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Partial characterization of an exopolysaccharide secreted by a marine bacterium, *Vibrio neocaledonicus* sp. nov., from New Caledonia

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Abstract :

Aims : Exopolysaccharides (EPS) are industrially valuable molecules with numerous useful properties. This study describes the techniques used for the identification of a novel *Vibrio* bacterium and preliminary characterization of its EPS.

Methods and Results : Bioprospection in marine intertidal areas of New Caledonia followed by screening for EPS producing brought to selection of the isolate NC470. Phylogenetic analysis (biochemical tests, gene sequencing and DNA–DNA relatedness) permitted to identify NC470 as a new member of the *Vibrio* genus. The EPS was produced in batch fermentation, purified using the ultrafiltration process and analysed by colorimetry, Fourier Transform Infrared spectroscopy, gas chromatography, Nuclear Magnetic Resonance and HPLC-size exclusion chromatography. This EPS exhibits a high *N*-acetyl-hexosamines and uronic acid content with a low amount of neutral sugar. The molecular mass was 672×10^3 Da. These data are relevant for possible technological exploitation.

Conclusions : We propose the name *Vibrio neocaledonicus sp. nov* for this isolate NC470, producing an EPS with an unusual sugar composition. Comparison with other known polymers permitted to select applications for this polymer.

Significance and Impact of the Study : This study contributes to evaluate the marine biodiversity of New Caledonia. It also highlights the biotechnological potential of New Caledonia marine bacteria.

Keywords : biopolymers ; Biotechnology ; Exopolysaccharide ; fermentation biotechnology

37 **Introduction**

38 Marine bacteria, whether attached or free-living, are found in almost all parts of the marine
39 world, from the artic oceans to deep-sea hydrothermal vents (Nichols et al. 2005). They have
40 developed various strategies to survive in extreme conditions, such as metabolic pathways
41 adaptation including the production of protective structures like biofilms. Biofilms are highly
42 hydrated matrix formed by extracellular polymeric substances such as exopolysaccharides
43 (EPS), proteins, lipids and extracellular DNA (Flemming et al. 2007).
44 Polysaccharides commonly found in plants, are also produced by some microorganisms where
45 they act as nutrient stores components in the cell walls (Work 1961); some are secreted in
46 form of EPS. Polysaccharides have numerous interesting properties relevant to industrial
47 applications; they can be used as stabilizers, emulsifiers, thickeners, gelling agents,
48 coagulating agents and for their water retention capacity (Rasmussen et al. 2007). They are
49 currently used in diverse applications in various sectors including food and beverage
50 industries (Suresh Kumar et al. 2007), health industry (Okutani 1984; Okutani 1992), and
51 industrial waste treatment and mining industries (Iyer et al. 2004; 2005). Polysaccharides can
52 be chemically modified to impart or improve particular functional properties (Guezennec et
53 al. 1998a) further increasing their exploitation potential. Bacteria present the advantage to
54 grow rapidly and can be used for rapid fermentative production of polysaccharides. The
55 polymers produced by batch fermentation are highly reproducible from a batch production to
56 another; they are not sensitive to marine pollution, crop failure, or climatic events.
57 Consequently, these systems are valuable biotechnological resources. Bacterial
58 polysaccharides have a wide variety of chemical arrangement and properties which are not
59 found elsewhere, they often outperform polysaccharides from other sources, e.g., algae
60 (alginates, carrageenans) or crustacean (chitin) (Rasmussen et al. 2007).

61 Bacteria excrete polysaccharides which then forms a protective coat on their cell surfaces
62 against environmental stresses including variations in abiotic factors such as temperature, pH,
63 salinity or UV (Boyle and Reade 1983). EPS also play a role in protection against antibiotics
64 (Høiby et al. 2009) and heavy metals(Gutnick and Bach 2000). Indeed, hydrated biofilms
65 offer a buffered micro-environment in which bacteria can live, adapt, resist to stressful
66 conditions, and thereby ensure their survival and proliferation (Decho and Herndl 1995).
67 Aiming at the discovery of novel biopolymers and biomolecules of biotechnological
68 significance, it is now widely accepted that microorganisms from unusual environments not
69 only provide valuable resources for exploiting novel biotechnological processes but also serve
70 as models for investigating how biomolecules are stabilized when subjected to changing
71 conditions.
72 Bioprospection in microbial mats present on some French Polynesian atolls in the Pacific
73 Ocean led to the discovery of new bacterial species able to produce biopolymers with unusual
74 chemical structures (Moppert et al. 2009). In that Pacific Ocean seems to be a promising
75 source of new bacterial molecules and other marine biotechnological developments, such as
76 novel drugs and healthcare products (Guezennec et al. 2011). A similar program started in
77 New Caledonia in April 2010 with the aim to isolate marine bacteria and evaluate their
78 biotechnological potential.
79 As part of this program, the present work describes a new strain, designated NC470 and able
80 to produce under laboratory conditions an EPS. A preliminary chemical characterization of
81 this biopolymer is also reported.
82

83 **Materials and methods**

84 *Bioprospection and sampling*

85 New Caledonia is a Melanesian archipelago in the South West Pacific. It possesses one of the
86 largest lagoons in the world and has exceptionally diverse marine areas, including reef flats,
87 mangrove swamps and sandy beaches. During bioprospection, samples of waters, sediments,
88 intertidal rocks, invertebrates, plants, fish, and biofilms found on inorganic substrates
89 (Guezennec et al. 1998b) were collected, mainly in the western part of the main island of New
90 Caledonia (Figure 1). Strain NC470 was isolated from a biofilm found on an invertebrate
91 animal (Holothuroidea) in St-Vincent Bay (S 21° 55' 631" / E 166° 04' 840").

92 *Isolation and culture of the bacterial strain*

93 The samples collected were used to inoculate liquid Zobell (ZoBell 1941) medium at pH 7.6
94 and incubated at 28°C for 24 h. Successful cultures were subcultured on a solid medium under
95 the same conditions; this procedure was repeated several times until pure cultures were
96 obtained and the isolates were stored at -80°C in 20% glycerol (v/v).

97 *Physiological and biochemical characterization*

98 Tests to identify optimal growth conditions were performed in 20 ml tubes with 8 ml of
99 Zobell liquid medium. The tubes were inoculated (10%, v/v) with a primary bacterial culture
100 in the exponential phase of growth and incubated with rotary shaking (Unitron, Infors, Massy,
101 France) at 200 rpm. The environmental conditions at the sampling site include a temperature
102 range of 28 to 30°C; pH 7 to 8; and, 37 to 38‰ salinity. Thus the growth rates were
103 determined by studying the following range of culture conditions:

104 - Temperature from 20 to 45°C, with 5°C steps.
105 - pH from 6 to 9, with 0,5 steps, in 50 mmol l^{-1} MOPS (3-(N-morpholino)
106 propanesulfonic acid, pH 6 to 6.5) and Tris (pH 7 to 9) buffers.
107 - Salinity from 20 to 70 g l^{-1} with 10 g l^{-1} step using NaCl.

108 Bacterial growth was measured every hour by spectrometry (Uvikon XS, Secoman, Ales,
109 France) at λ=600 nm, until the end of the exponential phase of growth.
110 API 50CH, API 20NE, API 20E, ATB G- and ATB PSE tests kits were used to determine
111 metabolic properties and antibiotic susceptibilities.
112 *Phylogenetic analyses*
113 Genomic DNA was isolated using the QIAmp® kit (QIAGEN S.A., Courtaboeuf, France) and
114 the extracted DNA was adjusted to 20 ng μl^{-1}. The 16S ribosomal RNA (16S rRNA) gene was
115 amplified by PCR using two primers: SADIR (5'-AGAGTTTGATCATGGCTCAGA-3') and
116 S17REV (5'-GTTACCTTGTTACGACTT-3') (Cambon-Bonavita et al. 2002). The PCR
117 involved of a first denaturation at 95°C, then a 35 cycles of 94°C for 2 min, 55°C for 30 s and
118 72°C for 90 s, followed by a final extension of 72°C for 7 min and ended with cooling to 4°C
119 (Verity, Applied Biosystems).
120 The primers described by Sawabe (Sawabe et al. 2007) were used for multilocus sequence
121 typing (MLST) study using the housekeeping genes *gapA*, *gyrB*, *pyrH* and *topA*. The PCR
122 program used was the same as for 16S rRNA but with annealing temperature of 58°C, 53°C,
123 53°C and 58°C for *gapA*, *gyrB*, *pyrH* and *topA*, respectively.
124 Sequence reads on the ABI 3130 xl were made at the regional genomic core research facilities
125 for life science in New-Caledonia ''Plate-Forme du Vivant de Nouvelle-Calédonie (PFV-
126 NC).
127 The 16S sequence obtained was compared to the GeneBank sequence database
128 (http://blast.ncbi.nlm.nih.gov) using the BLAST algorithm to determine the bacterial genus of
129 NC470. Ninety-six type strains from this genus (Balcázar et al. 2010; Beaz-Hidalgo et al.
130 2010; Rameshkumar et al. 2010; Sheu et al. 2010; Chimetto et al. 2011a; Wang et al. 2011;
131 Gomez-Gil et al. 2012) were used for phylogenetic analyses, type strain DNA sequences of
132 were aligned with that of NC470 using the BioEdit software package (Hall 1999).

133 The phylogenetic analyses were performed using a combination of three methods and
134 distances corrected according to the Kimura two-parameter model (Kimura 1985). The
135 neighbor-joining (Saitou and Nei 1987), maximum parsimony (Kluge and Farris 1969) and
136 maximum-likehood (Felsenstein 1992) methods were implanted in the Phylo_win program
137 (Galtier et al. 1996) with bootstrap values determined after 500 replications. The tree was
138 constructed with the njplot program (Perrière and Gouy 1996).
139 The strains and sequence accession numbers of 16S rRNA, *gapA*, *gyrB*, *pyrH* and *topA* genes
140 of the species most closely related to NC470 are listed in Table 1.
141 The DNA base composition was determined by a fluorimetric method involving quantitative
142 PCR (Light-Cycler 2 from Roche diagnostic): the thermal denaturation of DNA was
143 determined, in duplicate, as described elsewhere (Xu et al. 2000; Gonzalez and Saiz-Jimenez
144 2002). The T*m* values of 12 *Vibrio* type species with a known % G+C were used to construct
145 a standard curve equation that allows the %G+C of NC470 to be determined. Using these
146 results, DNA-DNA hybridization study was performed between NC470 and the three most
147 closely related type strains as assessed from the 16SrRNA sequences and the concatenated
148 MLST gene study. The method used was based on that developed for the *Vibrio* genus
149 (Moreira et al. 2010) and the work of De Ley (De Ley 1970) for optimal DNA renaturation
150 (T*or*). Aliquots of 10µl of each DNA preparation (adjusted to 50 ng μl^{-1}) were mixed and
151 heated to 99°C for 10 min. The samples were then incubated for 8 h at 70°C and the
152 temperature decreased 10°C per hour to 25°C. The samples were then cooled to 4°C and
153 tested by quantitative PCR apparatus as explained previously to obtain the denaturation curve.
154 The DNA-DNA relatedness was determined from the ΔT*m* value for the hybrid formed
155 between the DNAs from NC470 and the type strain

156 *Production of exopolysaccharides*

157 EPS was produced by culturing NC470 for 72 h in a 3.5 l fermenter (Minifors, Infors, Massy,

158 France) containing 3 l of Zobell medium, supplemented with 30 g l^{-1} glucose.

159 Each batch of culture medium was inoculated at 10% (v/v) with a suspension of cells in

160 exponential growth. The temperature was maintained at 30°C and the pH was adjusted to 7.5

161 by automatic addition of NaOH (0.2 mol l^{-1}) or HCl (0.2 mol l^{-1}). Foaming was avoided by

162 adding Struktol (40 µl l^{-1}). The air flow was set to 30 l h^{-1} and the agitation rate was

163 controlled to maintain dissolved O_2 at approximately 40% saturation.

164 *Isolation, purification and characterisation of the EPS*

165 Cells were removed from the medium after 72 h of culture by centrifugation at 15500 rpm

166 (Sorvall Evolution RC, Saint Herblain, France). The EPS was then purified from the culture

167 supernatant by ultrafiltration using a Sartocon Slice system (Sartorius Stedim Biotech,

168 Aubagne, France) equipped with a 100 kDa filter, and lyophilized.

169 *Chemical analyses*

170 The total neutral carbohydrate and hexuronic acid content were determined by the phenol-

171 sulfuric (DuBois et al. 1956) and the meta-hydroxydiphenyl (Blumenkrantz and Asboe-

172 Hansen 1973) methods, respectively. Hexosamines were assayed by staining with the Ehrlich

173 reagent and spectrophotometry (Belcher et al. 1954) using N-acetyl-glucosamine and N-

174 acetyl-galactosamine as standards. Protein content was determined by the BCA method

175 (Lowry et al. 1951) with bovine serum albumin as the standard.

176 The molar ratio of monosaccharides was estimated after acidic methanolysis of the EPS using

177 3 mol l^{-1} of MeOH/HCl, 4 h at 100°C, followed by gas chromatography (GC) analyses of the

178 trimethylsilyl derivatives. Methyl glycosides were converted to trimethylsilyl derivatives as

179 described elsewhere (Montreuil et al. 1986) and analyzed by GC using. myo-inositol

180 (1 mg ml^{-1}) as the internal reference (Rougeaux et al. 1996; Rougeaux et al. 2001). GC

181 analysis was performed on an Agilent Technologies GC 7890A Series fitted with a HP1 fused
182 silica column (60m x 0.32mm) with helium as carrier gas. The temperature program was 50°C
183 for 1 min, then increasing 20°C per min up to 120°C, 2°C per min up to 250°C, 50°C per min
184 up to 280°C and 5 min at 280°C.
185 The attenuated total reflectance Fourier Transform Infrared spectroscopy (ATR-FTIR)
186 spectrum was recorded on a Nicolet iS10 ThermoScientific (Saint Herblain, France) FTIR
187 spectrometer. The exopolysaccharide sample was deposited on a germanium disk. Scans were
188 performed in the wave frequency range of 600 to 4000 (cm^{-1}).
189 *Nuclear Magnetic Resonance (NMR) analysis.*
190 The EPS was dissolved in D_2O for 1D-NMR analysis in a Bruker DRX 500 spectrometer
191 equipped with a 5 mm TBI 1H/(BB)13C probehead at 50°C. The chemical shifts are
192 expressed in parts per million with reference to the external standard TSP (tetrasilyl
193 propionate deutero acid).
194 *High performance size exclusion chromatography*
195 Size exclusion chromatography (SEC) experiments were carried out at room temperature in
196 water (0.1 mol l^{-1} $NaNO_3$ + 50 mg l^{-1} NaN_3 as bacteriostatic agents) using a Thermo Sep
197 pump operating at a flow rate of 1 ml min^{-1} in combination with an automatic Gilson 234
198 injector (injection volume = 0.3 ml with polymer concentration ~ 0.2 g l^{-1}). A dual flow
199 refractive index detector (RI171 from Shodex) and a UV detector (UV2000 from Spectra
200 Physics) operating at 280 nm were used for detection. A column set constituted of one
201 PW6000 TSK column (17 µm, 7.5 x 600 mm) and of a guard DUPONT zorbax Bio series GF
202 450 column (9.4 x 250 mm) filled with hydroxylated polyether to prevent polymer adsorption
203 was used for separation. The columns were calibrated with pectin. Molecular weights were
204 read from the refractometer trace and are expressed as pectin equivalents.

205

206 **Results**

207 *Bioprospection and sampling*

208 Sampling areas were selected to be physically and chemically diverse; a collection of 493
209 marine bacterial isolates was obtained. Those isolates were then screened for their ability to
210 produce EPS (see below): 58% of the isolates produced EPS, and 10% showed a very mucoid
211 aspect on marine agar medium supplemented with 30 g l^{-1} glucose.

212 *Morphology*

213 One of the strains isolated, strain NC470, is a mobile, facultative anaerobe, and a non-
214 luminescent, non-pigmented, Gram-negative rod. Three-day-old colonies on Zobell-glucose
215 were opaque, smooth and gummy colonies of about 1.2 cm in diameter. In the absence of
216 glucose, hemi-translucent white swarming colonies measured about 0.9 cm in diameter
217 formed after three days of growth.

218 *Cultural characteristics*

219 Strain NC470 was selected for its ability to show a mucoid phenotype after 2 days on Zobell
220 medium supplemented with glucose 30 g l^{-1} at 28°C.

221 The optimal growth conditions were identified as being a temperature of 35°C, a pH of 7 and
222 a salinity of 50 g l^{-1} .Under these conditions the doubling time was 30 min.

223 *Metabolic properties*

224 The API 50CH, API 20NE, API 20E kits showed positive responses for: catalase, oxydase,
225 reduction of nitrate to nitrite, indole production, gelatinase, cytochrome oxidase, lysine
226 decarboxylase, acetoïne production and ornithin decarboxylase. The biochemical and
227 nutritional tests showed that the NC470 isolate can use a wide range of carbohydrate
228 substrates as the sole carbon source: N-acetyl-glucosamine, gluconate, malate, inositol,
229 potassium glucuronate, D-glucose, D-mannitol, sucrose and amygdalin.

230 NC470 was resistant to ampicillin, ticarcillin, ticarcillin-clavulanic acid, piperacillin,
231 cefalotin, cefuroxime, tobramycin, and colistin as determined by ATB G- and ATB PSE.

232 *Phylogenetic analyses*

233 BLAST analysis of the 16S rRNA sequence indicated that NC470 belonged to the gamma
234 subdivision of the *Proteobacteria* phylum and was included a member of the *Vibrionaceae*
235 family, order *Vibrionales*. The 16S rRNA sequence was aligned with and compared to 96 type
236 *Vibrio* strains. The three phylogenetic methods used (neighbor-joining, maximum parsimony
237 and maximum likelihood) placed NC470 in a group that includes *Vibrio natriegens* as the
238 most closely related species (Figure 3A) with a percentage of similarity of 95.7%. MLST with
239 four housekeeping genes of NC470 showed a similarity with other *Vibrio* species between
240 71.4 and 90.9%, 61.3 and 95.4%, 61.9 and 81.8% and 65.8 and 95.3%, for the *gapA, gyrB,*
241 *pyrH* and *topA* genes. A phylogenetic tree was constructed with the concatenated sequence of
242 16S rRNA, *gapA*, *gyrB, pyrH* and *topA* genes (Figure 3B) in which NC470 is closest to
243 *Vibrio diabolicus* (92.2% similarity) and *Vibrio alginolyticus* (90.4% similarity).

244 The 16S rRNA (1420 bp) *gapA* (727 bp), *gyrB* (851 bp), *pyrH* (535 bp) and *topA* (711bp)
245 sequences of NC470 and the *Vibrio diabolicus gapA* (717 bp) and *topA* (657 bp), and
246 *Vibrio parahaemolyticus topA* (662 bp) used for this phylogenetic analysis were deposited in
247 NCBI Genebank. The accession Numbers are provided in Table 1.

248 The %G+C was estimated by the Tm value of *Vibrio* type strains that led to a standard curve
249 conforming to the equation %G+C = 0.3677 T*m* + 15.682 such that the %G+C of NC470 was
250 estimated to be 45.9% +/- 0.21 (mean *Tm* = 82.4 +/- 0.56) (supplementary fig 1). The DNA-
251 DNA relatedness between NC470 and the three closest species was determined by
252 hybridization: the ΔT*m* values were 5.7°C for *Vibrio alginolyticus*, 6°C for *Vibrio diabolicus*
253 and 7.6°C for *Vibrio natriegens* (supplementary fig 2).

254 *Production of the EPS*

255 During batch fermentation of NC470, EPS production began at the end of the exponential
256 phase of growth and continued during the entire stationary phase. The concentration of EPS
257 was reaching 2 g l^{-1} (dry weight) at the end of the experiment (72 h).

258 *Characterization of the EPS*

259 The gross chemical composition of the EPS produced by NC470 is listed in Table 2 as well as
260 some other EPS from unusual environments (Loaëc et al. 1997; Cambon-Bonavita et al. 2002;
261 Ortega-Morales et al. 2007; Guezennec et al. 2011). Hexosamines predominated with
262 concentrations up to 40.5% while uronic acids and neutral sugars accounted for 10% and 5%
263 of the total sugars respectively. The low amount of protein (2.5%) indicated a good efficiency
264 of the purification protocol applied to this polymer.

265 FTIR on the EPS produced by strain NC470 showed a broad band beyond 3000 cm^{-1} resulting
266 from O-H and C-H bond stretching absorption bands at 3291 cm^{-1} and 2930 cm^{-1} respectively,
267 and an intense absorption band at 1652 cm^{-1} with a shoulder at 1732 cm^{-1} due to the presence
268 of carboxylic groups on the uronic acids. Moreover, the bands at 1558 and 1645 cm^{-1} were
269 assigned to amino I and amino II groups of osamines, as confirmed by the low amount of
270 protein (2.5%). The absence of a doublet at 1250-1230 cm^{-1} indicated that no sulfate groups
271 were present in this EPS (figure 2A).

272 GC analysis of the monosaccharides as per-*O*-trimethylsilyl methylglycosides showed N-
273 acetyl glucosamine, N-acetyl-galactosamine, glucose, galacturonic acid and glucuronic acid
274 as the main constituents in a molar ratio of 1.5:0.7:1.6:1.1:0.3 (figure 2B). The 1D NMR
275 spectra of the NC 470 EPS in native state showed a complex anomeric region with at least
276 four signals and confirmed the presence of a methyl at 2.08 ppm of acetyl groups, which can
277 be assigned to N-acetylation of the hexosamines (figure 2C).

278 HPSEC showed that the polysaccharide molecular weight (Mw) was of approximately 672 x
279 10^3 Da, with a polydispersity (Ip) of 1.7, thus indicating an homogeneous biopolymer (figure
280 2D). One should note that the lack of low molecular weight polymer might be explained by
281 the ultrafiltration method used that selected polymers higher than 100 kDa.
282

283 **Discussion**

284 Bioprospection in the New Caledonian lagoons aimed to constitute a bank of marine bacteria
285 isolated from these atypical environments. During this program, more than 490 bacterial
286 isolates were collected from various sources along the west coast of New Caledonia.

287 The bacterial isolate NC470 was obtained from the surface of an invertebrate integument. On
288 the basis of morphological and biochemical data, this aero-anaerobic, mesophilic and
289 heterotrophic bacterium clearly belongs to the *Vibrio* genus. Phylogenetic analyses of
290 16S rRNA sequences demonstrate that strain NC470 was different from any other known
291 *Vibrio* species with less than 97% of sequence identity the closest species. According to
292 Stackebrandt (Stackebrandt and Goebel 1994) this degree of identity is clearly lower than the
293 cut-off defining interspecific relationships, and therefore strain NC470 appears to be a novel
294 *Vibrio* species.

295 MLST with four housekeeping genes- *gapA*, *gyrB*, *pyrH* and *topA* (Thompson et al. 2005;
296 Sawabe et al. 2007)- confirmed that NC470 is a new *Vibrio* species. According to Thompson
297 *et al.* (Thompson et al. 2009), a *Vibrio* species is defined as a group of strains that share >95%
298 of DNA identity in MLST. The concatenated 16S rRNA, *gapA*, *gyrB*, *pyrH* and *topA* gene
299 sequences clearly defined a monophyletic cluster including NC470, *Vibrio alginolyticus* and
300 *Vibrio diabolicus* with which it displays similarity of 90.4% and 92.2%, respectively. The
301 study of concatenated gene for phylogenetic analysis has been previously described in recent
302 article on *Vibrio* genus (Chimetto et al. 2011a; Chimetto et al. 2011b; Gomez-Gil et al. 2012).
303 The %G+C of NC470 was estimated as being 45.9% calculated using type strain denaturation
304 curve. DNA-DNA relatedness was further investigated by measuring the ΔTm value for
305 hybrids between NC470 and type strains denaturation curves. All the hybrids obtained
306 showed a ΔTm above 5°C. According to the recommendation of the *ad hoc* committees of
307 1987 and 2002 (Wayne et al. 1987; Stackebrandt et al. 2002) the cut-off for interspecies

308 discrimination for this type of study is 5°C. Therefore, we suggest that NC470 should be
309 considered to be a new member of the *Vibrio* genus for which the name
310 *Vibrio neocaledonicus* is proposed. The type strain *Vibrio neocaledonicus* NC470 has been
311 deposited in the Collection de l'Institut Pasteur (Institut Pasteur, Paris: reference number CIP
312 110538T).

313 This study was part of a larger project to assess the potential of New Caledonian marine
314 bacteria for biotechnological applications. Under non optimized culture conditions, the yield
315 of EPS reached 2 g l^{-1} dry weight. EPS synthesized under laboratory conditions by strain
316 NC470 was mainly characterized by high content of hexosamines (as N-acetyl-glucosamine
317 and N-acetyl galactosamine) accounting for up to 40% of the EPS dry weight. To date only a
318 few marine bacteria have been described to produce EPS with hexosamines as the major
319 constituents with the exception of members of the *Vibrio* genus. *Vibrio diabolicus* strain
320 HE800 *(*Rougeaux et al. 1996) isolated from a *Alvinella pompejana* worm tube collected from
321 a deep-sea hydrothermal field of the East Pacific Rise (Raguenes et al. 1997) produced an
322 EPS characterized by equal amounts of uronic acid and hexosamines (N-acetyl glucosamine
323 and N-acetyl galactosamine) in a molar ratio of approximately 1:0.5:0.5, respectively. EPS
324 from *Vibrio alginolyticus*, a marine fouling bacterium revealed the presence of glucose
325 aminoarabinose, aminoribose and xylose in the molar ratio of 2:1:9:1 (Muralidharan and
326 Jayachandran 2003). More recently a new EPS bacterial producer was isolated from a
327 microbial mat located on atolls in French Polynesia. This biopolymer was characterized by
328 the presence of N-acetyl glucosamine, N-acetyl galactosamine, glucuronic acid, glucose and
329 galactose in a molar ratio of 2:1:1:0.1.0.2 (personal data).

330 *Vibrio parahaemolyticus* was shown to produce an EPS consisting of four major sugars, *i.e*
331 glucose, galactose, fucose and N-acetylglucosamine (Enos-Berlage and McCarter 2000,
332 Kavita et al. 2011). *Vibrio cholerae* is also an EPS producing bacterium with as major

333 components N-acetyl-D-glucosamine, D-mannose, 6-deoxy-D-galactose, and D-galactose in a
334 molar ratio of 7.4:10.2:2.4:3.0 (Wai et al. 1998). EPS from other *Vibrio* sp such as,
335 *Vibrio harveyi* (Bramhachari and Dubey 2006) and *Vibrio vulnificus* (Reddy et al. 1992) also
336 were also characterized by the presence of amino sugars.

337 The presence of high content of hexosamines and uronic acids raise interesting questions
338 about the biotechnological potential of (Gomez-Gil et al. 2012)such EPS. The high molecular
339 weight linear bacterial exopolysaccharide produced by *Vibrio diabolicus* strain HE 800
340 appears to be a strong bone-healing material. Introducing this new glycosylaminoglycan-like
341 (GAG) polysaccharide in critical size defects in bone in rats induces a nearly complete
342 healing within no longer than 2 weeks with a total restore of the anatomy of the defect with
343 trabecular and cortical structure This hyaluronic acid-like EPS constitutes a material which
344 potentiates bone repair and its particular activity has to be related its original physicochemical
345 characteristics (Zanchetta et al. 2003a; b). Exopolysaccharide produced by strain NC 470 also
346 has a composition similar to both other well-known biologically active glycosaminoglycans
347 (GAGs) and to the extracellular matrix. Both of these bioactive molecule polysaccharides and
348 the extracellular matrix are known to play a major role in the first healing steps and during
349 bone or injury healing (Jerdan et al. 1991; Esford et al. 1998; Lipscombe et al. 1998).

350 Mining represents the major economic activity in New Caledonia (Dalvi et al. 2004), and
351 generates residues laden with heavy metals that are released into the environment
352 (http://www.goronickel-icpe.nc/global/pages/02-impacts/section). Therefore, alternative
353 solutions to heavy metal discharge would be of great interest from an industrial and ecological
354 point of view. In that bacterial EPS can serve as an alternative source of low-cost biosorbents.
355 EPS contain ionisable functional groups such as carboxyl, amine, sulfate and to a lesser extent
356 hydroxyl groups that enable these biopolymers to bind heavy metals (Loaëc et al. 1997).

357 Preliminary experiments conducted on EPS produced NC470 strain showed a high
358 metal binding capacity with values up to 370 mg Cu(II) g^{-1} EPS and up to
359 70 mg Ni(II) g^{-1} EPS. Similar high uptake capacities were found for hexosamine rich
360 exopolysaccharide produced by a marine bacterium isolated from a microbial mat in a
361 Polynesian atoll with 400 mg Cu(II) g^{-1} EPS and 65mg Ni(II) g^{-1} EPS respectively
362 (Guezennec et al. 2011). Further structural elucidation will allow a better understanding of the
363 mechanism of this affinity and also determine the potential commercial value of this EPS as a
364 biosorbent for a variety of heavy metals.
365
366
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378

Bibliography

Balcázar, J.L., Pintado, J. and Planas, M. (2010) Vibrio hippocampi sp. nov., a new species isolated from wild seahorses (Hippocampus guttulatus). *FEMS Microbiology Letters* **307**, 30-34.

Beaz-Hidalgo, R., Diéguez, A.L., Cleenwerck, I., Balboa, S., Doce, A., de Vos, P. and Romalde, J.L. (2010) Vibrio celticus sp. nov., a new Vibrio species belonging to the Splendidus clade with pathogenic potential for clams. *Systematic and Applied Microbiology* **33**, 311-315.

Belcher, R., Nutten, A.J. and Sambrook, C.M. (1954) The determination of glucosamine. *Analyst* **79**, 201-208.

Blumenkrantz, N. and Asboe-Hansen, G. (1973) New method for quantitative determination of uronic acids. *Analytical Biochemistry* **54**, 484-489.

Boyle, C.D. and Reade, A.E. (1983) Characterization of Two Extracellular Polysaccharides from Marine Bacteria. *Applied and Environmental Microbiology* **46**, 392-399.

Bramhachari, P.V. and Dubey, S.K. (2006) Isolation and characterization of exopolysaccharide produced by Vibrio harveyi strain VB23. *Letters in Applied Microbiology* **43**, 571-577.

Cambon-Bonavita, M.A., Raguénès, G., Jean, J., Vincent, P. and Guezennec, J. (2002) A novel polymer produced by a bacterium isolated from a deep-sea hydrothermal vent polychaete annelid. *Journal of Applied Microbiology* **93**, 310-315.

Chimetto, L., Cleenwerck, I., Moreira, A.P.B., Brocchi, M., Willems, A., De Vos, P. and Thompson, F. (2011a) Vibrio variabilis sp. nov. and Vibrio marinus sp. nov., isolated from Palythoa caribaeorum. *International Journal of Systematic and Evolutionary Microbiology* **61**, 3009-3015.

Chimetto, L.A., Cleenwerck, I., Alves, N., Silva, B.S., Brocchi, M., Willems, A., De Vos, P. and Thompson, F.L. (2011b) Vibrio communis sp. nov. isolated from marine animals (Mussismilia hispida, Phyllogorgia dilatata, Palythoa caribaeorum, Palythoa variabilis and Litopenaeus vannamei). *International Journal of Systematic and Evolutionary Microbiology* **61**, 362-368.

Dalvi, A.D., Bacon, W.G. and Osborne, R.C. (2004) Past and future of nickel laterite projects. *International Laterite Nickel Symposium 2004*, 27.

410 De Ley, J. (1970) Reexamination of the Association Between Melting Point, Buoyant
411 Density, and Chemical Base Composition of Deoxyribonucleic Acid. *Journal of Bacteriology*
412 **101**, 738-754.
413 Decho, A.W. and Herndl, G.J. (1995) Microbial activities and the transformation of organic
414 matter within mucilaginous material. *Science of The Total Environment* **165**, 33-42.
415 DuBois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. (1956) Colorimetric
416 Method for Determination of Sugars and Related Substances. *Analytical Chemistry* **28**, 350-
417 356.
418 Esford, L.E., Maiti, A., Bader, S.A., Tufaro, F. and Johnson, P. (1998) Analysis of CD44
419 interactions with hyaluronan in murine L cell fibroblasts deficient in glycosaminoglycan
420 synthesis: a role for chondroitin sulfate. *Journal of Cell Science* **111**, 1021-1029.
421 Felsenstein, J. (1992) Evolutionary trees from DNA sequences: a maximum likelihood.
422 *Evolution* **46**, 159-173.
423 Flemming, H.-C., Neu, T.R. and Wozniak, D.J. (2007) The EPS Matrix: The "House of
424 Biofilm Cells". *Journal of Bacteriology* **189**, 7945-7947.
425 Galtier, N., Gouy, M. and Gautier, C. (1996) SEAVIEW and PHYLO_WIN: two graphic
426 tools for sequence alignment and molecular phylogeny. *Computer applications in the*
427 *biosciences : CABIOS* **12**, 543-548.
428 Gomez-Gil, B., Roque, A., Chimetto, L., Moreira, A.P.B., Thompson, F.L. and Lang, E.
429 (2012) Vibrio alfacsensis sp. nov., isolated from marine organisms. *International Journal of*
430 *Systematic and Evolutionary Microbiology* **In press**.
431 Gonzalez, J.M. and Saiz-Jimenez, C. (2002) A fluorimetric method for the estimation of G+C
432 mol% content in microorganisms by thermal denaturation temperature. *Environmental*
433 *Microbiology* **4**, 770-773.
434 Guezennec, J, Pignet, P, Lijour, Y, Gentric, E, Ratiskol, J, Colliec, J. and S (1998a) Sulfation
435 and depolymerization of a bacterial exopolysaccharide of hydrothermal origin. *Carbohydrate*
436 *Polymers* **37**, 14-24.
437 Guezennec, J., Moppert, X., Raguenes, G., Richert, L., Costa, B. and Simon-Colin, C. (2011)
438 Microbial mats in French Polynesia and their biotechnological applications. *Process*
439 *Biochemistry* **46**, 16-22.
440 Guezennec, J., Ortega-Morales, O., Raguenes, G. and Geesey, G. (1998b) Bacterial
441 colonization of artificial substrate in the vicinity of deep-sea hydrothermal vents. *FEMS*
442 *Microbiology Ecology* **26**, 89-99.

443 Gutnick, D.L. and Bach, H. (2000) Engineering bacterial biopolymers for the biosorption of
444 heavy metals; new products and novel formulations. *Applied microbiology and biotechnology*
445 **54**, 451-460.
446 Hall, T. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis
447 program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**, 95-98.
448 Høiby, N., Bjarnsholt, T., Givskov, M., Molin, S. and Ciofu, O. (2009) Antibiotic resistance
449 of bacterial biofilms. *International Journal of Antimicrobial Agents* **35**, 322-332.
450 Iyer, A., Mody, K. and Jha, B. (2004) Accumulation of hexavalent chromium by an
451 exopolysaccharide producing marine Enterobacter cloaceae. *Marine Pollution Bulletin* **49**,
452 974-977.
453 Iyer, A., Mody, K. and Jha, B. (2005) Biosorption of heavy metals by a marine bacterium.
454 *Marine Pollution Bulletin* **50**, 340-343.
455 Jerdan, J.A., Michels, R.G. and Glaser, B.M. (1991) Extracellular matrix of newly forming
456 vessels--an immunohistochemical study. *Microvascular Research* **42**, 255-265.
457 Kimura, M. (1985) *The Neutral Theory of Molecular Evolution.*: Cambridge: Cambridge
458 University Press.
459 Kluge, A.G. and Farris, J.S. (1969) Quantitative Phyletics and the Evolution of Anurans.
460 *Systematic Zoology* **18**, 1-32.
461 Lipscombe, R.J., Nakhoul, A.M., Sanderson, C.J. and Coombe, D.R. (1998) Interleukin-5
462 binds to heparin/heparan sulfate. A model for an interaction with extracellular matrix. *Journal*
463 *of Leukocyte Biology* **63**, 342-350.
464 Loaëc, M., Olier, R. and Guezennec, J. (1997) Chelating properties of bacterial
465 exopolysaccharides from deep-sea hydrothermal vents. *Carbohydrate Polymers* **35**, 65-70.
466 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) Protein measurement
467 with the Folin phenol reagent. *Journal of Biological Chemistry* **193**, 265-275.
468 Montreuil, J., Bouquelet, S., Debray, H., Fournet, B., Spik, G. and Strecker, G. (1986)
469 *Glycoproteins*. Oxford IRL Press.
470 Moppert, X., Le Costaouec, T., Raguenes, G., Courtois, A., Simon-Colin, C., Crassous, P.,
471 Costa, B. and Guezennec, J. (2009) Investigations into the uptake of copper, iron and
472 selenium by a highly sulphated bacterial exopolysaccharide isolated from microbial mats.
473 *Journal of Industrial Microbiology & Biotechnology* **36**, 599-604.
474 Moreira, A.P.B., Pereira, N. and Thompson, F.L. (2010) Usefulness of a real time PCR
475 platform for GC content and DNA-DNA hybridization similarity estimations in vibrios.
476 *International Journal of Systematic and Evolutionary Microbiology* **61**, 2379-2383.

477 Muralidharan, J. and Jayachandran, S. (2003) Physicochemical analyses of the
478 exopolysaccharides produced by a marine biofouling bacterium, Vibrio alginolyticus. *Process*
479 *Biochemistry* **38**, 841-847.
480 Nichols, C.A.M., Guezennec, J. and Bowman, J.P. (2005) Bacterial Exopolysaccharides from
481 Extreme Marine Environments with Special Consideration of the Southern Ocean, Sea Ice,
482 and Deep-Sea Hydrothermal Vents: A Review. *Marine Biotechnology* **7**, 253-271.
483 Okutani, K. (1984) Antitumor and immunostimulant activities of polysaccharide produced by
484 marine bacterium of the genus Vibrio. *Bulletin of the Japanese Society of Scientific Fisheries*
485 **50**, 1035-1037.
486 Okutani, K. (1992) Antiviral activities of sulfated derivatives of a fucosamine-containing
487 polysaccharide of marine bacterial origin. *Nipon Suisan Gakkai* **58**, 927-930.
488 Ortega-Morales, B.O., Santiago-García, J.L., Chan-Bacab, M.J., Moppert, X., Miranda-Tello,
489 E., Fardeau, M.L., Carrero, J.C., Bartolo-Pérez, P., Valadéz-González, A. and Guezennec, J.
490 (2007) Characterization of extracellular polymers synthesized by tropical intertidal biofilm
491 bacteria. *Journal of Applied Microbiology* **102**, 254-264.
492 Perrière, G. and Gouy, M. (1996) WWW-query: An on-line retrieval system for biological
493 sequence banks. *Biochimie* **78**, 364-369.
494 Raguenes, G., Christen, R., Guezennec, J., Pignet, P. and Barbier, G. (1997) Vibrio diabolicus
495 sp. nov., a New Polysaccharide-Secreting Organism Isolated from a Deep-Sea Hydrothermal
496 Vent Polychaete Annelid, Alvinella pompejana *International Journal of Systematic*
497 *Bacteriology* **47**, 989-995.
498 Rameshkumar, N., Sproer, C., Lang, E. and Nair, S. (2010) Vibrio mangrovi sp. nov., a
499 diazotrophic bacterium isolated from mangrove-associated wild rice (Poteresia coarctata
500 Tateoka). *FEMS Microbiology Letters* **307**, 35-40.
501 Rasmussen, R.S., Morrissey, M.T. and Steve, L.T. (2007) Marine Biotechnology for
502 Production of Food Ingredients. *Advances in Food and Nutrition Research* **52**, 237-292.
503 Reddy, G.P., Hayat, U., Abeygunawardana, C., Fox, C., Wright, A.C., Maneval, D.R., Jr.,
504 Bush, C.A. and Morris, J.G., Jr. (1992) Purification and determination of the structure of
505 capsular polysaccharide of Vibrio vulnificus M06-24. *Journal of Bacteriology* **174**, 2620-
506 2630.
507 Rougeaux, H., Guezennec, M., Che, L.M., Payri, C., Deslandes, E. and Guezennec, J. (2001)
508 Microbial Communities and Exopolysaccharides from Polynesian Mats. *Marine*
509 *Biotechnology* **3**, 181-187.

510 Rougeaux, H., Pichon, R., Kervarec, N., Raguénès, G.H.C. and Guezennec, J. (1996) Novel
511 bacterial exopolysaccharides from deep-sea hydrothermal vents. *Carbohydrate Polymers* **31**,
512 237-242.
513 Saitou, N. and Nei, M. (1987) The neighbor-joining method: a new method for reconstructing
514 phylogenetic trees. *Molecular Biology and Evolution* **4**, 406-425.
515 Sawabe, T., Kita-Tsukamoto, K. and Thompson, F.L. (2007) Inferring the Evolutionary
516 History of Vibrios by Means of Multilocus Sequence Analysis. *Journal of Bacteriology* **189**,
517 7932-7936.
518 Sheu, S.-Y., Jiang, S.-R., Chen, C.A., Wang, J.-T. and Chen, W.-M. (2010) Vibrio
519 stylophorae sp. nov., isolated from the reef-building coral Stylophora pistillata. *International*
520 *Journal of Systematic and Evolutionary Microbiology* **61**, 2180-2185.
521 Stackebrandt, E., Frederiksen, W., Garrity, G.M., Grimont, P.A.D., Kräpfer, P., Maiden,
522 M.C.J., Nesme, X., Rossello-Mora, R., Swings, J., Trüper, H.G., Vauterin, L., Ward, A.C. and
523 Whitman, W.B. (2002) Report of the ad hoc committee for the re-evaluation of the species
524 definition in bacteriology. *International Journal of Systematic and Evolutionary Microbiology*
525 **52**, 1043-1047.
526 Stackebrandt, E. and Goebel, B.M. (1994) Taxonomic Note: A Place for DNA-DNA
527 Reassociation and 16S rRNA Sequence Analysis in the Present Species Definition in
528 Bacteriology. *International Journal of Systematic Bacteriology* **44**, 846-849.
529 Suresh Kumar, A., Mody, K. and Jha, B. (2007) Bacterial exopolysaccharides – a perception.
530 *Journal of Basic Microbiology* **47**, 103-117.
531 Thompson, C., Vicente, A., Souza, R., Vasconcelos, A., Vesth, T., Alves, N., Ussery, D., Iida,
532 T. and Thompson, F. (2009) Genomic taxonomy of vibrios. *BMC Evolutionary Biology* **9**,
533 258.
534 Thompson, F.L., Gevers, D., Thompson, C.C., Dawyndt, P., Naser, S., Hoste, B., Munn, C.B.
535 and Swings, J. (2005) Phylogeny and Molecular Identification of Vibrios on the Basis of
536 Multilocus Sequence Analysis. *Applied and Environmental Microbiology* **71**, 5107-5115.
537 Wai, S.N., Mizunoe, Y., Takade, A., Kawabata, S.-I. and Yoshida, S.-I. (1998) Vibrio
538 cholerae O1 Strain TSI-4 Produces the Exopolysaccharide Materials That Determine Colony
539 Morphology, Stress Resistance, and Biofilm Formation. *Applied and Environmental*
540 *Microbiology* **64**, 3648-3655.
541 Wang, H., Liu, J., Wang, Y. and Zhang, X.-H. (2011) Vibrio marisflavi sp. nov., isolated
542 from seawater. *International Journal of Systematic and Evolutionary Microbiology* **61**, 568-
543 573.

544 Wayne, L.G., Brenner, D.J., Colwell, R.R., Grimont, P.A.D., Kandler, O., Krichevsky, M.I.,
545 Moore, L.H., Moore, W.E.C., Murray, R.G.E., Stackebrandt, E., Starr, M.P. and Trüper, H.G.
546 (1987) Report of the ad hoc committee on reconciliation of approaches to bacterial
547 systematics. *International Journal of Systematic Bacteriology* **37**, 463-464.
548 Work, E. (1961) The Mucopeptides of Bacterial Cell Walls. A Review. *Journal of General*
549 *Microbiology* **25**, 167-189.
550 Xu, H.X., Kawamura, Y., Li, N., Zhao, L., Li, T.M., Li, Z.Y., Shu, S. and Ezaki, T. (2000) A
551 rapid method for determining the G+C content of bacterial chromosomes by monitoring
552 fluorescence intensity during DNA denaturation in a capillary tube. *International Journal of*
553 *Systematic and Evolutionary Microbiology* **50**, 1463-1469.
554 Zanchetta, P., Lagarde, N. and Guezennec, J. (2003a) A New Bone-Healing Material: A
555 Hyaluronic Acid-Like Bacterial Exopolysaccharide. *Calcified Tissue International* **72**, 74-79.
556 Zanchetta, P., Lagarde, N. and Guezennec, J. (2003b) Systemic Effects on Bone Healing of a
557 New Hyaluronic Acid-Like Bacterial Exopolysaccharide. *Calcified Tissue International* **73**,
558 232-236.
559 ZoBell, C.E. (1941) Studies on marine bacteria. The cultural requirements of heterotrophic
560 aerobes. *Journal of Marine Research* **4**, 42-75.
561
562

563 Table 1: Sequence accession numbers of strains used for phylogenetic analyses

Species	Strain	16S rRNA	gapA	gyrB	pyrH	topA
Vibrio alginolyticus	ATCC 17749	*X56576*	*DQ907274*	*AB298202*	*FM202578*	*DQ907472*
Vibrio azureus	LC2-005	*AB428897*				
Vibrio campbellii	ATCC 25920	*X56575*	*EF596565*	*EU130500*	*FM202551*	*DQ907475*
Vibrio cholerae	CECT 514[T]	*X76337*	*DQ907273*	*FM202624*	*FM202582*	*DQ907478*
Vibrio communis	R-40496	*GU078672*	*AB609125*	*GU078680*	*GU078691*	*GU078704*
Vibrio diabolicus	HE800	*X99762*	***JQ934821****	***JQ972871****	*JF739414*	***JQ934822****
Vibrio harveyi	NCIMB1280[T]	*AY750575*	*EF596145*	*AB298221*	*FM202541*	*GQ428295*
Vibrio jasicida	TCFB 0772[T]	*AB562592*	*AB562597*	*AB562598*	*AB562600*	*AB562602*
Vibrio mytili	CECT 632	*X99761*	*DQ907293*	*AB298231*	*GU266287*	*DQ907499*
Vibrio natriegens	ATCC 14048[T]	*X74714*	*DQ907294*	*AB298232*	*FM202573*	*DQ907500*
Vibrio parahaemolyticus	ATCC 17802	*AF388386*	*DQ449618*	*AB298239*	*EU118240*	***JQ934827****
Vibrio rotiferianus	LMG 21460	*AJ316187*	*DQ449619*	*AB298244*	*FM202568*	*DQ907515*
Vibrio sagamiensis	LC2-047	*AB428909*				
Vibrio xiamenensis	G21[T]	*GQ397859*				
Vibrio neocaledonicus sp. nov	NC470	***JQ934828****	***JQ934823****	***JQ934823****	***JQ934825****	***JQ934826****

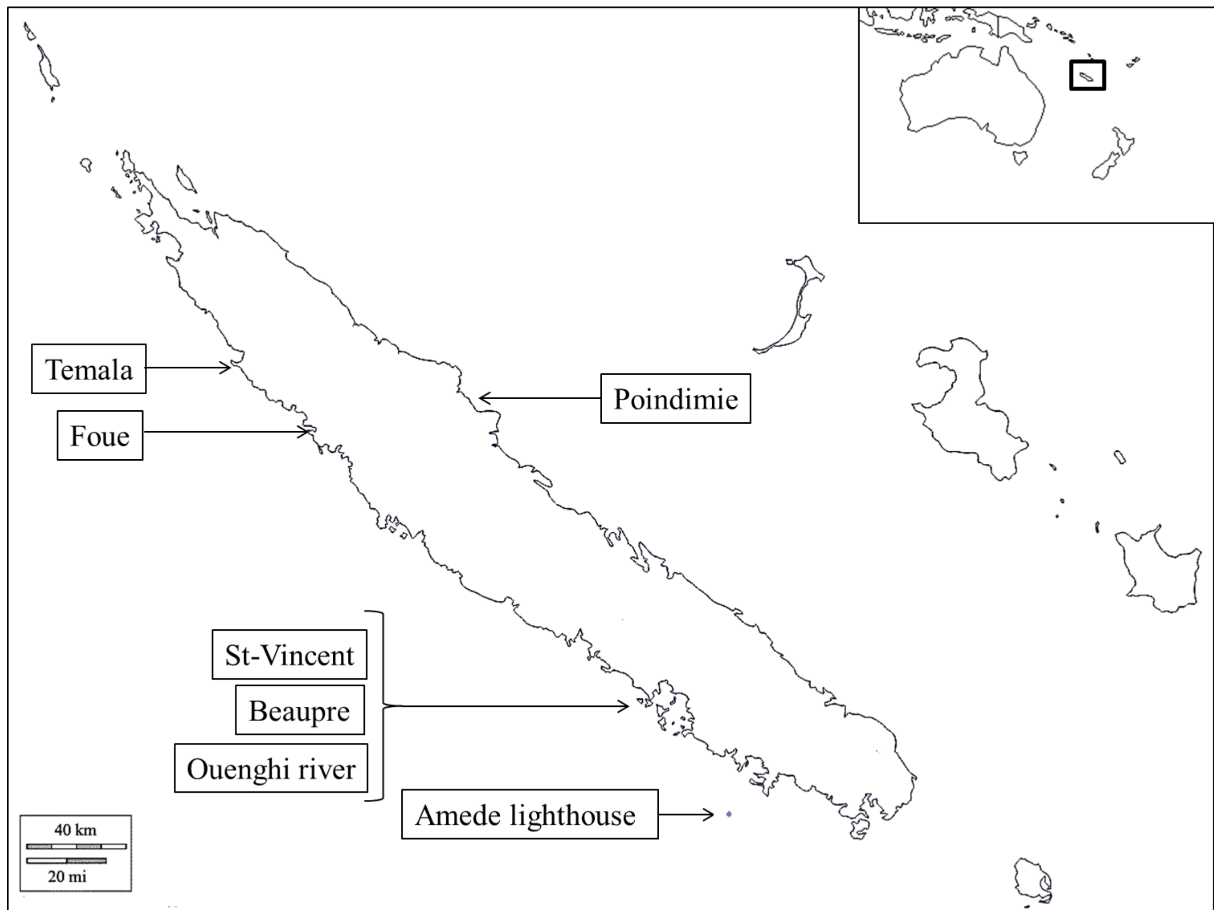
564 *sequences submitted to GeneBank data base

565 Table 2: Gross chemical composition of NC470 exopolysaccharide and comparison with other microorganisms (% w/w)

EPS	Protein	Hexosamines	Hexuronic acids	Neutral sugars	Reference
NC470	2,5	40,5	10	5	Present study
Vibrio diabolicus HE800	2,5	33	32	2,5	(Loaëc et al. 1997)
Vibrio sp (Mo 245)	2	30	27	11	(Guezennec et al. 2011)
Microbacterium sp (MC6B-22)	8,9	21,15	14,7	5,5	(Ortega-Morales et al. 2007)
Alteromonas infernus	5.5	-	37	51	(Cambon-Bonavita et al. 2002)

566 Figure 1: Map of New Caledonia showing the sites sampled during the 2010 bioprospection

567 program.



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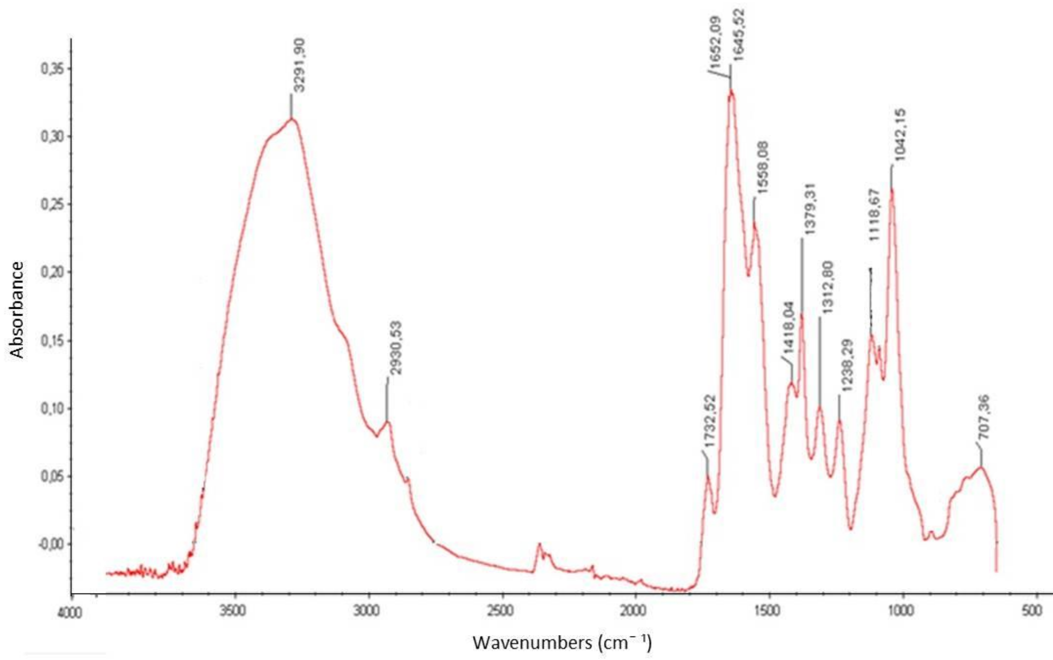
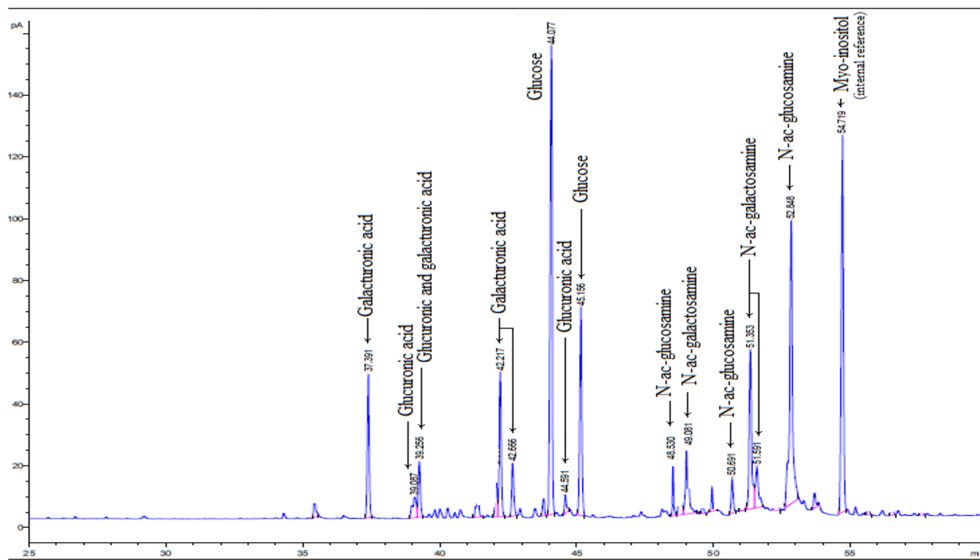
570 Figure 2: The chemical analysis performed on the EPS produced by NC470 exposing four

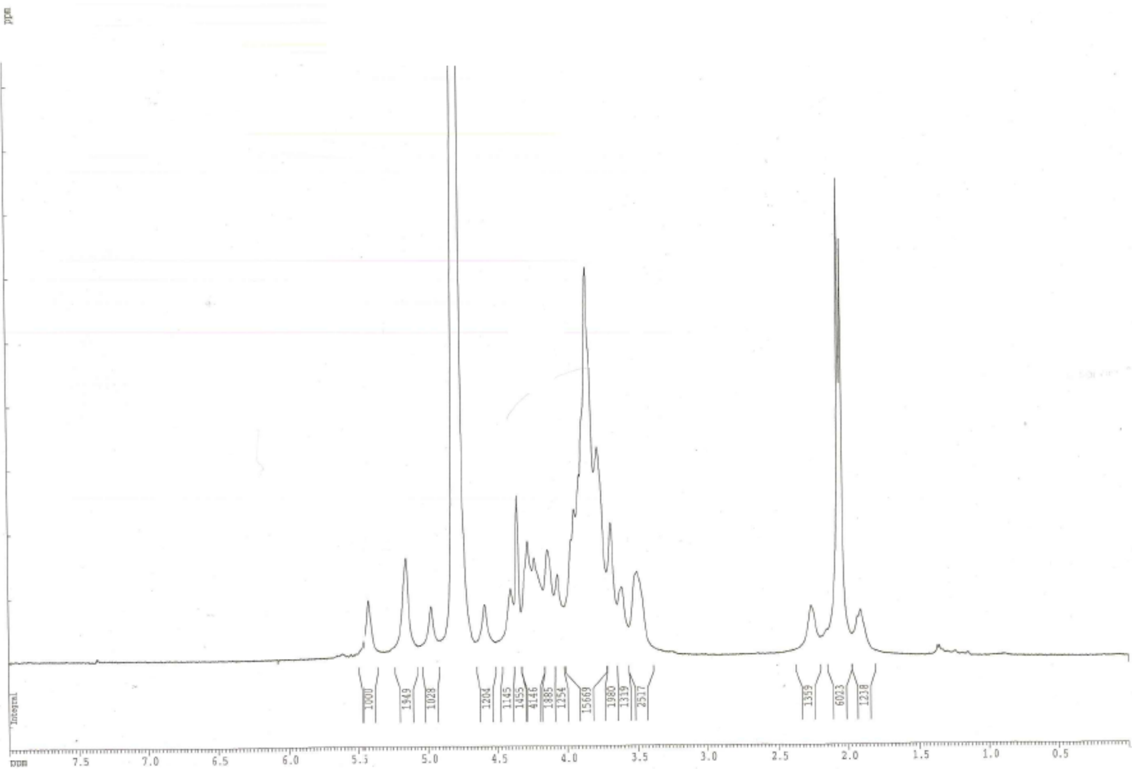
571 panel data with a) FTIR spectra, b) GC spectra, c) 1D RMN spectra and d) SEC plots.



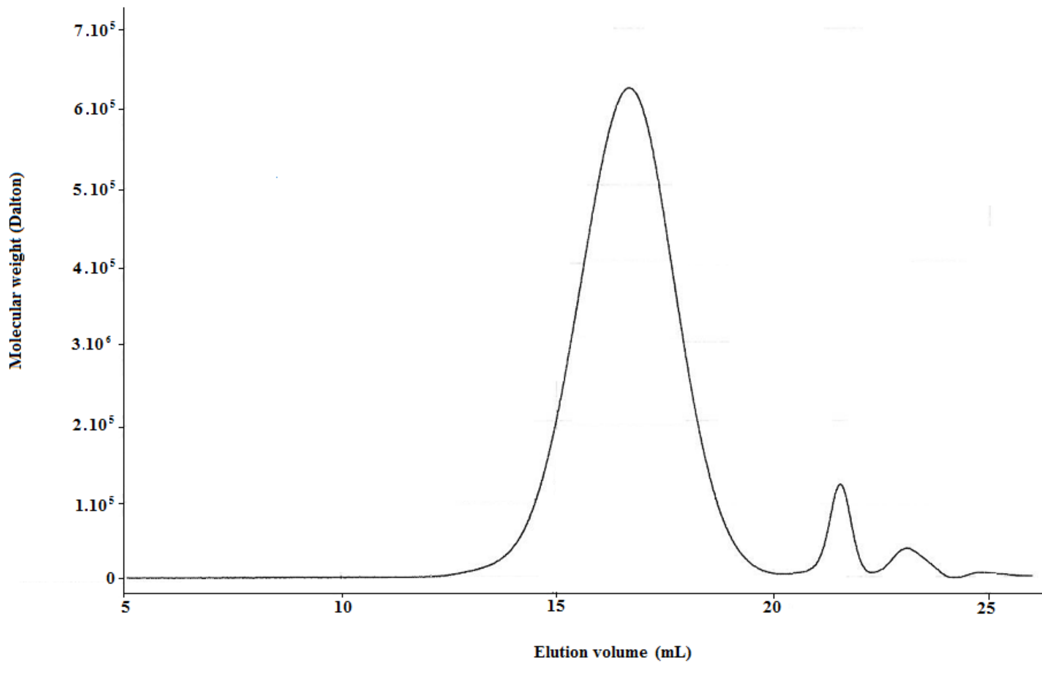
572 A)



573 B)



574 C)

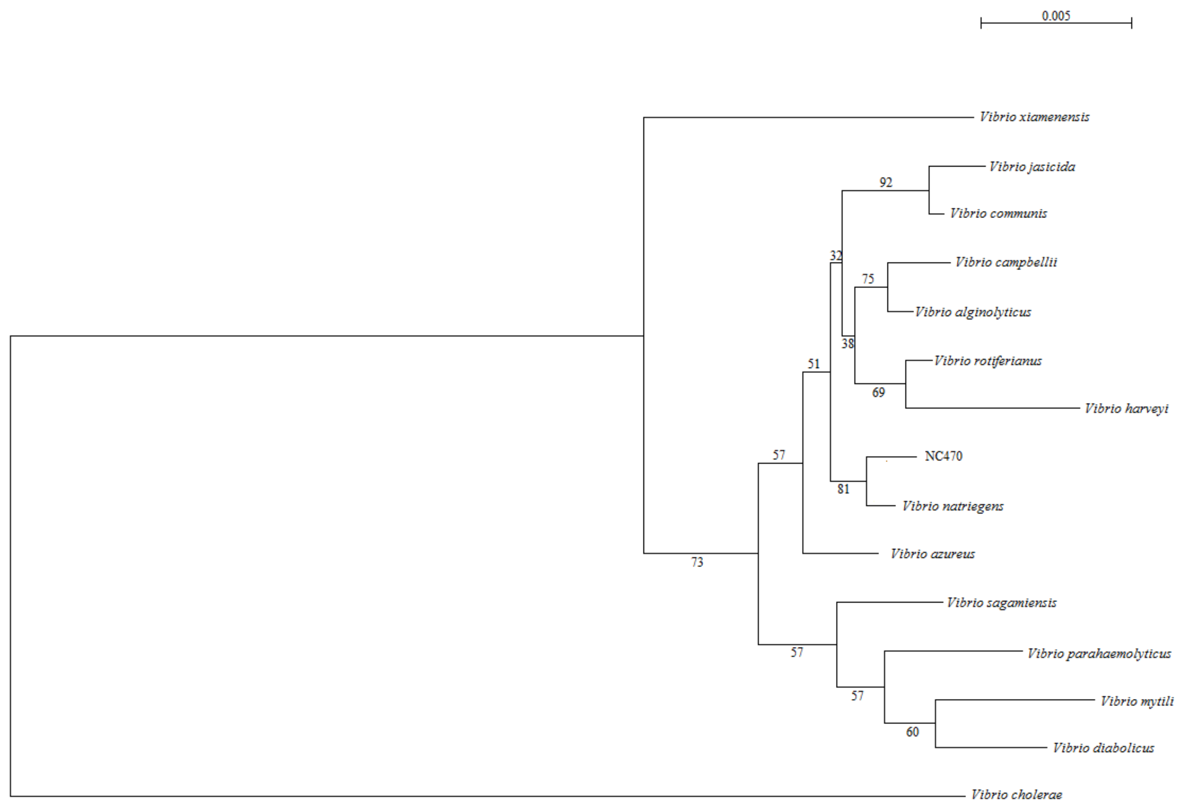


575 D)

576

577 Figure 3: Neighbor-joining phylogenetic tree using a subset of the *Vibrio* genus and showing
578 the position of NC470 on basis of the 16S rRNA gene sequence (A) and a second tree based
579 on the analysis of a concatenated 16S rRNA, *gapA, gyrB, pyrH* and *topA* sequence analysis
580 (B). Evolutionary distances were evaluated using the Kimura-2 parameter. Bootstrap values
581 are indicated at nodes and the level is based on 500 repetitions. *Vibrio cholerae* was used as
582 an out group to root the tree.

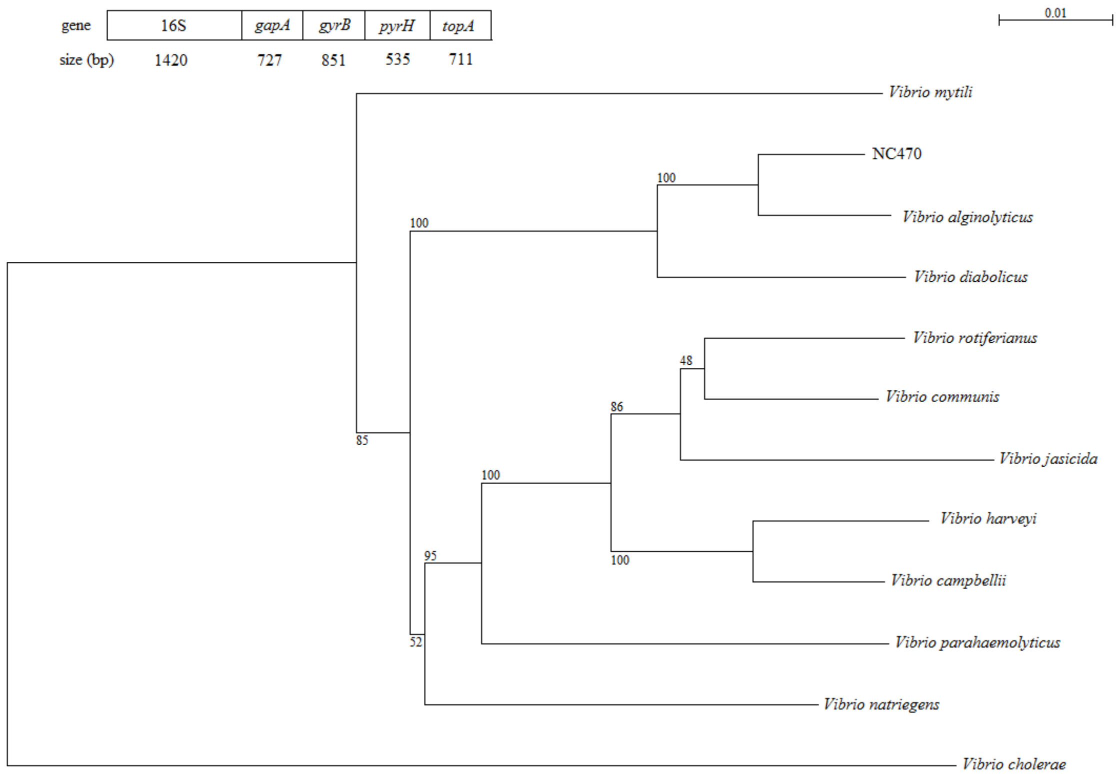
583 A) 16S rRNA gene sequence analysis



584

585 B) Analysis of five concatenated gene sequences (16S, *gapA, gyrB, pyrH* and *topA 4244*

586 *bp*) of NC470



587

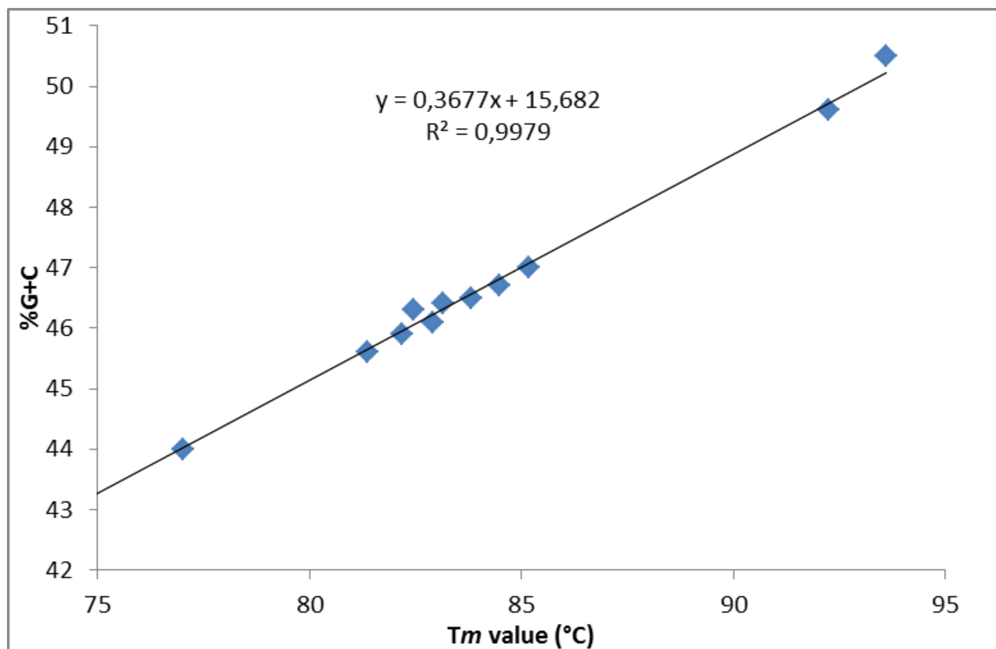
588 Supplementary figure S1: Estimation of %G+C of NC470 using the standard curve equation

589 determined from the T*m* values (measured twice: T*m*1 and T*m*2) of 12 *Vibrio* type species.

Strain	Acession number	T*m* 1	T*m* 2	T*m* mean	%G+C
NC470		81,85	82,97	**82,41**	45,98
Vibrio alginolyticus	CIP 103336	82,51	83,3	**82,905**	**46,09**
Vibrio campbellii	LMG 11216	82,5	87,9	**85,2**	**47**
Vibrio coralliilyticus	CIP 102760	79,7	83,0	**81,4**	**45,6**
Vibrio diabolicus	HE800	90,6	93,9	**92,2**	**49,6**
Vibrio fischeri	ATCC 700601	60,7	62,3	**61,5**	**38,3**
Vibrio harveyi	CIP 103192	82,5	85,2	**83,8**	**46,5**
Vibrio natriegens	DSM 759	81,1	83,8	**82,5**	**46,3**
Vibrio neptunis	CIP 108274	81,4	83,0	**82,2**	**45,9**
Vibrio proteolyticus	ATCC 15338T	93,3	93,9	**93,6**	**50,5**
Vibrio penaecida	KH1	84,29	84,68	**84,485**	**46,7**
Vibrio tubiashii	CIP 102760	75,7	78,4	**77,0**	**44**
Vibrio vulnificus	ATCC 27562 T	82,5	83,8	**83,1**	**46,4**

Type species (rows *Vibrio alginolyticus* to *Vibrio vulnificus*)

590



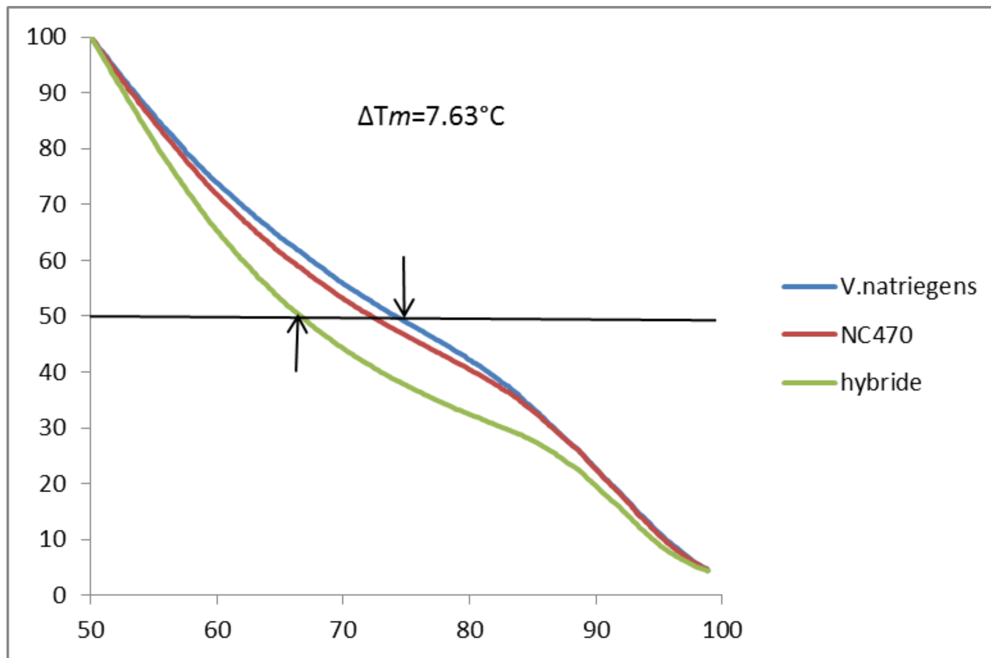
591

592 <u>Supplementary figure S2</u>: Determination of DNA-DNA relatedness between NC470 and the

593 three closest species.

594 A) Melting curve generated using the Light Cycler 2 for ΔT*m* determination. Comparison

595 between NC470 and *Vibrio natriegens* provide a ΔT*m* of **7.63°C**.



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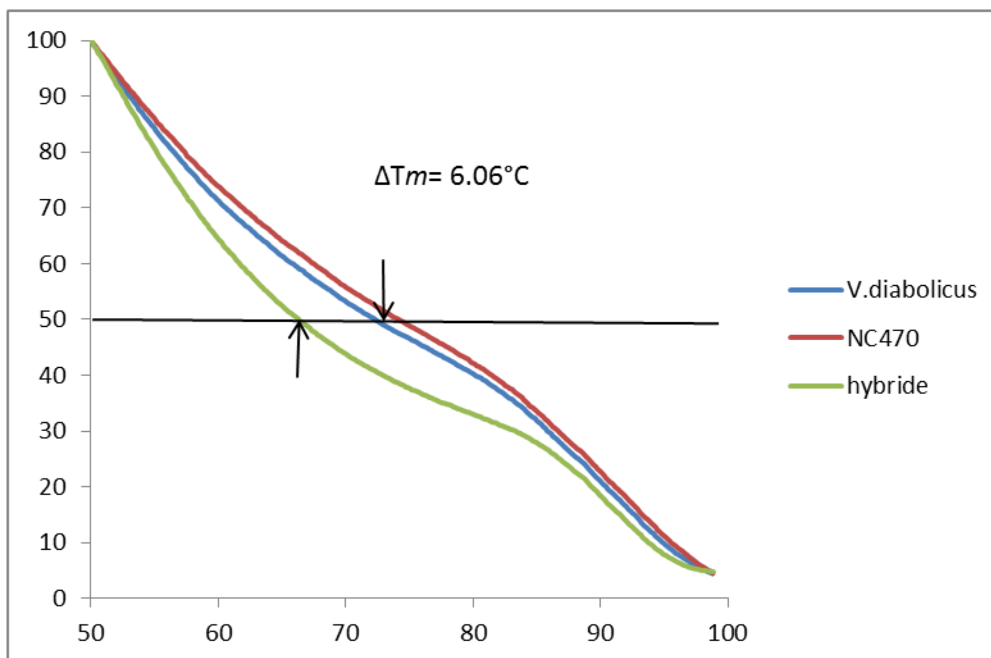
597 B) Melting curve generated using the Light Cycler 2 for ΔT*m* determination. Comparison

598 between NC470 and *Vibrio diabolicus* provides a ΔT*m* of **6.06°C**.



599

600

601 C) Melting curve generated using the Light Cycler 2 for ΔTm determination. Comparison

602 between NC470 and *Vibrio alginolyticus* provides a ΔTm of **6.06°C**.

603