Supplementary information for "Oxyfunctionalization of pyridine derivatives using whole cells of *Burkholderia* sp. MAK1"

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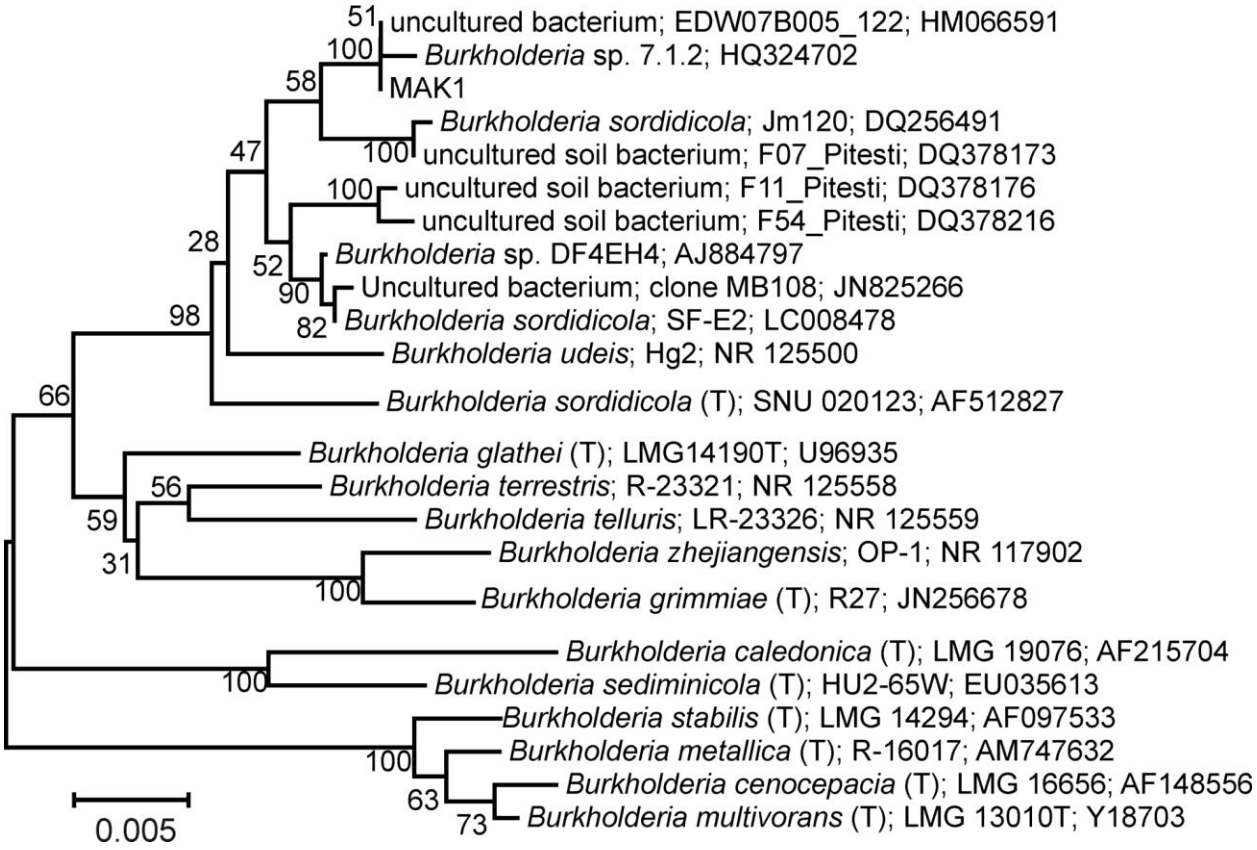


Figure S-I. Phylogenetic tree (constructed using the neighbour-joining method) showing the phylogenetic position of strain MAK1, based on 16S rRNA gene sequences.

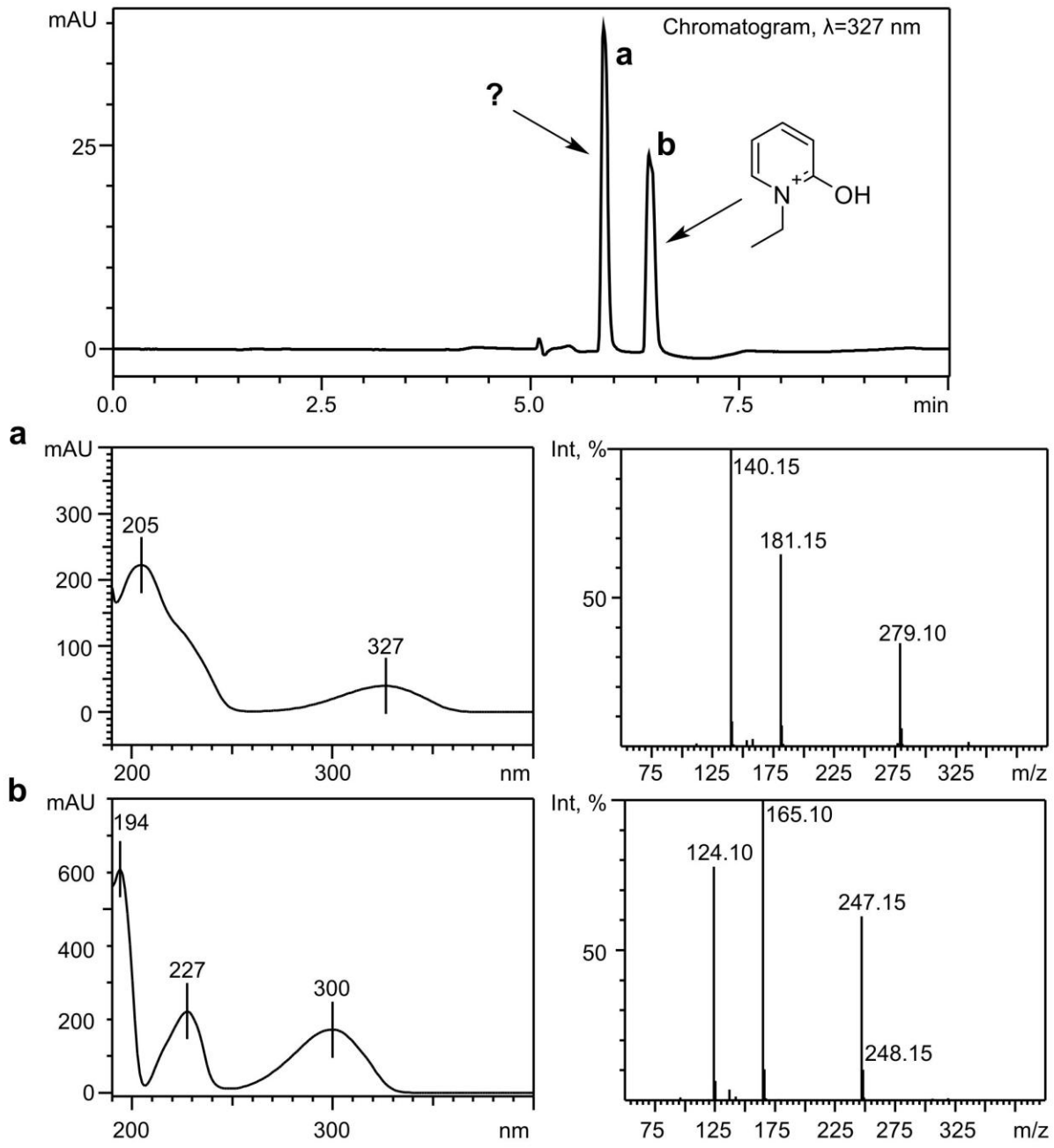


Figure S-II. Transformation of *N*-ethyl-2-hydroxypyridine by Burkholderia sp. MAK1. Analysis of the reaction mixture was performed by HPLC-MS. Chromatogram, UV-Vis spectra and mass spectra of (a) the product and (b) the substrate are presented.

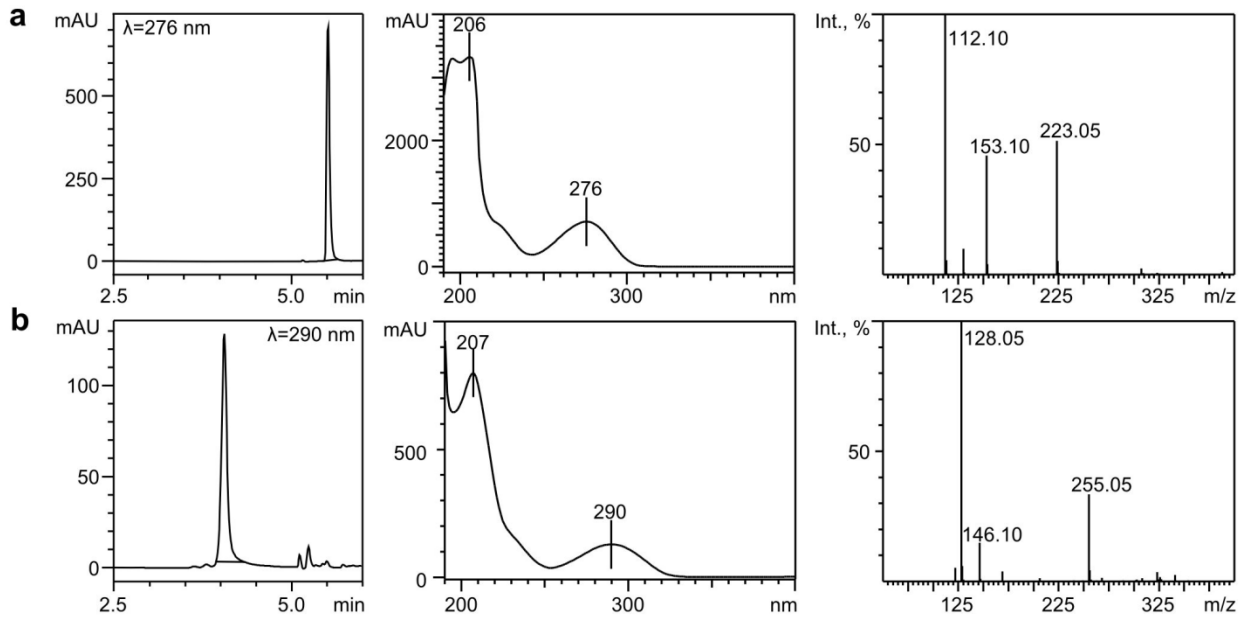


Figure S-III. Regioselective oxidation of 2,4-dihydroxypyridine by the cells of *Burkholderia* sp. MAK1. Analyses of the reaction mixture were performed by HPLC-MS (a) immediately after the addition of the substrate and (b) after 20 hours of incubation at 30 °C. Chromatograms and UV-Vis and mass spectra of the peaks are presented.

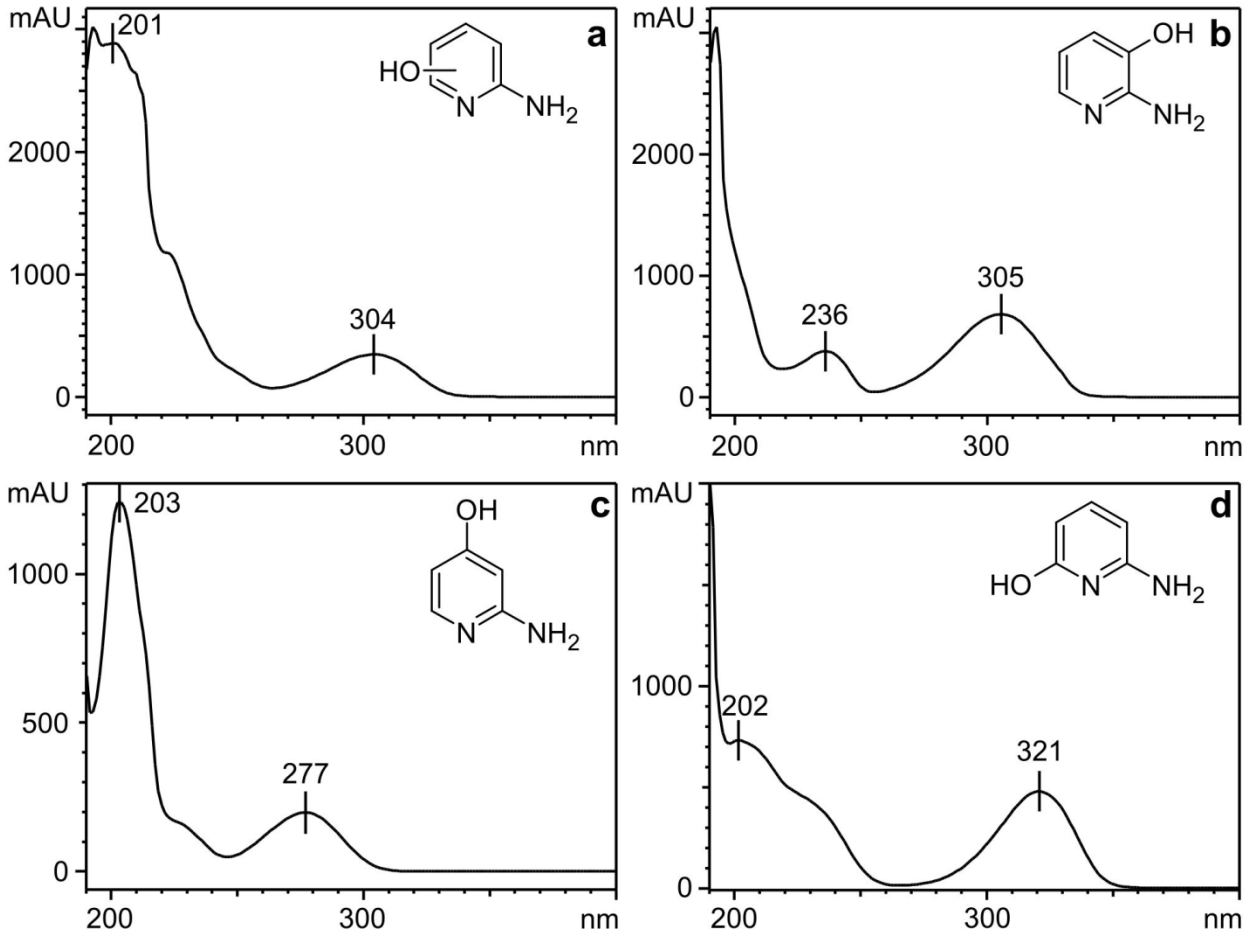


Figure S-IV. UV spectra of 2-aminohydroxypyridines. The UV spectrum of (a) 2-aminopyridine hydroxylated by *Burkholderia* sp. MAK1 cells and UV spectra of (b) 2-amino-3-hydroxypyridine, (c) 2-amino-4-hydroxypyridine, (d) 2-amino-6-hydroxypyridine standard solutions. The spectra were obtained by HPLC-MS. Note: the standard of 2-aminopyridin-5-ol is commercially unavailable.

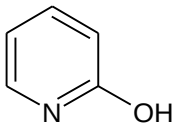
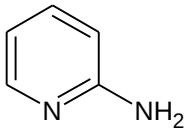
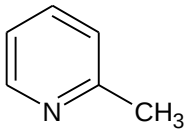
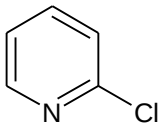
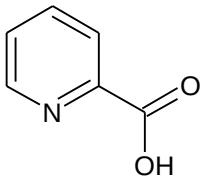
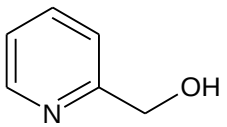
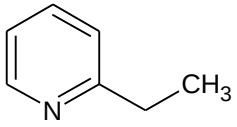
Table S-1. Biochemical characterization of *Burkholderia* sp. MAK1.

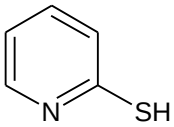
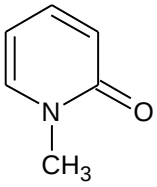
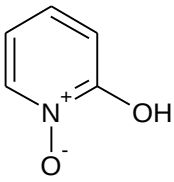
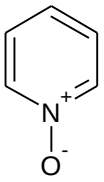
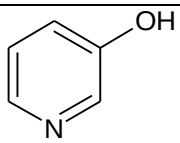
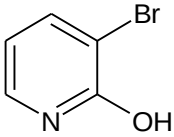
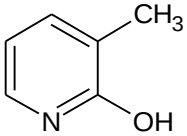
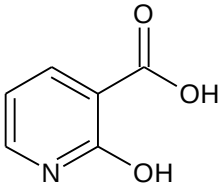
According to API™ ZYM test (bioMerieux, USA), *Burkholderia* sp. MAK1 is alkaline phosphatase (2-naphthyl phosphate), esterase (C 4) (2-naphthyl butyrate), esterase (C 8) (2-naphthyl caprylate), leucine arylamidase (L-leucyl-2-naphthylamide), acid phosphatase (2-naphthyl phosphate), naphthol-AS-BI-phosphohydrolase (naphthol-AS-BI-phosphate), β-galactosidase (2-naphthyl-β-D-galactopyranoside), and *N*-acetyl-β-glucosaminidase (1-naphthyl-*N*-acetyl-β-D-glucosaminide) positive. *Burkholderia* sp. MAK1 cells show weak activities of lipase (C 14) (2-naphthyl myristate), valine arylamidase (L-valyl-2-naphthylamide), cystine arylamidase (L-cystyl-2-naphthylamide), α-galactosidase (6-Br-2-naphthyl-α-D-galactopyranoside), α-glucosidase (2-naphthyl-α-D-glucopyranoside), and α-fucosidase (2-naphthyl-α-L-fucopyranoside). The microorganism is negative for trypsin (*N*-benzoyl-DL-arginine-2-naphthylamide), α-chymotrypsin (*N*-glutaryl-phenylalanine-2-naphthylamide), β-glucuronidase (naphthol-AS-BI-β-D-glucuronide), β-glucosidase (6-Br-2-naphthyl-β-D-glucopyranoside), and α-mannosidase (6-Br-2-naphthyl-α-D-mannopyranoside).

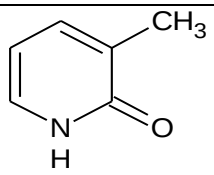
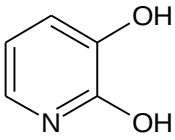
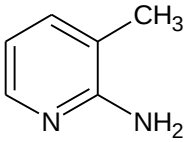
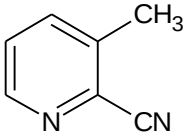
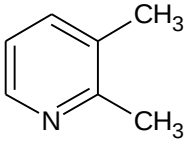
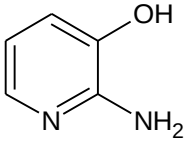
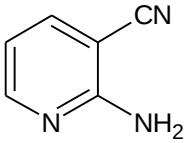
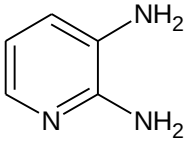
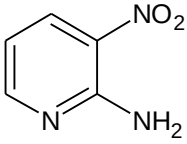
According to API™ 50 CH test (bioMerieux, USA), *Burkholderia* sp. MAK1 is able to metabolize the following carbohydrates: D-arabinose, L-arabinose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, N-acetylglucosamine, amygdalin, esculin ferric citrate, salicin, D-cellobiose, D-maltose, D-lactose, D-melibiose, D-saccharose, D-trehalose, inulin, D-raffinose, amidon (starch), glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, D-arabitol, and potassium gluconate.

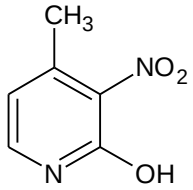
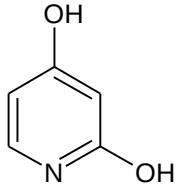
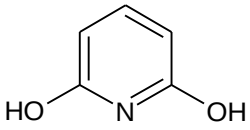
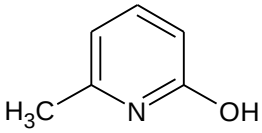
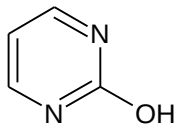
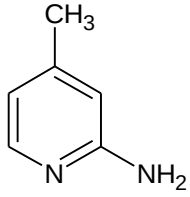
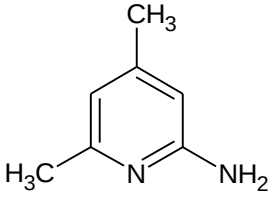
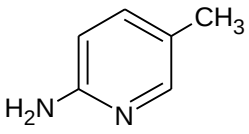
Burkholderia sp. MAK1 cells grow on D-glucose (0.1 %), D-galactose (0.1 %), *myo*-inositol (0.1 %), D-mannitol (0.1 %), D-mannose (0.1 %), D-sorbitol (0.1 %), D-fructose (0.1 %), D-xylose (0.1 %), succinate (0.1 %), and 2-hydroxypyridine (0.1 %) as sole carbon source but not on D-trehalose, D-ribose, 2-deoxy-D-glucose, D-melibiose, D-melezitose, sucrose, D-raffinose, D-maltose, D-cellobiose, and D-maltotriose.

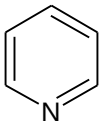
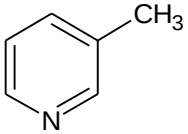
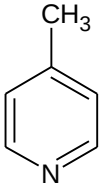
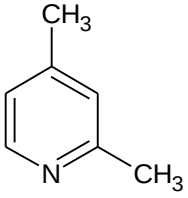
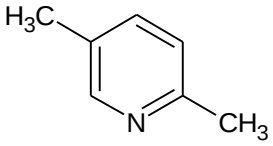
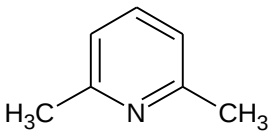
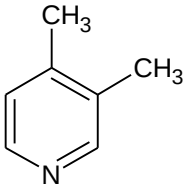
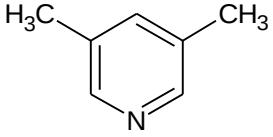
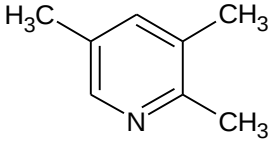
Table S-2. The list of compounds which were tested as potential substrates for transformation by whole cells of *Burkholderia* sp. MAK1.

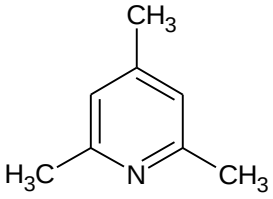
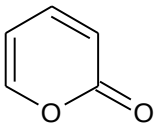
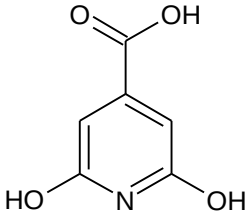
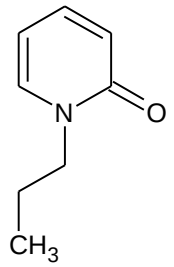
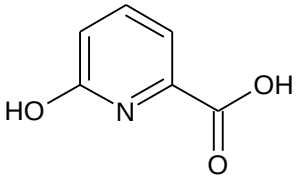
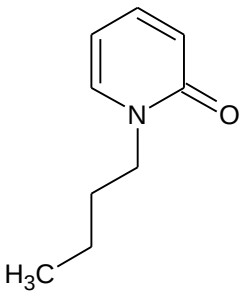
Substrate			Product
Name	Structure	$[M+H]^+$	$[M+H]^+$
Pyridin-2-ol		96	Substrate consumption occurs, no products detected
Pyridin-2-amine		95	111
2-Methylpyridine		94	110
2-Chloropyridine		114	130
Pyridine-2-carboxylic acid		124	No reaction observed
2-Pyridylmethanol		110	126 Slow transformation
2-Ethylpyridine		108	124

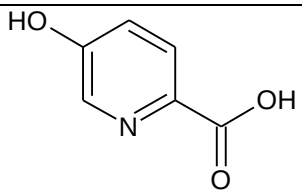
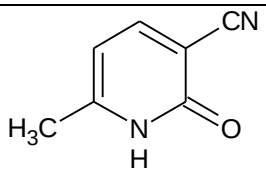
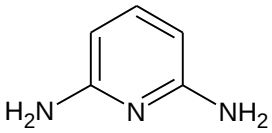
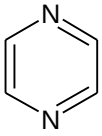
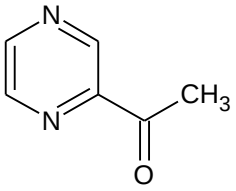
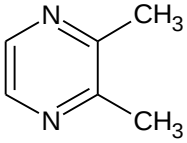
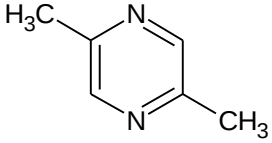
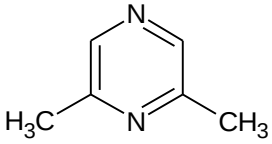
Pyridine-2-thiol		112	-
1-Methylpyridin-2-one		110	126
Pyridin-2-ol *N*-oxide		112	Substrate consumption occurs, no products detected
Pyridine-*N*-oxide		96	-
Pyridin-3-ol		96	112
3-Bromopyridin-2-ol		174 and 176	Substrate consumption occurs, no products detected
3-Methylpyridin-2-ol		110	Substrate consumption occurs, no products detected
2-Hydroxypyridine-3-carboxylic acid		140	-

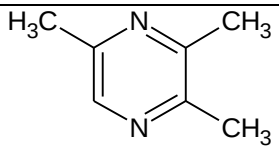
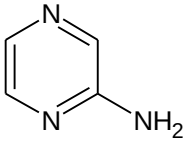
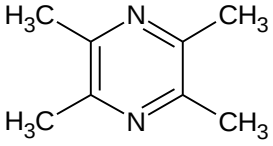
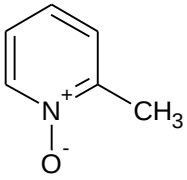
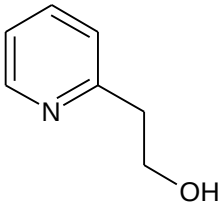
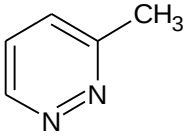
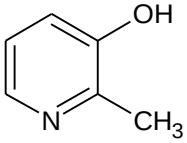
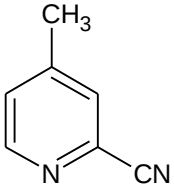
3-Methoxypyridin-2-ol		126	-
Pyridin-2,3-diol		112	Substrate consumption occurs, no products detected
3-Methylpyridin-2-amine		109	125
3-Methylpyridine-2-carbonitrile		119	-
2,3-Dimethylpyridine		108	124 Traces of the product
2-Aminopyridin-3-ol		111	215
2-Aminopyridine-3-carbonitrile		161 (as a cluster with acetonitrile)	-
Pyridin-2,3-diamine		110	214
3-Nitropyridin-2-amine		140	156

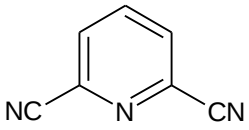
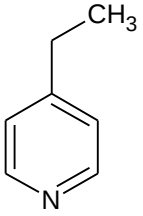
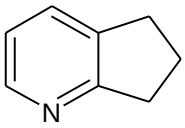
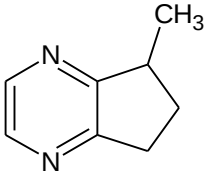
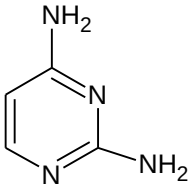
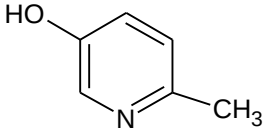
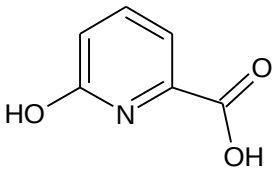
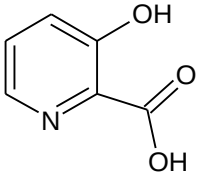
4-Methyl-3-nitro-pyridin-2-ol		155	125
Pyridin-2,4-diol		112	128
Pyridin-2,6-diol		112	Substrate consumption occurs, blue color, no products detected
6-Methyl-pyridin-2-ol		110	Substrate consumption occurs, no products detected
Pyrimidin-2-ol		97	-
4-Methylpyridin-2-amine		109	125
4,6-Dimethylpyridin-2-amine		123	-
3-Methylpyridin-6-amine		109	125 Slow transformation

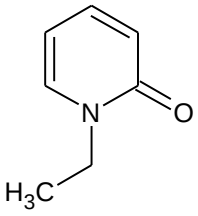
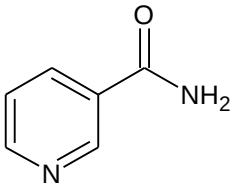
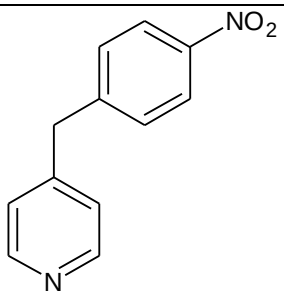
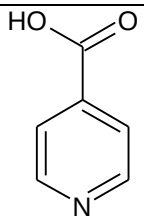
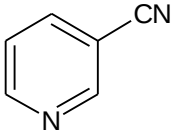
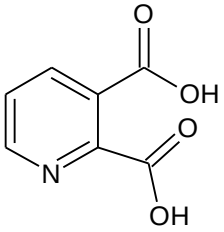
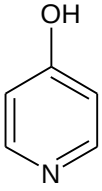
Pyridine		80	96
3-Methylpyridine		94	110
4-Methylpyridine		94	110
2,4-Dimethylpyridine		108	124
2,5-Dimethylpyridine		108	124
2,6-Dimethylpyridine		108	-
3,4-Dimethylpyridine		108	124
3,5-Dimethylpyridine		108	124
2,3,5-Trimethylpyridine		122	138 Traces of the product

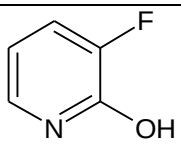
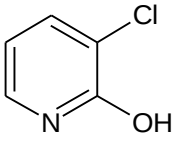
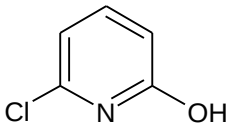
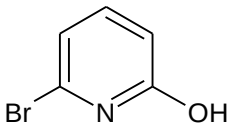
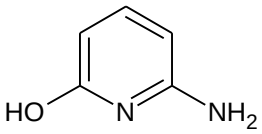
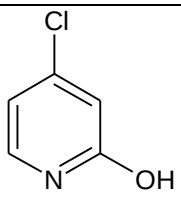
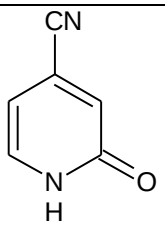
2,4,6-Trimethylpyridine		122	-
Pyran-2-one		97	Substrate consumption occurs, no products detected
2-Hydroxy-6-oxo-1H-pyridine-4-carboxylic acid		156	-
1-Propylpyridin-2(1*H*)-one		138	154 Traces of the product
6-Hydroxypyridine-2-carboxylic acid		140	-
1-Butylpyridin-2(1*H*)-one		152	-

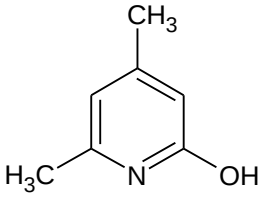
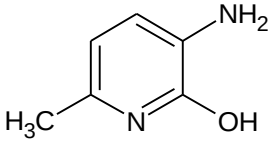
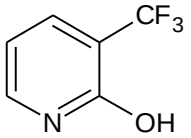
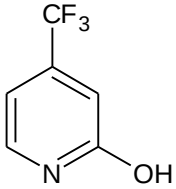
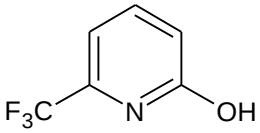
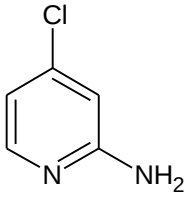
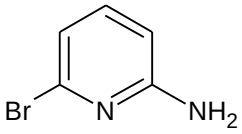
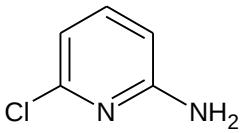
5-Hydroxypyridine-2-carboxylic acid		140	-
2-Hydroxy-6-methylpyridine-3-carbonitrile		135	Substrate consumption is slow, no products detected
Pyridin-2,6-diamine		110	Substrate consumption occurs, no products detected
Pyrazine		81	97; 113
1-Pyrazin-2-ylethanone		288	125
2,3-Dimethylpyrazine		109	125 Traces of the product
2,5-Dimethylpyrazine		109	125 Traces of the product
2,6-Dimethylpyrazine		109	125

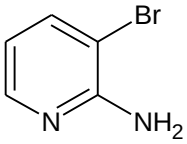
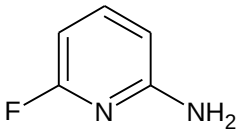
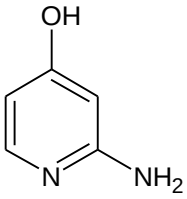
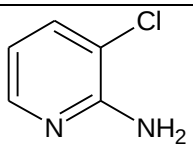
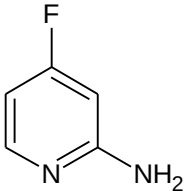
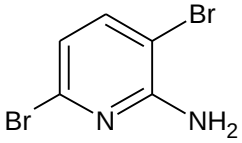
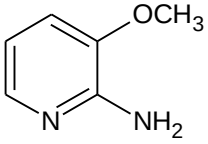
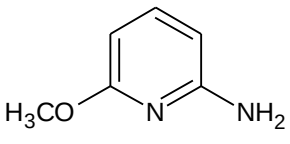
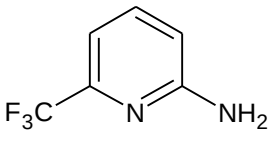
2,3,5-Trimethylpyrazine		123	139 Traces of the product
Pyrazin-2-amine		96	112
Tetramethylpyrazine		137	-
Pyridin-2-ol *N*-oxide		110	-
2-(2-Pyridyl)ethanol		124	140 Slow transformation
3-Methylpyridazine		95	-
2-Methylpyridin-3-ol		110	126
4-Methylpyridine-2-carbonitrile		119	-

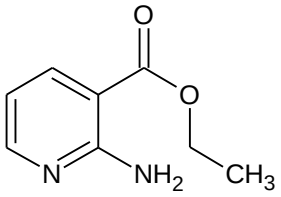
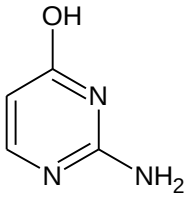
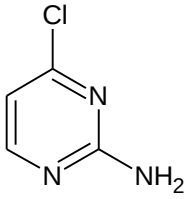
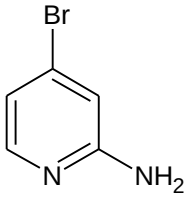
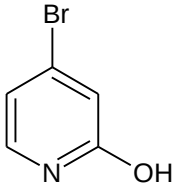
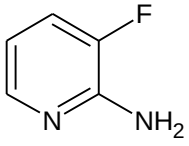
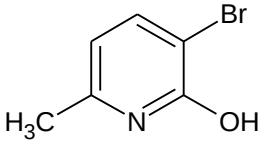
Pyridine-2,6-dicarbonitrile		No substrate detected	-
4-Ethylpyridine		108	124
6,7-Dihydro-5H-cyclopenta[b]pyridine		120	136
5-Methyl-6,7-dihydro-5H-cyclopenta[b]pyrazine		135	-
Pyrimidin-2,4-diamine		111	127
2-Methylpyridin-5-ol		110	-
6-Hydroxypyridine-2-carboxylic acid		140	-
3-Hydroxypyridine-2-carboxylic acid		140	-

1-Ethylpyridin-2(1*H*)-one		124	140
Pyridine-3-carboxamide		123	140
4-[(4-Nitrophenyl) methyl]pyridine		215	-
Pyridine-4-carboxylic acid		124	-
Pyridine-3-carbonitrile		105	121
Pyridine-2,3-dicarboxylic acid		168	-
Pyridin-4-ol		96	-

3-Fluoropyridin-2-ol		114	Substrate consumption occurs, no products detected
3-Chloropyridin-2-ol		130	Substrate consumption occurs, no products detected
6-Chloropyridin-2-ol		130	Substrate consumption occurs, green, no products detected
6-Bromopyridin-2-ol		174 and 176	Substrate consumption occurs, green, no products detected
2-Aminopyridin-6-ol		111	Substrate consumption occurs, several products formed
4-Chloropyridin-2-ol		130	-
2-Oxo-1H-pyridine-4-carbonitrile		No substrate detected	-

4,6-Dimethylpyridin-2-ol		124	-
3-Amino-6-methyl-pyridin-2-ol		125	279
3-(Trifluoromethyl) pyridin-2-ol		164	$[M-H]^-$: 178
4-(Trifluoromethyl) pyridin-2-ol		165	-
6-(Trifluoromethyl) pyridin-2-ol		165	Substrate consumption occurs, no products detected
4-Chloropyridin-2-amine		129	145
6-Bromopyridin-2-amine		173 and 175	189 and 191
6-Chloropyridin-2-amine		129	145

3-Bromopyridin-2-amine		173 and 175	189 and 191
6-Fluoropyridin-2-amine		113	Substrate consumption occurs, no products detected
2-Aminopyridine-4-ol		111	-
3-Chloropyridin-2-amine		129	145
4-Fluoropyridin-2-amine		113	129
3,6-Dibromopyridin-2-amine		No substrate detected	?
3-Methoxypyridin-2-amine		125	-
6-Methoxypyridin-2-amine		125	-
6-(Trifluoromethyl) pyridin-2-amine		-	-

Ethyl 2-aminopyridine-3-carboxylate		167	183 Traces of the product
2-Aminopyrimidin-4-ol		112	128
4-Chloropyrimidin-2-amine		130	146 Traces of the product
4-Bromopyridin-2-amine		173 and 175	189 and 191
4-Bromopyridin-2-ol		174 and 176	-
3-Fluoropyridin-2-amine		113	129 Two peaks with the same molecular mass
3-Bromo-6-methylpyridine-2-ol		188 and 190	-

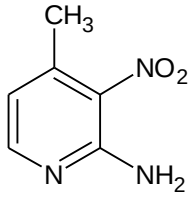
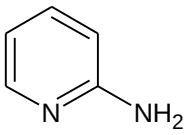
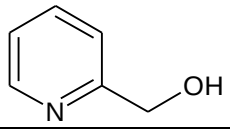
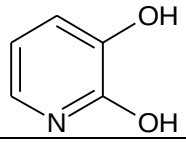
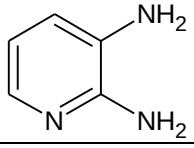
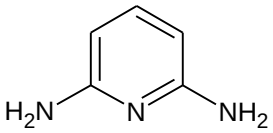
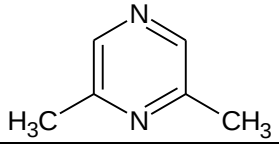
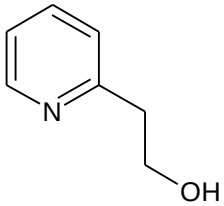
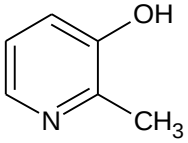
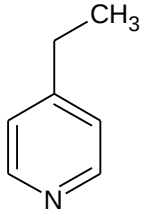
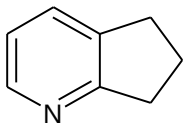
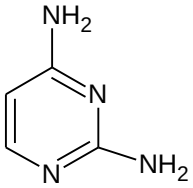
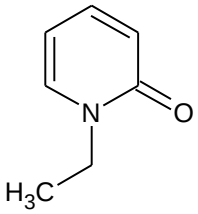
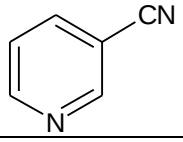
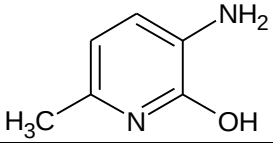
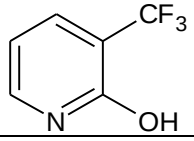
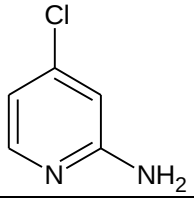
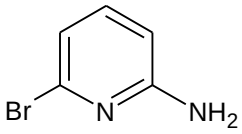
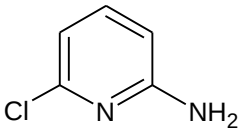
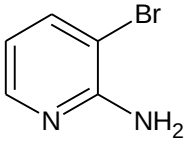
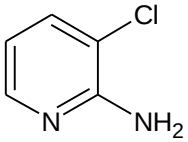
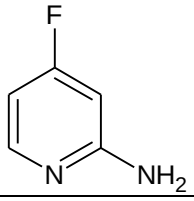
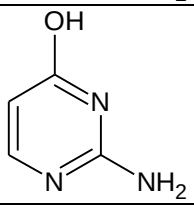
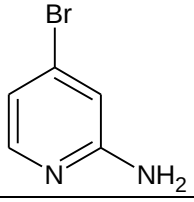
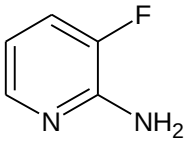
4-Methyl-3-nitro-pyridin-2-amine		154	-

Table S-3. The efficiency of conversion of selected compounds by whole cells of *Burkholderia* sp. MAK1.

Name	Structure	Conversion, %
Pyridin-2-amine		99
2-Pyridylmethanol		21
Pyridin-2,3-diol		97
Pyridin-2,3-diamine		88
Pyridin-2,6-diamine		94
2,6-Dimethylpyrazine		63
2-(2-Pyridyl)ethanol		17
2-Methylpyridin-3-ol		2
4-Ethylpyridine		10
6,7-Dihydro-5H-cyclopenta[b]pyridine		13

Pyrimidin-2,4-diamine		31
1-Ethylpyridin-2(1*H*)-one		17
Pyridine-3-carbonitrile		27
3-Amino-6-methyl-pyridin-2-ol		88
3-(Trifluoromethyl) pyridin-2-ol		46
4-Chloropyridin-2-amine		96
6-Bromopyridin-2-amine		53
6-Chloropyridin-2-amine		89
3-Bromopyridin-2-amine		48
3-Chloropyridin-2-amine		88

4-Fluoropyridin-2-amine		70
2-Aminopyrimidin-4-ol		95
4-Bromopyridin-2-amine		48
3-Fluoropyridin-2-amine		43