

**NMR-based metabolomic analysis of the physiological role of the electron-bifurcating FeFe-hydrogenase Hnd in *Solidesulfovibrio fructosivorans* under pyruvate fermentation**

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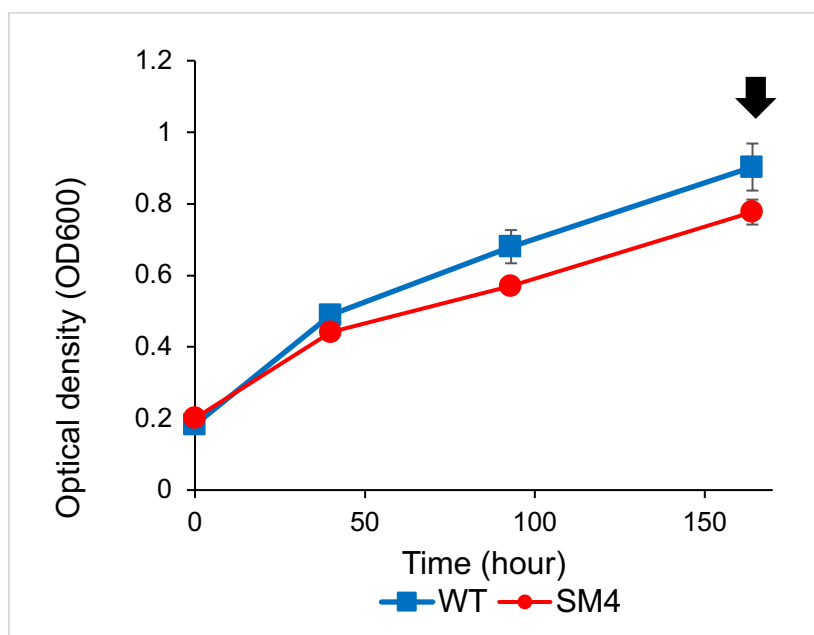
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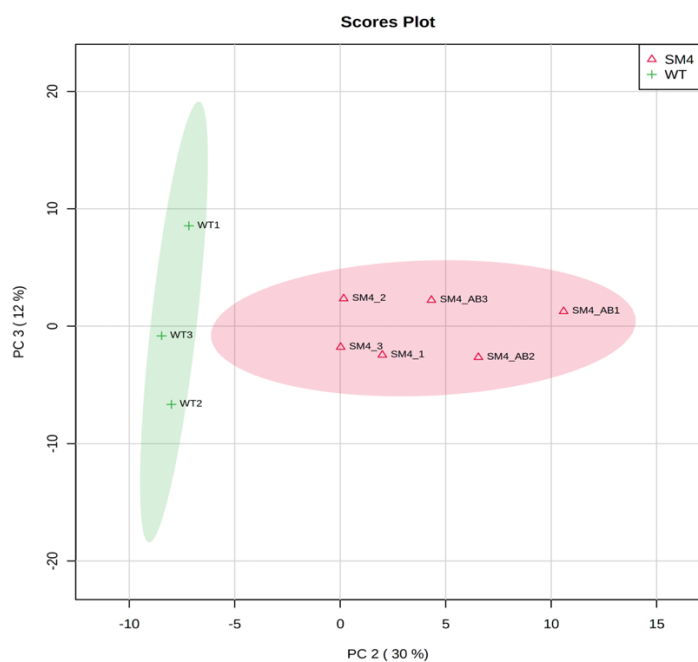
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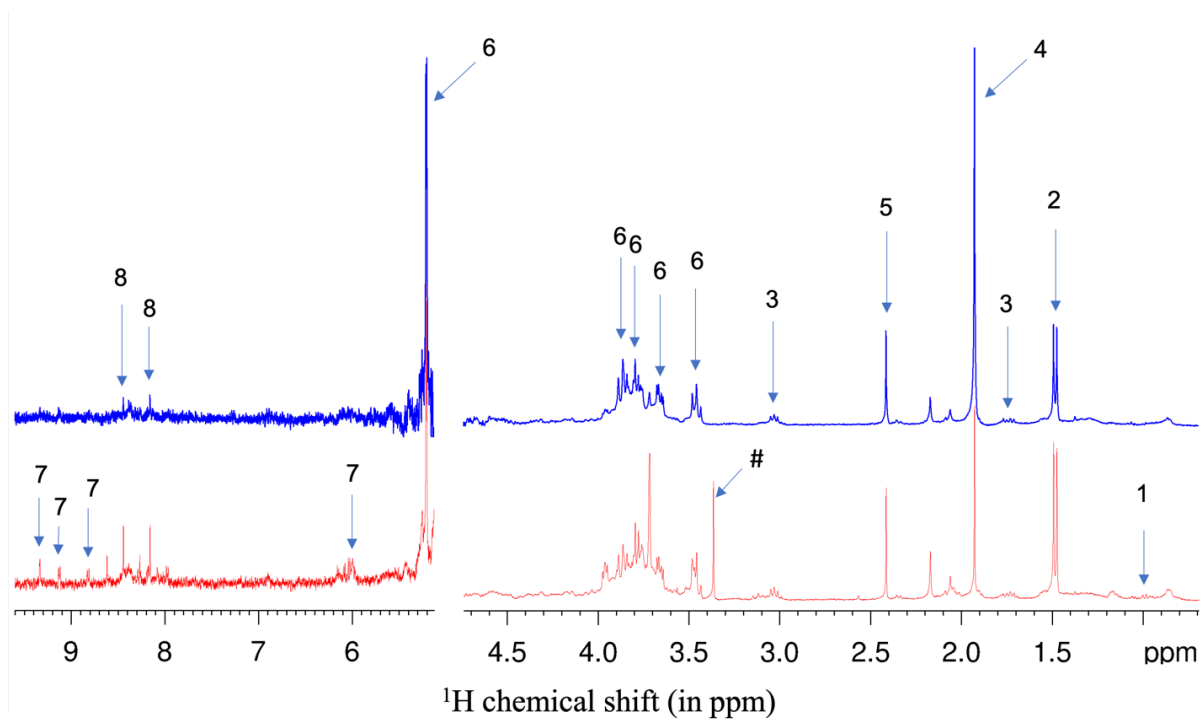
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**Figure S1** Growth curves for *S. fructosivorans* WT and SM4 strains cultivated under fermentative conditions (PS0). The average value of OD<sub>600</sub> for each sample group is reported according to the time (in hour). Error bars were calculated as standard error means. The arrow indicates the time of sample collection, i.e. 164 h.



**Figure S2** PCA score plot of <sup>1</sup>H NMR spectra from WT samples (WT), SM4 grown with antibiotics (SM4\_AB) and SM4 grown without antibiotics (SM4). SM4 samples, grown with or without antibiotics are clustered together validating the innocuity of the antibiotics on the overall bacterium metabolism.



**Figure S3** Representative <sup>1</sup>H HRMAS NMR spectra of *S. fructosivorans* cells (WT (blue), SM4 (red)) after 164 h growth; # = methanol from antibiotic. Assignments: 1: Valine; 2: Alanine; 3:lysine; 4: acetate; 5: succinate; 6: trehalose; 7: NAD(P)<sup>+</sup>; 8: AMP. Only major metabolites are represented on the figure. Assignments of all identified metabolites are reported in Table S1.

**Table S1** Assignments of identified metabolites

Metabolites	$\delta^1\text{H}$ ( $^{13}\text{C}$ )(in ppm)	Type <sup>a</sup>
Acetate	1.92(23.7)	intra/extra
Alanine	1.48(16.7) 3.77(51.1)	intra/extra
2-Aminobutyrate	0.97(12.5) 1.87 3.70	extra
Adenosine monophosphate (AMP)	6.13 8.25 8.62	intra
1-Butanol	0.86(15.1) 1.37 1.49	extra
Butyrate	0.88(16.5) 1.55 2.16	extra
Citramalate	1.34(27.7) 2.46 2.73 51.7	extra
1,3-Dihydroxyacetone	3.58 4.41	extra
Ethanol	1.17(19.6) 3.65	intra/extra
Formate	8.44(172)	extra
Glutamate	2.04 2.12 2.31 2.36 3.76	intra
Glycine	3.54(42.4)	extra
Inosine	6.09 8.22 8.35	intra
Isoleucine	0.93(11.5) 1.00(15.1) 1.25 1.45 1.98 3.65	intra/extra
Isobutyrate	1.06 2.37	extra
Lactate	1.32(25.7) 4.10	extra
Lysine	1.46 1.71 1.88 1.93 3.01 3.75	intra
Methanol	3.34(51.2)	intra/extra
3-methyl-2-oxovalerate	1.08 1.45 1.72 2.93	extra
Methylsuccinate	1.07(18.4) 2.10 2.51 2.61	extra
NAD(P) <sup>+</sup>	6.06 8.14 8.16 8.42 8.80 9.11 9.32	intra
2-Oxobutyrate	1.06(21.3) 2.76	extra
Propionate	1.04(11.2) 2.16(31.4)	extra
Pyruvate	2.36(28.1)	intra/extra
Succinate	2.39(35.4)	intra/extra
Trehalose	3.44 3.65 3.76 3.81 3.85 3.86 5.18	intra
Valine	0.98(15.6)1.03(18.7) 2.26(30.6) 3.59	intra/extra

<sup>a</sup> Sample types : intra = intracellular (intact bacteria); extra = extracellular (culture medium).

**Table S2** Fold change and corresponding p value of intracellular metabolites discriminating between *S. fructosivorans* WT and SM4 strains grown under pyruvate fermentation.

Metabolites	Fold change	P-value
		(SM4 vs WT)
NAD(P) <sup>+</sup>	3.4	5.10 <sup>-4</sup>
AMP	3.0	0.02
Alanine	2.4	0.006
Valine	2.3	0.03

**Table S3** Fold change and corresponding p value of extracellular metabolites discriminating between *S. fructosivorans* WT and SM4 strains

Metabolites	Fold change	P-value
	(SM4 vs WT)	(SM4 vs WT)
Ethanol	0.04	5.10 <sup>-10</sup>
Succinate	1.47	0.009
Propionate	2.13	5.10 <sup>-5</sup>
Citramalate	1.77	0.002
2-Aminobutyrate	1.70	5.10 <sup>-4</sup>
2-Oxobutyrate	2.23	0.003
Isoleucine	2.02	3.10 <sup>-7</sup>
Valine	2.6	5.10 <sup>-5</sup>