Supplementary Material for

Labelling strategy and membrane characterization of marine bacteria *Vibrio splendidus* by *in vivo* ^{2}H solid-state NMR

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(i) *Effect of labelling on bacterial growth*

Growth curves were acquired by periodic measurements of optical density (OD) at 600 nm for cultures enriched with exogenous fatty acids. Figure SI1 shows that supplementing the culture medium with either d_{31}-PA or the combination of d_{31}-PA and OA in the presence of Tween-20 did not induce major shifts in the delimitations of the growth phases. The transition between exponential and stationary phases was smooth, making delimitations hard to define. Typically, we define the mid-log phase occurring 15h to 16h after inoculation, if we consider stationary phase beginning around 25h and fully established after 30h of growth. Specific growth rates (μ) (see Materials & Methods) were calculated and showed no significant differences in the various labelling regimes of the cell growth (Fig. SI1). Similarly, whilst maximum optical densities in the enriched medium were slightly below those of the control, log phases durations were exactly the same (about 3 hours), indicating a good bacterial adaptation and no particular stress or toxic effect on growing bacteria in the ^2H enriched medium.

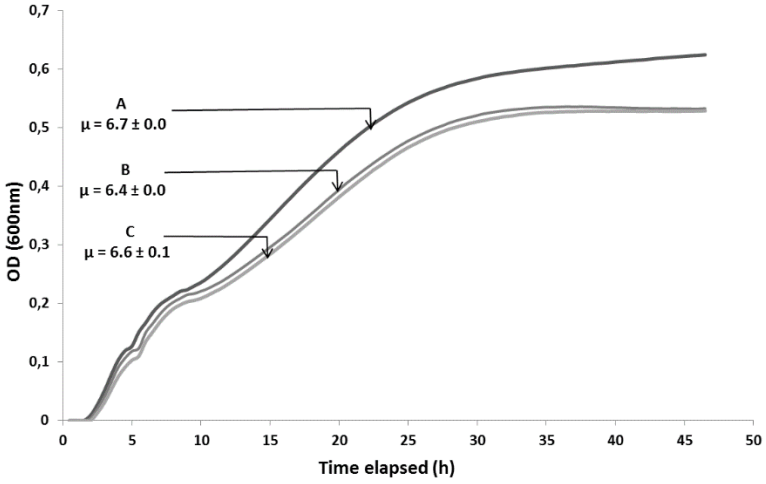

Figure SI1: Representative growth patterns of *V. splendidus* in culture medium (A), culture medium enriched with d_{31}-PA (B), with d_{31}-PA and OA (C) in presence of Tween-20. Cells were inoculated from cultures that had previously grown for 2 days. Specific growth rate (μ) was deduced from the first 25 hours of culture.

(ii) *Fatty acids contents*

Table SI1: FAs content in *V. splendidus* expressed in molar % per total FA content, deuterium fatty acid expressed in % per total palmitic acid and SFA/UFA ratio with cell growth phases under different labelling regimes: *V. splendidus* grown in contol LB-medium, in medium enriched with d_{31}-palmitic acid, and in medium enriched with d_{31}-palmitic acid and oleic acid. Bacteria were sampled during the exponential (Mid-log) and early stationary phase. Growth temperature was 25°C for all cultures.

	Control		medium + d^{31}-PA		medium + d^{31}-PA + OA	
Fatty Acid	**Mid-log**	**Stationary**	**Mid-log**	**Stationary**	**Mid-log**	**Stationary**
^{2}H-Palmitic acid ^{2}H-C16:0	0.09 (0.13)	n.d.	43.5 (0.6)	31.7 (0.9)	31.4 (4.7)	18.4 (1.8)
^{1}H-Palmitic acid ^{1}H-C16 :0	30.2 (0.9)	29.4 (0.7)	13.2 (3.7)	10.9 (1.2)	13.2 (1.9)	15.1 (0.4)
isotopic labeling %	n.a.	n.a.	77%	75%	69%	55%
Total Palmitic acid C16:0	30.3 (1.0)	29.4 (0.7)	56.7 (3.1)	42.6 (1.1)	44.6 (2.8)	33.4 (2.2)
Palmitoleic acid C16:1	52.11 (1.78)	51.2 (0.2)	21.38 (6.12)	17.7 (1.1)	13.7 (1.7)	49.1 (1.8)
Oleic acid C18:1	9.1 (1.0)	8.4 (0.5)	7.7 (4.9)	14.7 (1.1)	33.7 (3.3)	11.2 (1.2)
Lauric acid C12:0	1.35 (0.18)	1.17 (0.02)	3.14 (1.37)	2.12 (1.08)	0.96 (0.37)	0.83 (0.22)
Myristic acid C14:0	3.72 (0.65)	3.87 0.07)	4.57(0.74)	5.58 (0.42)	2.89 (0.79)	3.34 (1.33)
Pentadecanoic acid C 15:0	0.23 (0.18)	0.14 (0.10)	0.19 (0.07)	0.03 (0.04)	0.29 (0.22)	0.15 (0.14)
Heptadecanoic acid C17:0	0.26 (0.2)	0.13 (0.19)	0.21 (0.06)	n.d.	0.13 (0.11)	0.33 (0.36)
Stearic acid C18:0	1.22 (0.32)	2.65 (0.10)	1.92 (1.15)	0.67 (0.43)	0.97 (0.20)	1.18 (0.08)
Arachidic acid C20:0	0.05 (0.10)	n.d.	0.08 (0.12)	n.d.	0.12 (0.15)	n.d.
Myristoleic acid C14 :1	0.16 (0.24)	0.09 (0.13)	0.29 (0.11)	0.07 (0.10)	n.d.	0.16 (0.12)
cis-10-pentadecanoic acid C15:1	0.012 (0.027)	n.d.	0.11 (0.15)	n.d.	n.d.	n.d.
Cyclopropaneoctanoic acid-2-hydroxyl acid C17:1(cyC17:0)	0.19 (0.18)	n.d.	0.97 (0.52)	11.63 (0.48)	0.53 (0.45)	0.30 (0.40)
Stearidonic acid C18:4n	n.d.	n.d.	0.92 (0.86)	n.d.	0.24 (0.23)	n.d.
Alpha-linoleic acid C18:3n3	0.03 (0.08)	n.d.	0.96 (0.92)	n.d.	0.25 (0.21)	n.d.
Cyclopropaneoctanoic acid, 2-octyl acid C19:1(cyC19:0)	0.11 (0.16)	n.d.	0.32 (0.31)	4.95 (0.87)	0.52 (0.40)	n.d.
Eicosenoid FA C20:1n9 + C20:2 + C204n6 +C20:5n3	1.03 (1.18)	2.94 (0.06)	0.48 (0.48)	n.d.	n.d.	n.d.
Total SFA	37.16 (1.96)	37.37 (0.43)	66.86 (4.03)	50.06 (0.73)	51.06 (2.87)	39.24 (0.51)
Total UFA	62.73 (1.97)	62.63 (0.43)	33.15 (4.03)	49.94 (0.73)	48.94 (2.87)	60.76 (0.44)
SFA/UFA	**0.59 (0.05)**	**0.60 (0.01)**	**2.07(0.40)**	**1.04 (0.03)**	**1.05 (0.12)**	**0.65 (0.01)**

Values corresponds to means and respective standard deviations
"n.d."indicates that these measurements could not be detected
"n.a." indicates that these measurements are not applicable (unlabelled medium)

(iii) *NMR spectra of bacteria enriched with d_{31}-PA in presence of Tween-20*

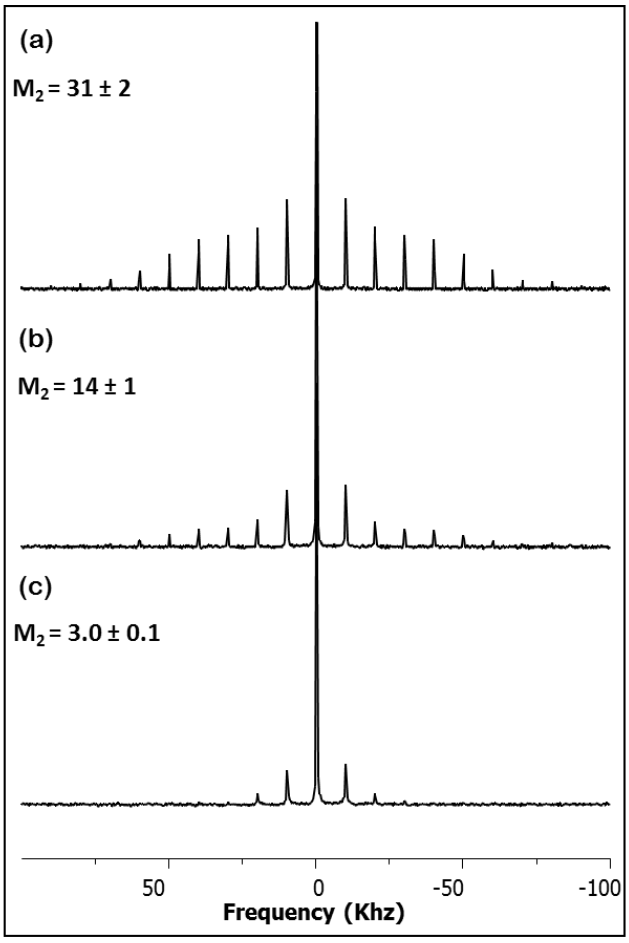

Figure SI2: ^{2}H MAS (10 kHz) SS-NMR spectra of intact *V. splendidus* harvested at three different cell growth times: (a) after 15 h in the mid-log phase ($OD_{600nm} \approx 0.3$), (b) after 22 h (±2h) at the beginning of the stationary stage ($OD_{600nm} \approx 0.5$), and (c) after 30 h at advanced stationary phase ($OD_{600nm} \approx 0.5$). Average second spectral moments M_2 are indicated (10^9 s^{-2}).