Supplementary materials for the article:

Hussain N. et al. Isolation, Molecular, and Metabolic Profiling of Benzene-Remediating Bacteria Inhabiting the Tannery Industry Soil.
2025, Vol. 74, No 1, 33–47

Table SI
Top BLAST sequence homology, % age homology, and accession numbers assigned to the present study of benzene-degrading bacteria.

#	Isolates (strains)	Top BLAST sequence homology	% Age homology	Accession No.
1	PUB1	*Paracoccus aestuarii*	100	OR272055
2	PUB2	*Bacillus tropicus*	100	OR272056
3	PUB3	*Bacillus albus*	100	OR272059
4	PUB4	*Bacillus subtilis*	100	OR272058
5	PUB6	*Bacillus cereus*	100	OR272060

Table SII
Morphological characteristics of benzene-degrading bacteria isolated in the present study.

Isolates	Color	Shape	Margins	Elevation	Texture
PUB1	Off white	Circular	Entire	Convex	Moist\creamy
PUB2	Off white	Circular	Entire	Raised	Creamy
PUB3	White	Round	Entire	Convex	Moist
PUB4	Off white	Round	Entire	Raised	Moist\creamy
PUB6	White	Round	Entire	Raised	Moist

Table SIII
RapID NF PLUS panel test codes and the results of isolated bacterial strains.

Isolates	URE	ADH	ODC	LDC	TET	LIP	KSF	SBL	GUR	ONPG	βXYL	βGLU	NAG	MAL	PRO	GGT	PYR	ADON	IND
PUB1	−	+	+	+	+	+	+	+	−	−	−	−	−	−	+	+	+	+	−
PUB2	−	−	+	+	−	+	+	+	+	−	−	−	−	−	+	+	+	+	−
PUB3	−	+	+	+	+	+	+	+	−	−	−	−	−	−	+	+	+	+	−
PUB4	−	−	−	−	+	+	+	+	−	−	−	−	−	−	−	−	+	+	−
PUB6	−	+	+	+	+	+	+	+	−	−	−	−	−	−	−	+	+	+	−

Table SIV

Zones of inhibition measured in present study isolates against different concentrations of antibiotics augmentin, amoxicillin, cephalexin and cefadroxil.

Isolates	Zones of inhibition (cm)											
	Augmentin			Amoxicillin			Cephalexin			Cefadroxil		
	5 µl	10 µl	15 µl	5 µl	10 µl	15 µl	5 µl	10 µl	15 µl	5 µl	10 µl	15 µl
PUB1	1.5	1.7	1.9	1.0	1.3	1.8	3.0	3.5	4.0	2.5	3.0	3.4
PUB2	2.0	2.5	2.9	1.5	2.0	2.4	1.9	2.4	2.9	1.0	1.9	2.4
PUB3	1.7	2.0	2.5	R	1.5	2.2	2.0	3.0	3.5	1.9	2.8	3.0
PUB4	R	R	R	R	R	1.8	3	3.4	4.0	1.0	3.0	3.4
PUB6	1.2	1.8	2.1	1.5	1.7	2.0	2.0	2.5	3.0	0.7	1.7	1.9

Table SV

Identification of benzene metabolic intermediates and products in present study bacteria identified on the basis of comparison of GC-MS spectra with spectra of compounds available in NIST Library.

#	Compounds identified	Molecular weight	Retention time (min)
1	Toluene	92.14	3.459
2	Benzene, chloro-	112.56	4.839
3	*p*-Xylene	106.16	5.293
4	*o*-Xylene	106.16	5.293
5	Cyclopentasiloxane, decamethyl	370.77	11.551
6	Cyclohexasiloxane, dodecamethyl	444.92	15.076
7	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl	577.2	15.076
8	3-Isopropoxy -1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	577.2	18.253
9	2-((5-nitrofuran-2-yl)methylene)hydrazine-1-carboxamide, 2TMS derivative	198.14	18.253
10	Trisiloxane, 1,1,1,5,5,5-hexamethyl-3,3-bis[(trimethylsilyl)oxy]-	384.8393	18.253
11	2,4-Dihydroxybenzoic acid, 3TMS derivative	370.6635	20.205
12	3,4-Dihydroxyphenylglycol, 4TMS derivative	458.8871	20.205
13	3,4-Dihydroxymandelic acid, 4TMS derivative	472.8706	20.205
14	2,5-Dihydroxybenzoic acid, 3TMS derivative	370.6635	21.109
15	Trimethylsilyl [2-(4-chlorophenyl)-4-phenyl-1,3-thiazol-5-yl]acetate	328.8	21.109
16	Cyclopentasiloxane, decamethyl	370.77	21.109
17	Cyclononasiloxane, octadecamethyl	667.4	23.265
18	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	577.2	23.265
19	Oxazepam, 2TMS derivative	286.71	23.580
20	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane	443.96	23.580

#	Compounds identified	Molecular weight	Retention time (min)
21	2-{[(2-Methylphenoxy)acetyl]amino}benzoic acid, 2TMS derivative		23.580
22	*n*-Hexadecanoic acid	256.4241	25.287
23	*n*-Hexadecanoic acid	256.42	25.420
24	Cyclononasiloxane, octadecamethyl	667.4	25.656
25	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	577.2	25.656
26	L-Ribulose, tetrakis(trimethylsilyl) ether, pentafluorobenzyloxime (isomer 1)		25.656
27	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane	443.96	25.789
28	Hexasiloxane, tetradecamethyl	458.9933	25.789
29	Trisiloxane, 1,1,1,5,5,5-hexamethyl-3,3-bis[(trimethylsilyl)oxy]-	384.8393	25.789
30	Cyclononasiloxane, octadecamethyl	667.4	27.774
31	Epinephrine-β-, 3TMS derivative		27.774
32	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	577.2	27.774
33	Octadecanoic acid	284.4772	28.079
34	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane	443.96	29.651
35	Cyclononasiloxane, octadecamethyl	667.4	29.651
36	Hexasiloxane, tetradecamethyl	458.9933	29.651
37	Cyclononasiloxane, octadecamethyl	667.4	31.363
38	Trisiloxane, 1,1,1,5,5,5-hexamethyl-3,3-bis[(trimethylsilyl)oxy]-	384.8393	31.363
39	Oxazepam, 2TMS derivative	286.71	31.363
40	Bis(2-ethylhexyl) phthalate	390.6	32.780
41	Cyclononasiloxane, octadecamethyl	667.4	32.973
42	*N*-Benzyl-*N*-ethyl-p-isopropylbenzamide	281.4	32.973
43	Oxazepam, 2TMS derivative	286.71	32.973
44	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane	443.96	34.476
45	Hexasiloxane, tetradecamethyl	458.9933	34.476
46	Mercaptoacetic acid, 2TMS derivative	92.11	34.476
47	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	390.5561	34.866
48	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	390.62	34.866
49	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl	577.2	35.882
51	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl	430.94	35.882
52	Formic acid, 1-(4,7-dihydro-2-methyl-7-oxopyrazolo[1,5-a]pyrimidin-5-yl)-, methyl ester	207.19	35.882
53	1,1,1,3,5,5,5-Heptamethyltrisiloxane	221.50	37.021
54	Methyltris(trimethylsiloxy)silane	310.68	37.021
55	6-Fluoroindole, TMS derivative	135.14	37.021

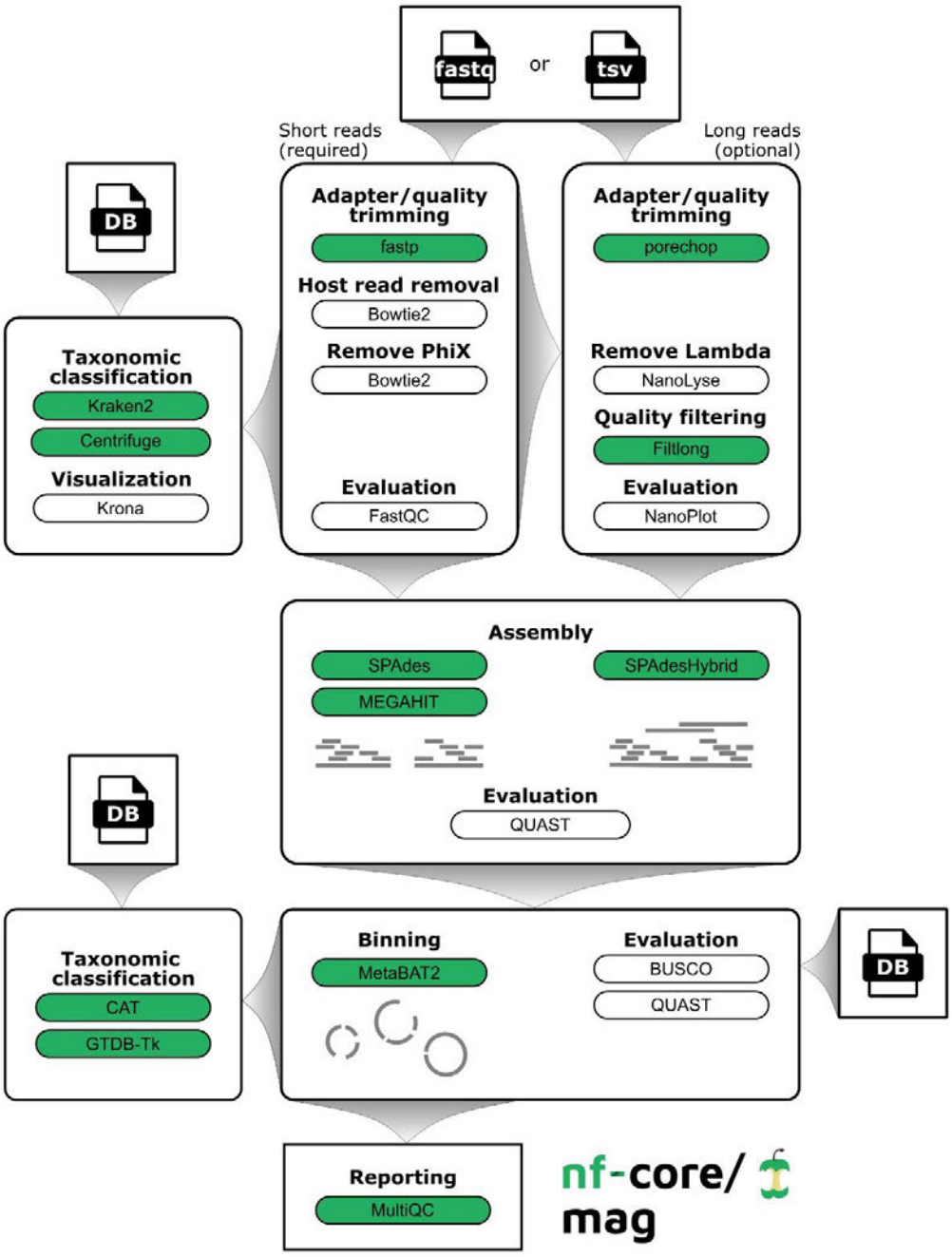


Fig. S1. Pipeline followed for identification of benzene metabolizing genes in bacterial consortium, comprising of present study benzene degrading isolates along with xylene and phenol metabolizing bacteria, subjected to whole-genome sequencing.

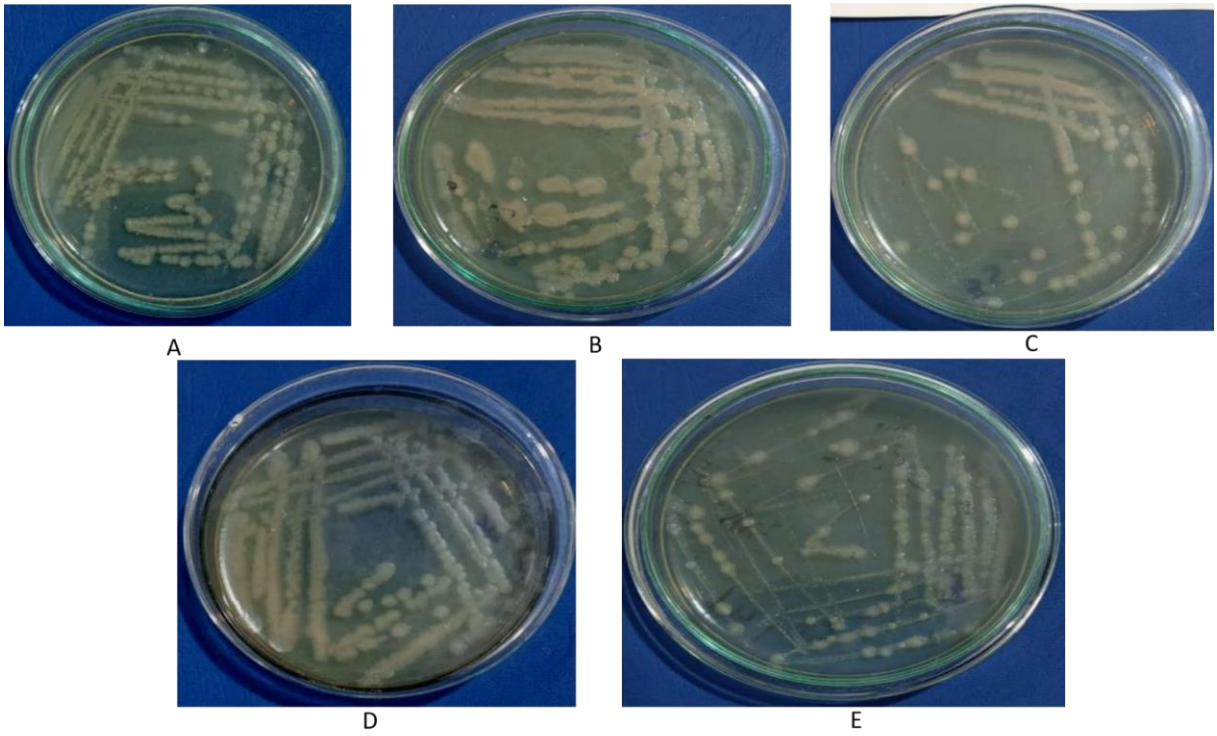
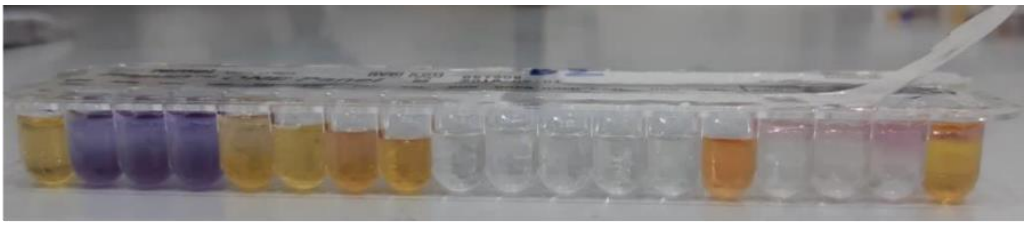
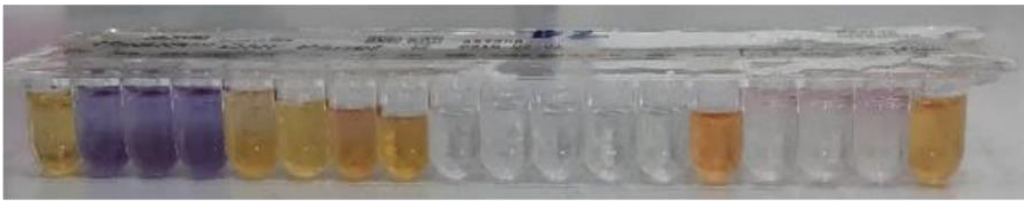


Fig S2. Streaked colonies of present study benzene degrading bacteria.
A) *Paracoccus aestuarii* PUB1, B) *Bacillus tropicus* PUB2, C) *Bacillus albus* PUB3, D) *Bacillus subtilis* PUB4, E) *Bacillus cereus* PUB6.



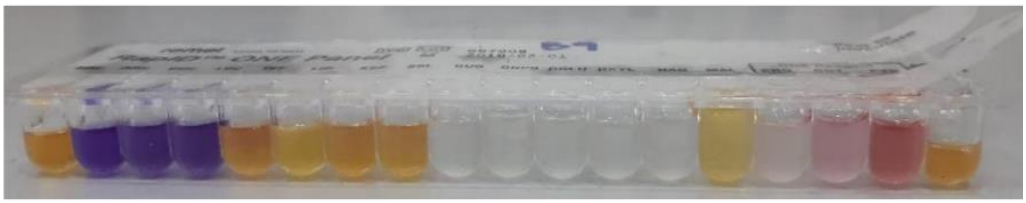
A

B



C

D



E

Fig. S3. Biochemical characterization of present study bacteria using RapID NF PLUS panel. A) *Paracoccus aestuarii* PUB1, B) *Bacillus tropicus* PUB2, C) *Bacillus albus* PUB3, D) *Bacillus subtilis* PUB4, E) *Bacillus cereus* PUB6.

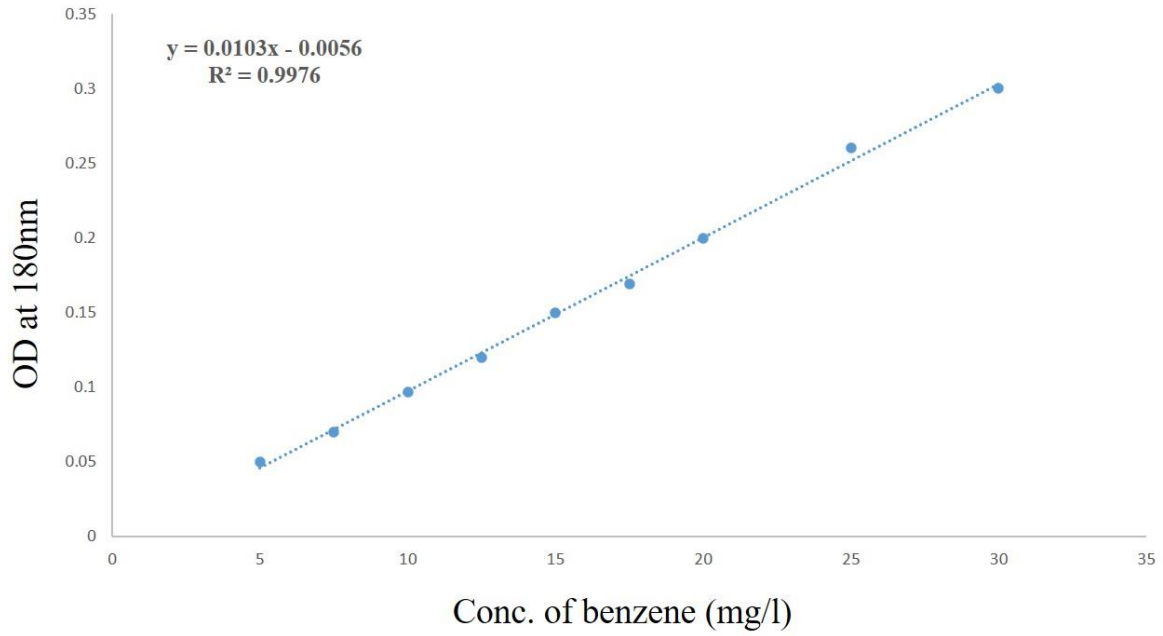


Fig. S4. Standard curve plotted to estimate the residual benzene in bacterial culture supernatant.

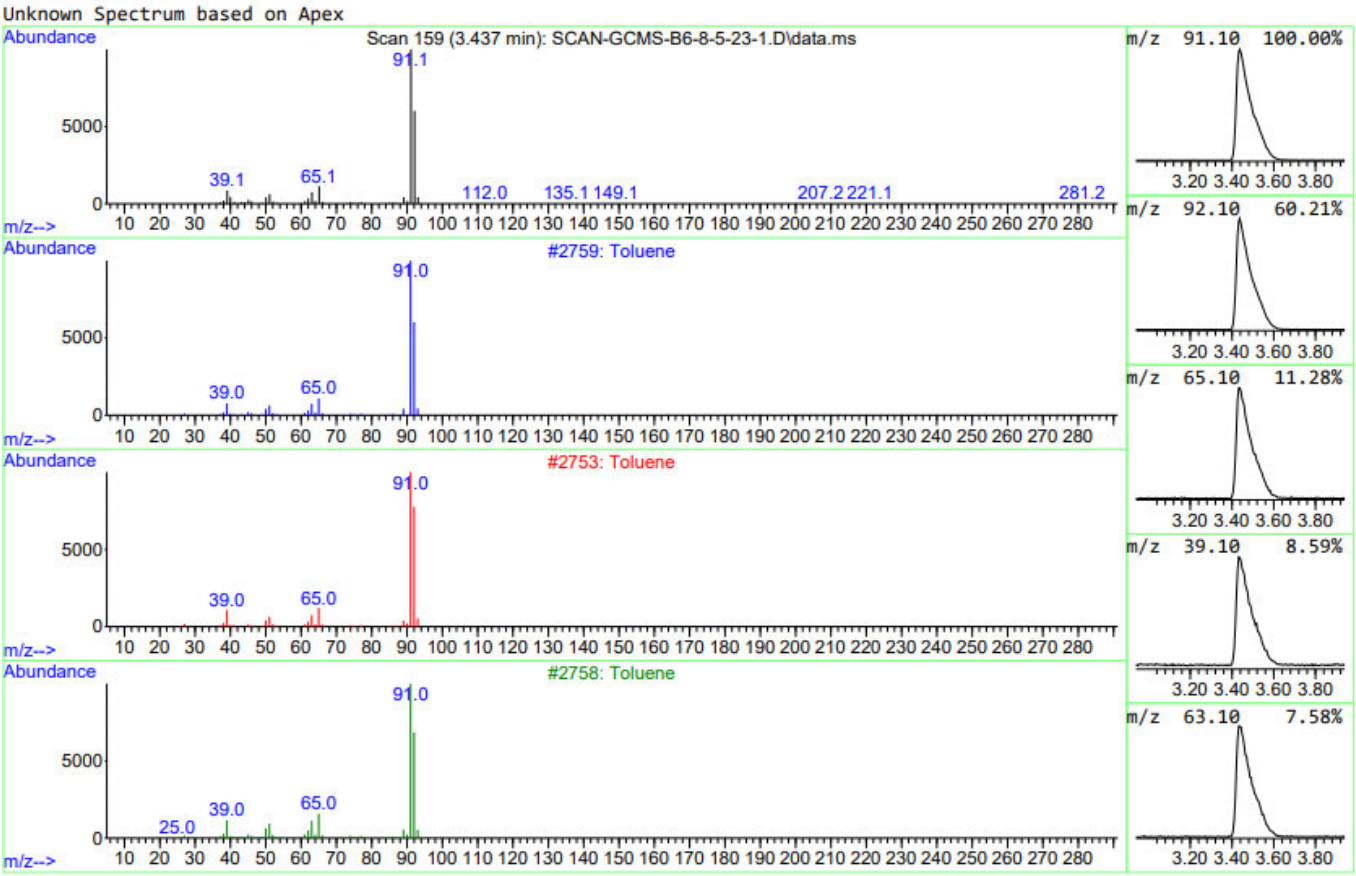


Fig S5a. GC-MS spectra of benzene degrading bacteria *Bacillus subtilis* PUB4.

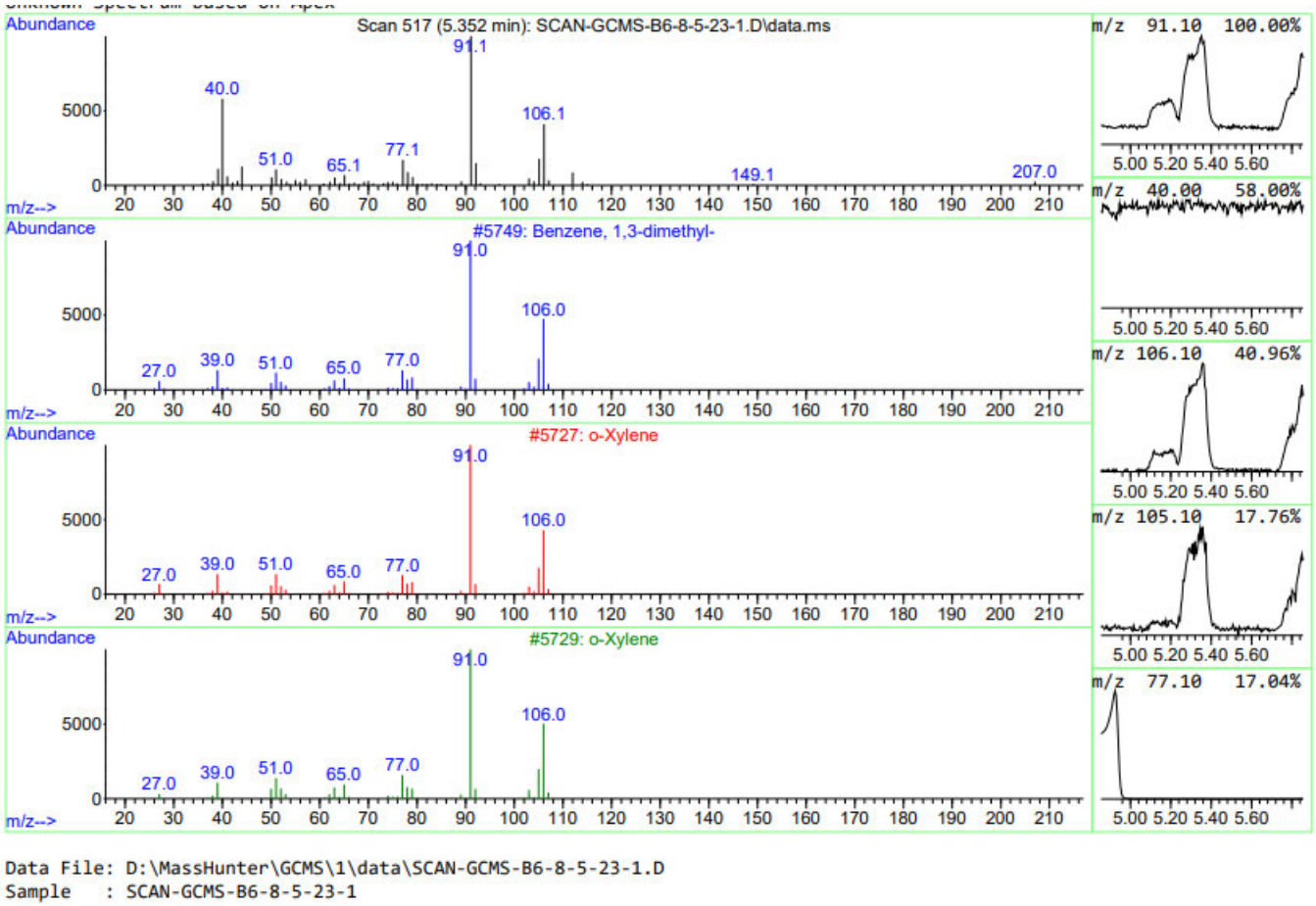


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Fig S5b. GC-MS spectra of benzene degrading bacteria *Bacillus subtilis* PUB4.

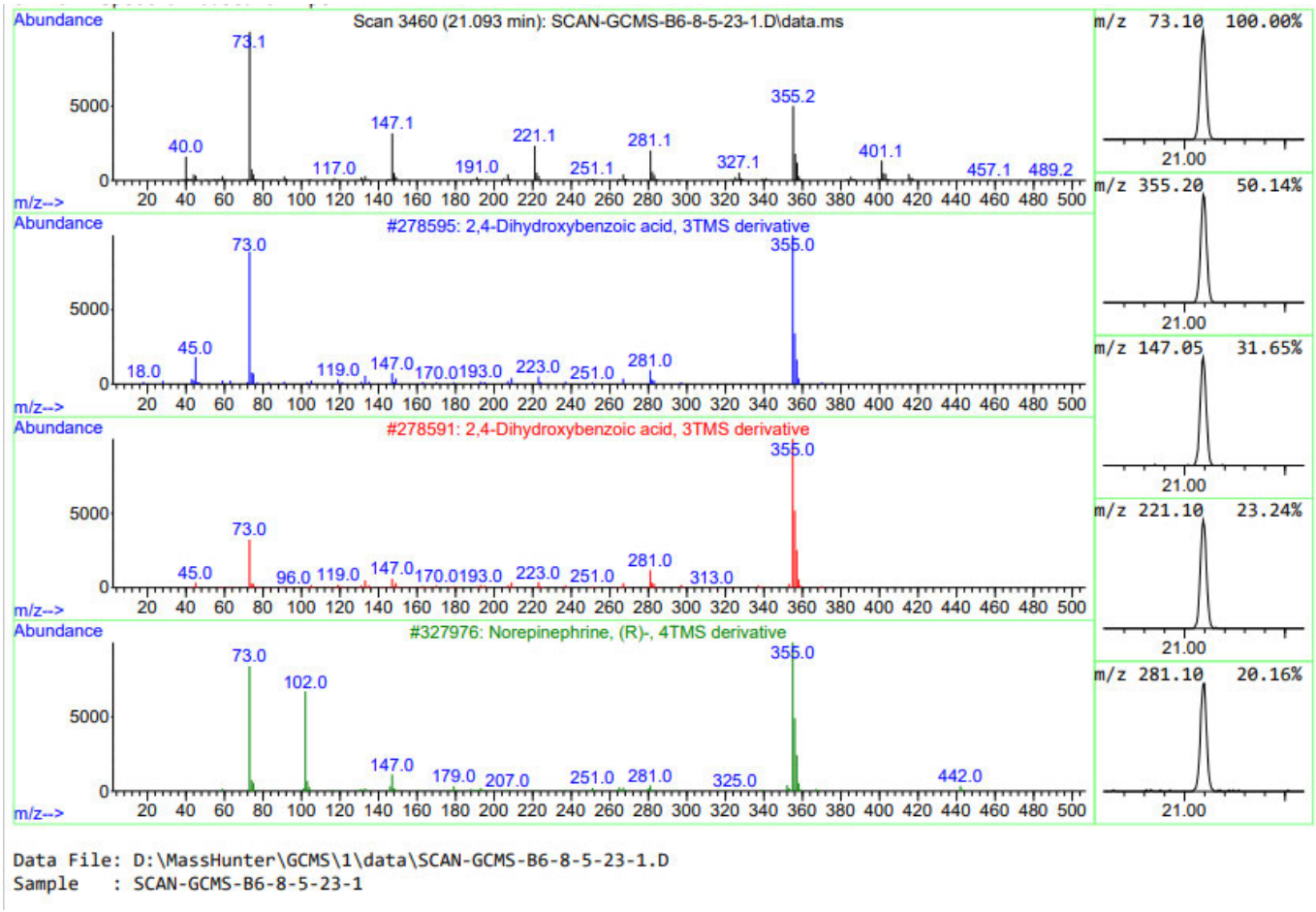

Fig S5c. GC-MS spectra of benzene degrading bacteria *Bacillus subtilis* PUB4.

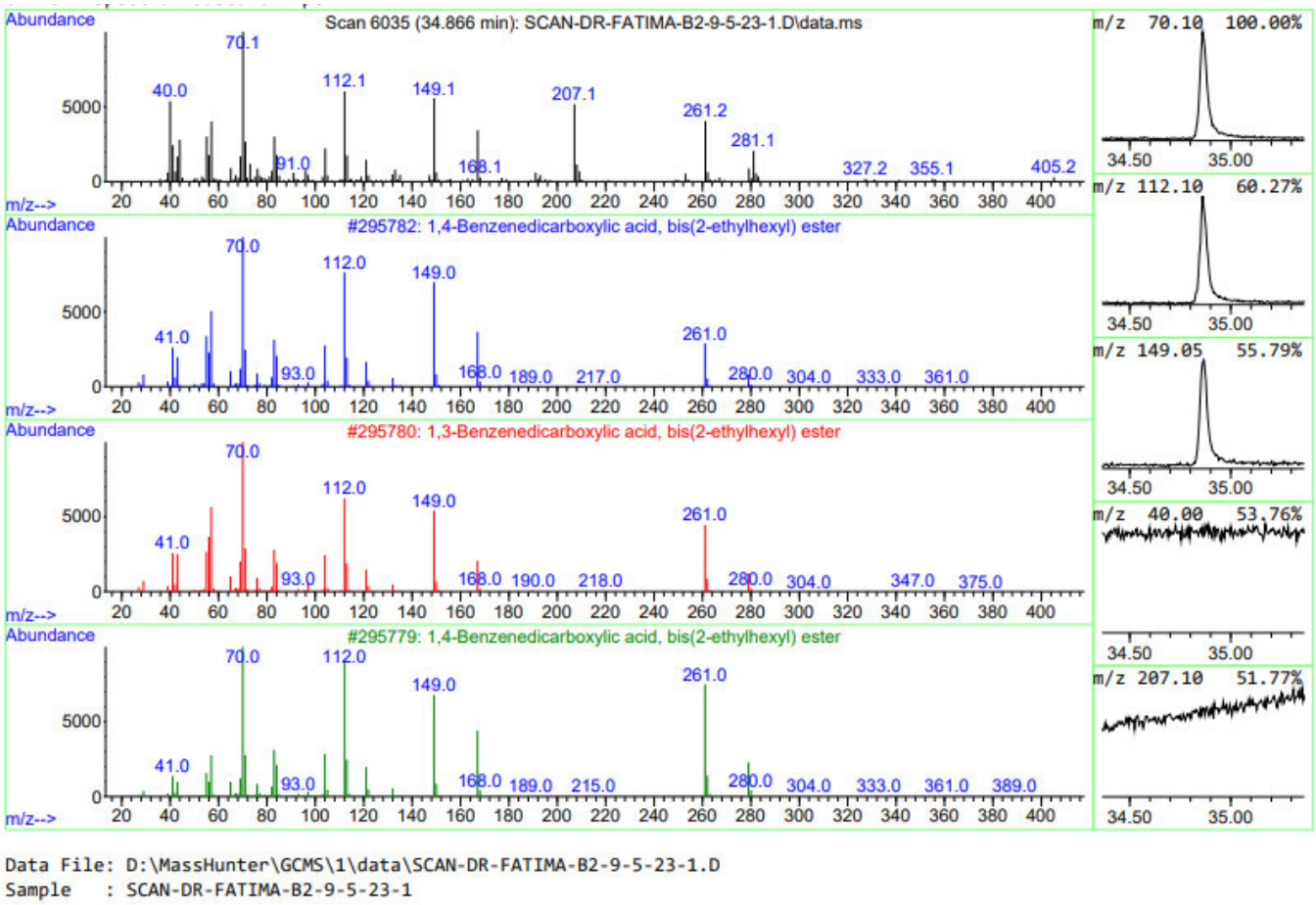

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Fig S5d. GC-MS spectra of benzene degrading bacteria *Paracoccus aestuarii* PUB1.

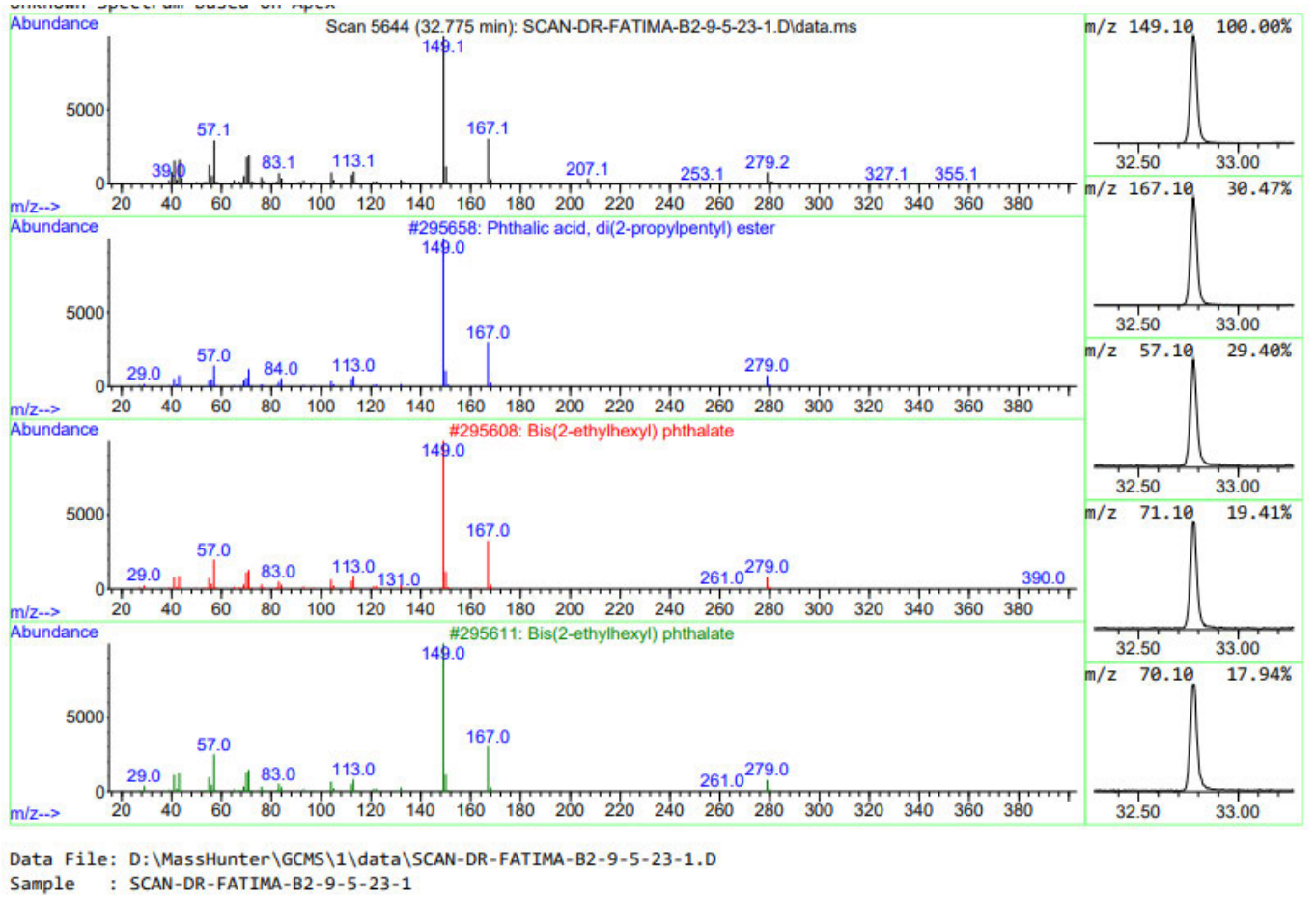

Fig S5e. GC-MS spectra of benzene degrading bacteria *Paracoccus aestuarii* PUB1.

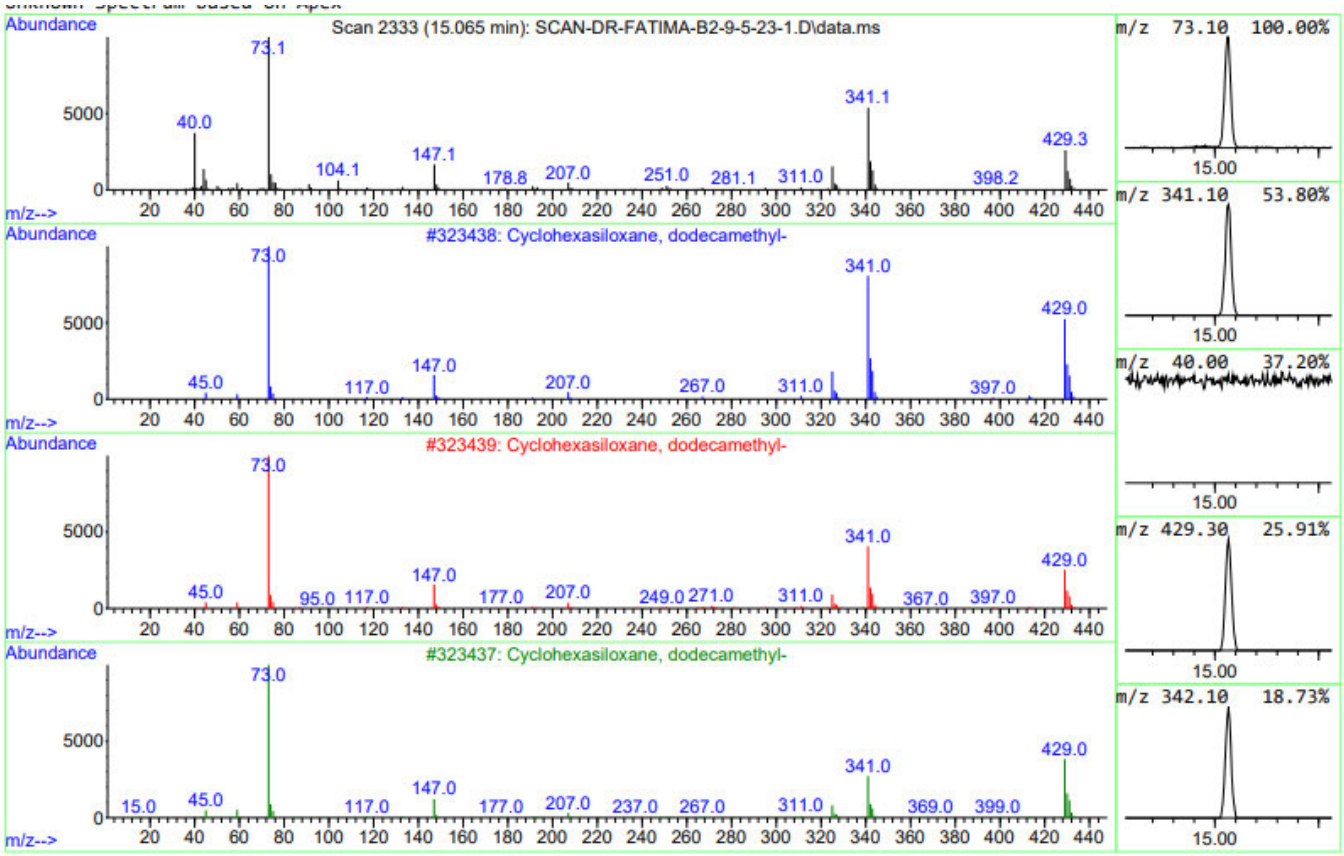

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Fig S5f. GC-MS spectra of benzene degrading bacteria *Paracoccus aestuarii* PUB1.

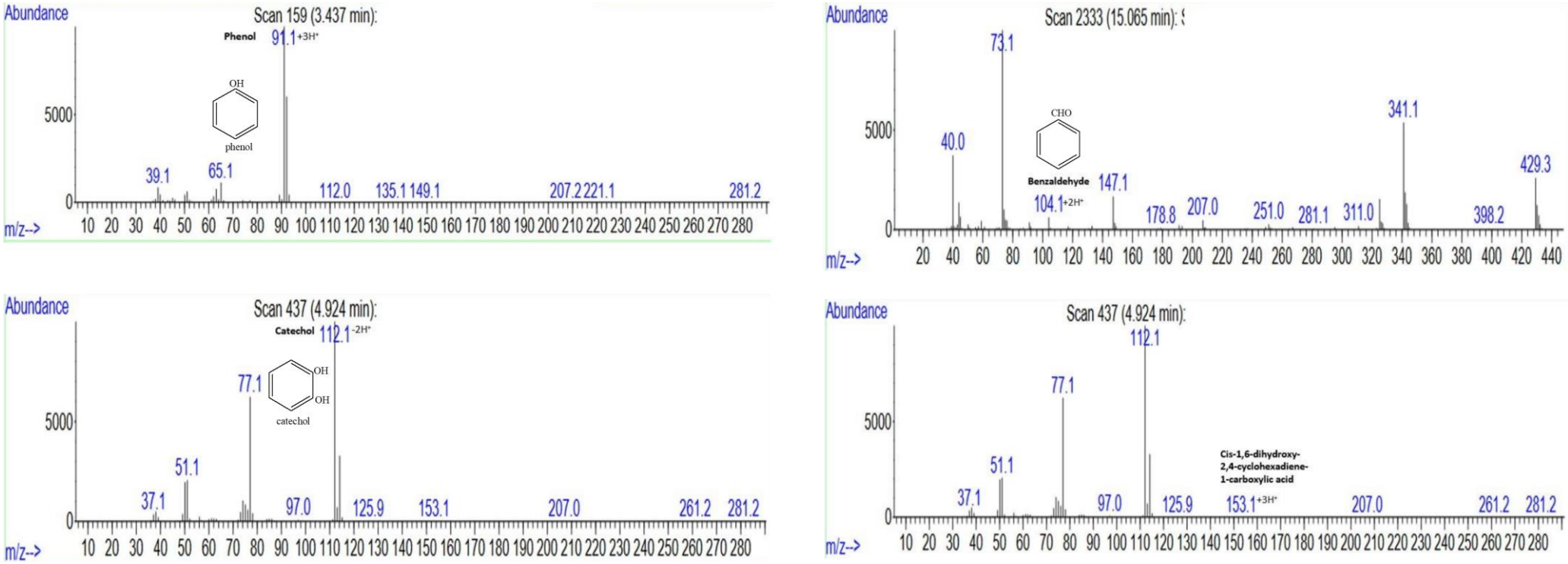

Fig. S6. GC-MS based identification of benzene metabolic intermediates in present study bacteria, through comparison of m/z ratios with molecular weights of earlier reported benzene metabolic intermediates.