxidoreductase	<i>Lactobacillus hayakitensis</i> DSM 18933	TS
KRM65245.1	KRM65245.1	oxidoreductase	<i>Lactobacillus agilis</i> DSM 20509	TS
WP_050611824.1	WP_050611824.1	aldo/keto reductase	<i>Lactobacillus agilis</i>	TS
WP_019206370.1	WP_019206370.1	aldo/keto reductase	<i>Lactobacillus ingluviei</i>	TS
KRL88436.1	KRL88436.1	organophosphate reductase	<i>Lactobacillus ingluviei</i> DSM 15946	TS
ASN60792.1	ASN60792.1	aldo/keto reductase	<i>Lactobacillus curvatus</i>	TS
WP_085844664.1	WP_085844664.1	aldo/keto reductase	<i>Lactobacillus curvatus</i>	TS
WP_054778020.1	WP_054778020.1	aldo/keto reductase	<i>Lactobacillus saniviri</i>	TS
BAN77682.1	BAN77682.1	conserved hypothetical protein	<i>Adlercreutzia equolifaciens</i> DSM 19450	TS
WP_041715350.1	WP_041715350.1	SDR family oxidoreductase	<i>Adlercreutzia equolifaciens</i>	TS
CDD77593.1	CDD77593.1	putative uncharacterized protein	<i>Cryptobacterium</i> sp. CAG:338	TS

CDD68244.1	CDD68244.1	putative uncharacterized protein	<i>Eggerthella</i> sp. CAG:368	TS
CDB33831.1	CDB33831.1	putative uncharacterized protein	<i>Eggerthella</i> sp. CAG:209	TS
CCY06514.1	CCY06514.1	putative uncharacterized protein	<i>Eggerthella</i> sp. CAG:1427	TS
CDD56334.1	CDD56334.1	putative uncharacterized protein	<i>Bacteroides pectinophilus</i> CAG:437	TS
WP_044925038.1	WP_044925038.1	short-chain dehydrogenase	<i>Anaerostipes hadrus</i>	TS
WP_008116638.1	WP_008116638.1	NAD(P)-dependent oxidoreductase	<i>Bacteroides pectinophilus</i>	TS
WP_077326212.1	WP_077326212.1	short-chain dehydrogenase	<i>Anaerostipes hadrus</i>	TS
OKZ9660.1	OKZ9660.1	short-chain dehydrogenase	Clostridiales bacterium Nov_37_41	TS
WP_055161728.1	WP_055161728.1	short-chain dehydrogenase	<i>Anaerostipes hadrus</i>	TS
WP_055258939.1	WP_055258939.1	short-chain dehydrogenase	<i>Anaerostipes hadrus</i>	TS
CCX88977.1	CCX88977.1	dehydrogenases with different specificities	<i>Clostridium</i> sp. CAG:590	TS
WP_009265642.1	WP_009265642.1	short-chain dehydrogenase	Lachnospiraceae bacterium 5_1_63FAA	TS

**TS = This study**