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Investigation of halotolerant marine *Staphylococcus* sp. CO100, as a promising hydrocarbon-degrading and biosurfactant-producing bacterium, under saline conditions

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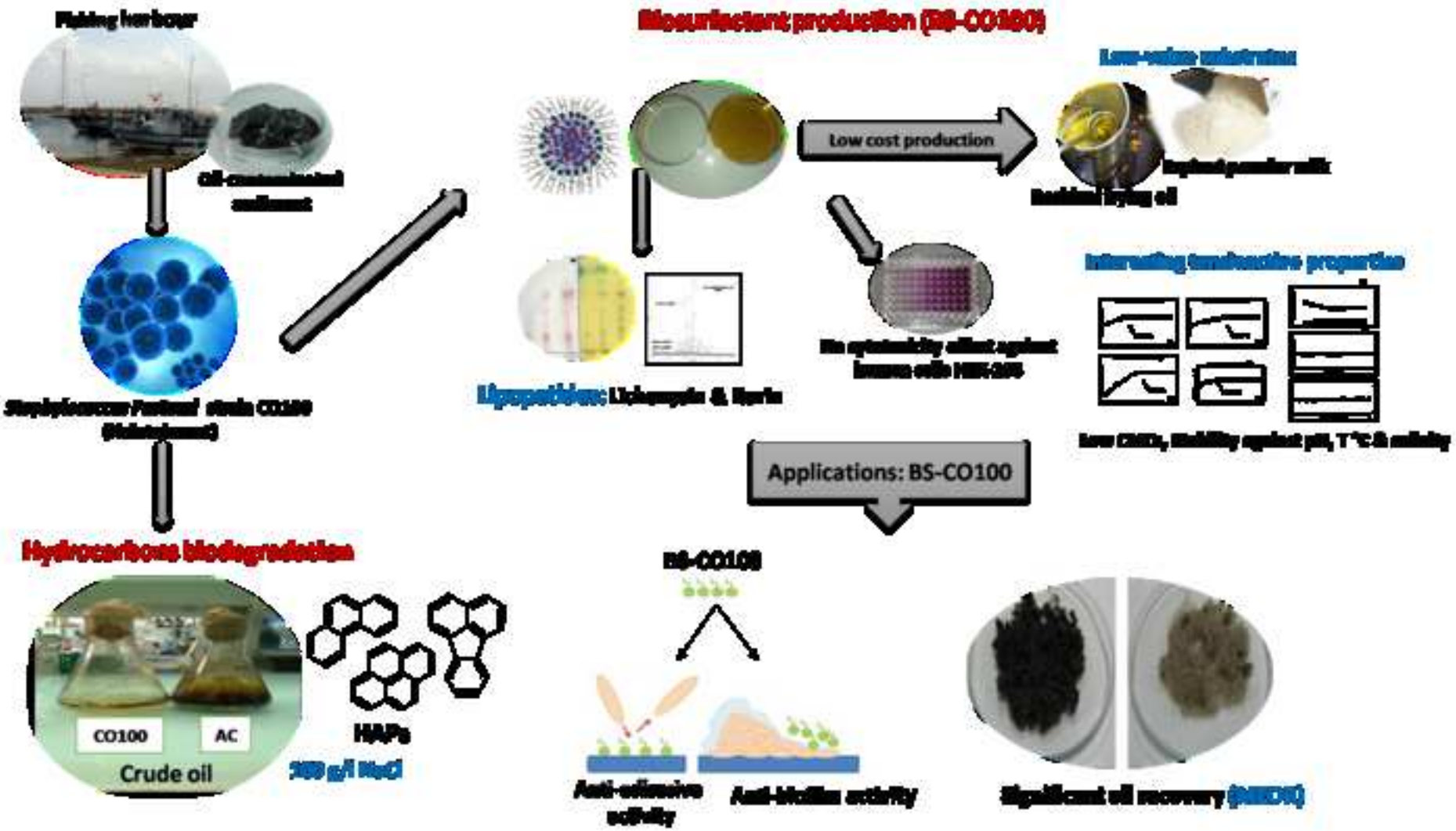
*Graphical Abstract



Highlights:

- Halotolerant isolate CO100 as a suitable candidate for hydrocarbons bioremediation.
- Production of biosurfactants BS-CO100 from low-value carbon sources.
- No cytotoxic effect of BS-CO100 toward human HEK293 cells.
- Promising applications of BS-CO100 in oil recovery and biofilm control.
- First report studying bioremediation capacities by *Staphylococcus pasteuri* species.

ABSTRACT

A halotolerant strain CO100 of *Staphylococcus* sp. was isolated from contaminated sediments taken from the fishing harbour of Sfax, Tunisia, as efficient hydrocarbonoclastic candidate. Strain CO100 exhibited high capacity to break down almost 72% of the aliphatic hydrocarbons contained in crude oil (1%, v/v), used as the sole carbon and energy source, after 20 days of culture, at 100 g/l NaCl, 37 °C and 180 rpm. The isolate CO100 displayed also its ability to grow on phenanthrene, fluoranthene and pyrene (100 mg/l), at 100 g/l NaCl. Moreover, the isolate CO100 showed a notable aptitude to synthesize an efficient tensioactive agent namely BS-CO100, on low-value substrates including residual frying oil (RFO) and expired milk powder (EPM), thus reducing the high cost of biosurfactant production. ESI/MS analysis designated that BS-CO100 belonged to lipopeptide class, in particular lichenysin and iturine members. Critical micelle concentrations (CMCs) of BS-CO100 were varying between 65 and 750 mg/l, depending of the purity of biosurfactant and the used carbon sources. BS-CO100 showed high steadiness against a wide spectrum of pH (4.3-12), temperature (4-121 °C) and salinity (0-300 g/l NaCl), supporting its powerful tensioactive properties under various environmental conditions. Likewise, BS-CO100 exhibited no cytotoxic effect toward human HEK293 cells, at concentrations within 125 and 1000 µg/ml. Furthermore, the biosurfactant BS-CO100 exhibited remarkable anti-adhesive and anti-biofilm activities, being able to avoid and disrupt the biofilm formation by certain pathogenic microorganisms. In addition, BS-CO100 was found to have more potential to remove hydrocarbons from contaminated soil, compared to some chemical surfactants. In light of these promising findings, strain CO100, as well as its biosurfactant, could be successfully used in different biotechnological applications including the bioremediation of oil-polluted areas, even under saline conditions.

Keywords:

Biofilm

Bioremediation

Biosurfactants

Halotolerant

Hydrocarbons

Staphylococcus

1. Introduction

Pollution of marine ecosystems by petroleum hydrocarbons is one of the serious global issues (Duran and Cravo-Laureau, 2016). Petroleum, which is a complex mixture of different hydrocarbons including aliphatics, cycolalkanes, mono and polyaromatics, asphaltenes and resins, is well known by its highly toxic, carcinogenic and persistent nature (Kuppusamy et al., 2020). Physical and chemical approaches are used to immobilize and destroy hydrocarbons reducing therefore their impact on environment (Li et al., 2020). Nevertheless, most of these physicochemical processes are expensive, not sufficiently efficient and environmentally polluting. Nowadays, biotech-based approaches are gaining an increasing interest as alternatives or complements to the physicochemical techniques (Azubuike et al., 2016). The bioremediation process, which is characterized by a non-polluting aspect and an absence of chemical by-products, is considered to be the main mechanism whereby most hazardous contaminants such as hydrocarbons are removed from the environment (Nikitha et al., 2017).

It has been reported that high salinities constitute a natural barrier for the degradation of hydrocarbons (Truskewycz et al., 2019). Indeed, the application of microbial technologies to treat saline or hypersaline environments is limited due to the harmful effects of the high salt concentration on microbial life, in particular the integrity of the cell membrane, the denaturation of enzymes, the poor oxygen solubility and desiccation (Fathepure, 2014). However, the search for halophilic or halotolerant microorganisms, capable of metabolizing hydrocarbons, has been enhanced significantly in recent decades. These microorganisms develop various mechanisms for their acclimation to the osmotic pressure caused by the raised salt concentrations of the environment (Louvado et al., 2015; Truskewycz et al., 2019) . Therefore, the use of organisms that tolerate high salt concentrations, in the bioremediation of

saline environments without a costly dilution of the soil and water laden with salt, could be a promising alternative in bioremediation (Fathepure, 2014).

In addition, the use of surfactants is a promising approach to enhance the biodegradation of hydrocarbons. These molecules are characterized by their potentiality to increase the solubilization and dispersion of oils and modify the affinity between the microbial cell and the organic compound (Geetha et al., 2018). Most commercially-available surfactants are of a chemical origin, causing a risk to the environment since they are generally toxic and not biodegradable (Ibrar and Zhang, 2020). Therefore, owing to the development of biotechnology, the production of biological surfactants has aroused a great interest among scientists. This is mainly because of the advantages presented by these biomolecules compared to synthetic surfactants, namely biodegradability, low toxicity, efficiency under extreme environmental conditions, etc (Banat et al., 2014b). Due to these promising characteristics, biosurfactants have been considerably applied in different fields including bioremediation, cosmetic, food processing and more (Santos et al., 2016). However, their high production costs highlight the need to optimize the production process to allow a possible application on an industrial scale (Banat et al., 2014b). Interestingly, biosurfactants can be synthesized from renewable carbon sources such as wastes food oils or dairy waste, allowing a significant reduction in production costs (Geetha et al., 2018). These alternative substrates which contained rich components as, fats, protein, organic acids, etc, provide great sources for microbial development and biosurfactant production. Moreover, the use of such economic substrates decreases not only the cost of surfactant production, but also the environmental contamination caused by the inappropriate elimination of such residues (Banat et al., 2014b).

It is known that the best adopted organisms for bioremediation are often the native species of polluted habitat. Indigenous microorganisms can survive and multiply in the presence of toxic substances, exhibiting broad capacities for biodegradation or synthesize of surfactants (Jeanbille et al., 2016). Although certain microbial strains have been selected for

98 their bioremediation capacity, they are generally less effective than indigenous populations.
99 The natural environments, terrestrial and marine, which are contaminated by hydrocarbons,
100 are considered, then, as potential biotopes for the isolation of microorganisms that can
101 degrade these pollutants (Jeanbille et al., 2016).

102 In this regard, our study focused on the isolation of a halotolerant bacterium CO100, from
103 contaminated marine sediments, marked by its high potentiality to break down hydrocarbons,
104 in the presence of high salt concentrations. Additionally, the production and the
105 characterization of biosurfactants from the isolate CO100, using low-value carbon sources,
106 along with the application of hydrocarbons recovery and biofilm control, were also
107 conducted. As far as we know, it seems this is the first report describing the biodegradation of
108 hydrocarbons and the production of biosurfactants by species of *Staphylococcus pasteuri*,
109 under high salinity conditions.

110 **2. Materials and methods**

111 *2.1. Sampling*

112 Top surface (1-5 cm) sediment samples were taken in January 2013 from the fishing
113 harbour of Sfax, Tunisia, Mediterranean sea (34°71′58.51″ N. 10°76′51.67″ E). The samples
114 were taken manually, transferred in sterile sampling bags and kept on the dark at 4 °C, for the
115 screening of halotolerant and hydrocarbonoclastic bacteria.

116 *2.2. Culture media*

117 The composition of the Luria-Bertani medium (LB) was (g/l): 10 peptone, 5 yeast extract,
118 and 10 NaCl. Nutrient broth medium (NB) was composed of (g/l): 15 peptone, 3 yeast extract,
119 1 glucose and 6 NaCl. Basal medium (BM) (g/l) contained in distilled water: 0.3 KH_2PO_4, 0.3
120 K_2HPO_4, 0.4 g NH_4Cl, 0.33 $MgCl_2.6H_2O$, 0.05 $CaCl_2.2H_2O$, 100 NaCl and 1 ml trace-element
121 solution (Hentati et al., 2019). Crude oil, collected from "Thyna Petroleum Services" (Sfax,
122 Tunisia) and polycyclic aromatic hydrocarbons (PAHs) (phenanthrene, fluoranthene, and

pyrene) purchased from Sigma Aldrich, were sterilized by autoclaving. Residual frying oil (RFO) was obtained from domestic oil wastes and sterilized by filtration (pore size 0.45 µm; Millipore). Expired milk powder (EPM), MODULAC, was provided by the Central Pharmacy (Sfax, Tunisia) and autoclaved to get sterilized.

2.3. Enrichment, isolation and characterization of oil-degrading bacteria

Samples of the studied contaminated sediment were served to the screening of aerobic and halotolerant oil-degrading bacterial strains, as described previously (Hentati et al., 2016). A 0.5 g of the polluted sediment (1%, w/v) was used to inoculate 50 ml of BM containing 1% (v/v) of crude oil, at 100 g/l NaCl, 37 °C and 180 rpm. After numerous enrichments, a stable microbial growth on crude oil 1% (v/v), obtained after four times of sub-culturing during 15 days under the same conditions, was used for the isolation of pure bacteria. Ten colonies were picked and a pure culture nominated CO100 was selected due to its high aptitude to develop on crude oil in liquid and solid BM, containing 100 g/l NaCl. The phenotypic and phylogenic studies were accomplished as stated by Hentati et al. (2016).

2.4. Biodegradation experiments

Biodegradation experiments were conducted in culture flasks containing 50 ml of BM, in the presence of 1% (v/v) crude oil, at 100 g/l NaCl, 37 °C and 180 rpm, with 3% (v/v) of inoculums. The aptitude of strain CO100 to metabolize crude oil was verified by monitoring the OD at 600 nm, by enumeration of Colony-Forming Unit (CFU) and by GC-MS analyses at times intervals of incubation of 0, 10 and 20 days (Hentati et al., 2016). The ability of strain CO100 to grow on phenanthrene, fluoranthene and pyrene (100 mg/l), containing in BM with 100 g/l NaCl, was also investigated by measuring the OD 600 nm. A comparison with biological and chemical controls was carried out, for all the biodegradation studies.

2.5. Biosurfactant production by strain CO100

147 The potential of strain CO100 to synthesize tensioactive agents was studied on nutrient
148 broth (NB) and by adding each of residual frying oil (RFO) (1%, v/v) and expired powder
149 milk (EPM) (20 g/l) into culture flasks containing BM, at 100 g/l NaCl, 37 °C and 180 rpm.
150 The surface tension (ST) measurement and the oil displacement test (ODT) were performed to
151 evaluate biosurfactants production in culture samples, according to the published procedures
152 (Hentati et al., 2019). The recuperation of biosurfactant, named BS-CO100, was carried out
153 after 2 days for RFO and EPM and after 3 days for NB, using Hentati et al. (2019)
154 approaches.

155 *2.6. Characterization of biosurfactant BS-CO100*

156 The critical micelle concentrations (CMCs) of biosurfactant BS-CO100 were calculated
157 by measuring the surface tension of serial dilutions of biosurfactants BS-CO100 produced on
158 NB, RFO and EPM, up to a constant value of ST. The effect of pH (2.4, 4.3, 6.5, 7.5, 8.6, 10
159 and 12), temperature (4, 30, 37, 55, 70 and 121 °C) and salinity (0, 10, 30, 60, 90, 120, 150,
160 200 and 300 g/l) on the BS-CO100 stability, was carried out using cell-free broth of strain
161 CO100 cultivated on RFO (Hentati et al., 2019).

162 The purification of crude BS-CO100, synthesized on NB, was carried out using silica gel
163 column and thin layer chromatography (TLC) methods according to Hentati et al. (2019).
164 Fractions obtained after purification were analyzed by electrospray ionization (ESI)
165 (LC/MSD-TOF, Agilent Technologies, Palo Alto, CA) (Jemil et al., 2019).

166 Cytotoxicity test was performed by treatment of human HEK293 cell line by different
167 doses of crude BS-CO100 (125-1000 µg/ml) produced on NB, using the colorimetric MTT
168 assay, as reported previously (Hadrich et al., 2015; Hentati et al., 2019).

169 *2.7. Application of BS-CO100 in hydrocarbons removal from polluted soil*

170 The BS-CO100 biosurfactant efficiency in oil recovery was determined by adding waste
171 motor oil (20%, v/w) to 10 g of soil samples, which were then exposed to the following

172 treatments: addition of 20 ml water (control) or 20 ml of each crude BS-CO100 solutions
173 synthesized on RFO and EPM, prepared at their CMCs (0.05%, w/v and 0,075, w/v,
174 respectively) or 20 ml of each cell-free broth of strain CO100 growing on RFO and EPM and
175 20 ml of the aqueous solutions of synthetic surfactants (Tritox X-100, SDS, Tween 20 and
176 Tween 80) adjusted at their CMCs. The specimens were kept during 24 h at 30 °C and 180
177 rpm, and then centrifuged at 6000 rpm for 20 min. The supernatant was extracted twice by
178 hexane (v/v). The amount of hydrocarbons removal from the soil after each treatment was
179 determined gravimetrically as described by Hentati et al., (2019).

180 *2.8. Biofilm control*

181 The application of biosurfactants BS-CO100 in the prevention (pre-treatment) and
182 elimination (post-treatment) of biofilms of some pathogenic microorganisms was performed
183 using the crystal violet staining method, as reported by Coronel-León et al. (2015). These tests
184 were performed on a polystyrene surface (microplate, 96 wells), using different
185 concentrations of BS-CO100 (0.125-4 µg/ml), produced on NB media. For biofilm formation,
186 various microorganisms were used including: Gram-negative bacteria (*Escherchia coli* ATCC
187 25922, *Klebsiella pneumoniae* ATCC 13883, *Salmonella typhimurium* ATCC 14028 and
188 *Pseudomonas aeruginosa* ATCC 27853); Gram-positive bacteria (*Bacillus cereus* ATCC
189 11778, *Enterococcus faecalis* ATCC 29216 and *Staphylococcus aureus* (MRSA) (Methicillin
190 resistant) ATCC 43300), as well as the yeast *Candida albicans* ATCC 10231.

191 *2.9. Statistical analysis*

192 All values represent the mean ± standard deviation (SD). Data were analyzed using One-
193 Way ANOVA" tests with "Tukey's multiple comparisons. Values of $p < 0.05$ were considered
194 statistically significant.

195 **3. Results and Discussion**

196 *3.1. Characterization of a halotolerant marine oil-degrading bacterium*

197 Strain CO100 was Gram positive, coccus, non-motile that occurred individually, in pairs
198 and in cluster. Colonies of strain CO100, formed after incubation during 24 h, appeared
199 creamy, smooth, circular and regular with 0.5 - 1 mm diameters. Moreover, strain CO100 was
200 catalase positive and oxidase negative. Strain CO100 was capable to grow at pH ranging
201 between 5.5 and 9. The growth was optimum at pH 7.2, but no growth was observed below
202 pH 4.2 and above pH 10.5. In addition, strain CO100 was capable of growing within
203 temperature range from 15 to 55 °C, with a growth optimum at 37 °C. An absence of growth
204 was reported at 10 and 55 °C. Furthermore, strain CO100 was able to grow at concentrations
205 of NaCl extending from 0 to 250 g/l NaCl. Strain CO100 exhibited an optimum growth in the
206 presence of 0-100 g/l NaCl. Nevertheless, it was unable to grow at 300 g/l NaCl. It is
207 therefore classified as a highly halotolerant strain.

208 The molecular analysis highlighted that strain CO100 could be closely related to
209 members of the genus *Staphylococcus*, especially to the species of *Staphylococcus pasteuri*
210 (Type strain ATCC 51129) (Chesneau et al., 1993), with a sequence similiraity of 99.4%. The
211 16S rRNA gene sequence of strain CO100, composed of 1473 nucleotides, was deposited in
212 the GenBank nucleotide database under accession number MT476860.

213 The resistance of microorganisms affiliated to the genus *Staphylococcus* to saline
214 conditions has been shown. An extremely halotolerant strain HPSSN35C of *Staphylococcus*
215 *arlettae*, isolated from saline soil, India, demonstrated its potentiality to grow within range of
216 NaCl concentrations extending from 0 to 6 M (Nanjani and Soni, 2014). Another CH1-8
217 strain of *Staphylococcus xylosus* isolated from fermented fish collected in the province of
218 Chumporna, Thailand, showed its potential to grow at NaCl concentration of 20% (m/v)
219 (Namwong and Tanasupawat, 2014). Recently, various haloversatile bacteria including
220 *Staphylococcus pasteuri* were detected from salt samples taken from Çamaltı Saltern, one of

221 the large seawater-based saltern located in Izmir, Turkey (Caglayan, 2019). Halotoerant
222 bacteria have the potentiality to prosper not only under broad range of salt concentrations, but
223 also in the absence of salt. This criterion makes them unique microorganisms extensively
224 recommended for different applications such as bioremediation.

225 *3.2. Oil-biodegradation potential of CO100 bacterium*

226 The potential of the isolated CO100 to metabolize crude oil (1%, v/v), without yeast
227 extract added, in the presence of 100 g/l NaCl, was studied. Fig. 1 illustrates the monitoring of
228 strain CO100 growth on crude oil, by measuring the OD at 600 nm, during 20 days. This
229 isolate showed a significant growth on crude oil compared to the abiotic control (Fig. 1).
230 Similarly, counting of viable cells indicated a positive development of strain CO100 in the
231 presence of this complex hydrocarbon (Fig. 1). Moreover, GC-MS analyses were
232 accomplished to evaluate the degradation of crude oil by strain CO100 after different time
233 intervals of incubation (0, 10 and 20 days) (Fig. 2). Results revealed that strain CO100 was
234 able of breaking down a broad range of aliphatic hydrocarbons contained in crude oil from
235 C_{13} to C_{29}, compared to abiotic control. Fig. 2 shows the remarkable decrease or the total
236 disappearance of the correspondent peak of each compound. Strain CO100 degraded almost
237 72% of aliphatic hydrocarbons existing in the crude oil, at 100 g/l NaCl, after 20 days of
238 incubation.

239 Polycyclic aromatic hydrocarbons (PAHs) are present in many saline environments
240 (Fathepure, 2014). The research of new hydrocarbonoclastic bacteria which support high salt
241 concentrations in order to eliminate these persistent compounds from the environment is of
242 great interest in bioremediation. Hence, the ability assessment of the bacterium CO100 to
243 grow on PAHs (100 mg/l): phenanthrene, fluoranthene and pyrene, as the sole carbon sources
244 and at 100 g/l of NaCl, was carried out. Results showed that the isolate CO100 was capable of
245 growing on these recalcitrant compounds, comparing to abiotic controls (Fig. S1). The

246 isolation of microorganisms belonging to the genus *Staphylococcus*, which is endowed with
247 hydrocarbon biodegradative potential, has been shown. In this context, the strain str. MN-005
248 of *Staphylococcus* sp. isolated from marine sediments contaminated by hydrocarbons, showed
249 the potential to degrade naphthalene (Zhuang et al., 2003). Another study reported the
250 isolation of hydrocarbonoclastic strains of *Staphylococcus* from oil-polluted coastal areas in
251 Japan (Chaerun et al., 2004). Moreover, the strain RD2 of *Staphylococcus pasteuri*, isolated
252 from hydrocarbon-contaminated soil taken from Guwahati Oil Refinery, India, was able to
253 degrade naphthalene (Dooley et al., 2014). Another strain BK37 of *Staphylococcus pasteuri*,
254 an indigenous bacterium from oil contaminated soil, was described as a *n*-alkane and PHA
255 degrader (Kiamarsi et al., 2019). Although the degradation of these compounds can be
256 relatively easy, in soils, fresh waters and in marine habitats with low salinity, their fate is
257 more difficult under moderate to high salinity conditions (3 to 30% salt) (Fathepure, 2014). In
258 fact, the solubility of hydrocarbons decreases with increasing salt concentration, hence a
259 decrease in bioavailability for biodegradation. The high salinity limits not only the access of
260 microorganisms to hydrocarbons, but also the availability of oxygen, since its solubility
261 decreases as the salinity augments (Truskewycz et al., 2019). In addition, high salt
262 concentrations tend to denature proteins, i.e. to break down the tertiary structure of proteins
263 which is essential for enzymatic activity (Fathepure, 2014). However, microorganisms, which
264 are capable of growing under such conditions, have physiological mechanisms that can
265 protect them from these fluctuations and allow their acclimation to the osmotic pressure
266 caused by the raised salinity of the environment (Fathepure, 2014). These microorganisms can
267 be effective depolluting agents capable of degrading hydrocarbons, under high salinity
268 conditions. The implication of the genus *Staphylococcus* in the process of biodegradation of
269 hydrocarbons was previously highlighted. However, only few studies have been carried out
270 on the degradation of these pollutants in the presence of high NaCl concentrations by
271 *Staphylococcus* strains. A concentration of 15% of NaCl has been tolerated by strains of

Bacillus and *Staphylococcus* used in batch fermentations of 1 m^3 to clean industrial wastewater contaminated with hydrocarbons (Patzelt, 2005).

3.3. Production and characterization of CO100 biosurfactant

3.3.1. Assessment of biosurfactant production by strain CO100 on low-value carbon sources

The hydrocarbonoclastic microorganisms are highly considered to be potential candidates for producing tensioactive agents. Despite the advantages of biological surfactants, their large-scale utilization is limited due to the high cost production. Many researches focused on the usage of inexpensive and renewable sources as substrates for cost reduction (Banat et al., 2014b). In this regard, the aptitude of strain CO100 to produce biosurfactants was investigated, using alternative carbon sources: residual frying oil (RFO) and expired powder milk (EPM). Fig.3 highlights that during the growth of strain CO100 on RFO and EPM, the surface tension decreased distinctly after one day of incubation and remained almost stable until the 7^{th} day: from 63.5 to 33-30 mN/m (Fig. 3a) and from 67 to 37-31 mN/m (Fig. 3b), respectively. Furthermore, the oil displacement test revealed the formation of clear halo zones with maximum diameter of 8.5 cm, from the 2^{nd} day to the 7^{th} day for RFO and from the 2^{nd} day to the 6^{th} day for LPP (Fig. 3). These findings supported the secretion of tensioactive agents by strain CO100. From the 8^{th} day, we noticed that the production of biosurfactants decreased (Increase of ST and diminution of halo zones diameters) (Fig. 3). This increase might be due to the degradation of biosurfactants in culture media or to the use of biosurfactants as substrates for cell survival (Patowary et al., 2017).

Hydrophobic substrates, including vegetable oils, were described as inducers and precursors for biosurfactant secretion. Strain CO100 showed a high capacity to produce biosurfactants in the presence of residual frying oil. A possible reason for this tendency is that species of *Staphylococcus* are recognized as lipase-producing microorganisms, facilitating the assimilation of fatty acids present in residual frying oil. It was mentioned that biosurfactants

297 can be produced using lipases, which catalyze fatty acids and sugar esterification (Colla et al.,
298 2010). A strain COM-4A of *Staphylococcus pasteuri*, isolated from grease and oil
299 contaminated areas, showed a remarkable capacity to produce lipase using a media based on
300 coconut oil mill waste (Kanmani et al., 2015). Moreover, the production of biosurfactants by
301 lactic acid bacteria was reported (Satpute et al., 2016). *Staphylococcus pasteuri* was identified
302 among various bacteria isolated from unpasteurized ewes' milk obtained from a farm located
303 in Slovakia (Pangallo et al., 2014). These findings might explain the tendency of strain
304 CO100 to produce biosurfactant using expired powder milk.

305 The recovery of biosurfactants produced by strain CO100 on RFO and EPM was carried
306 out after 2 days of incubation. The quantities of crude biosurfactants were almost 1.5 g/l for
307 RFO and 0.5 g/l for EPM. Strain 1E of *Staphylococcus* sp., cultivated on LB medium
308 containing olive oil, was able to produce around 2.1 g/l of lipopeptides biosurfactant
309 (Eddouaouda et al., 2012). Later, Chebbi et al. (2018) described the capacity of the strain SH6
310 of *Staphylococcus capitis*, to produce lipopeptides, with yields of around 50 and 100 mg/l,
311 using used motor oil and crude oil as substrates, respectively. Waste frying oil, owing to its
312 rich composition, and facile availability, could be used as an inexpensive substrate for the
313 production of biosurfactants. A significant amount of oils is used in most food industries,
314 resulting in the production of a large mass of residual oils (Hasanizadeh et al., 2017). The
315 immense majority of these oils are rejected as wastes after usage, causing numerous problems
316 with septic and sewer systems. In fact, when waste oils cool and settle, they congeal. This can
317 damage certain materials, clog up pipes and even affect the wildlife (Hasanizadeh et al.,
318 2017). On the other hand, these oils are carbon-rich and low-valued, which could be utilized
319 by numerous microorganisms as nutriments for growth. Similarly, as part of the recovery of
320 waste from the dairy industry and unsold or expired products, expired milk powder can be
321 used as a good source of carbon because of its composition, which is rich in proteins,
322 carbohydrates, lipid, etc, as well as its low cost (Charpiot, 2012). Bioconversion of these

wastes into value-added products, as biosurfactants, offers a double benefit. First, the waste will be removed from the environment. Second, the high cost of production of biosurfactants will be reduced.

3.3.2. ESI-MS identification of biosurfactant BS-CO100

Residual frying oil and expired powder milk, are consisted of mixed molecules (fatty substances, lipids, organic acids, etc), and can be trapped during biosurfactant extraction procedures, thereby decreasing the purification efficiency. Hence, to an effective identification of the nature of biosurfactants, it is better to use purer carbon sources. Nutrient broth, a culture medium composed of water-soluble nutrients (peptone, yeast extract and glucose), has been proven to be a good carbon source for the synthesis of the biosurfactants (Ali Khan et al., 2017). Furthermore, the yield of surfactants synthetized by the CO100 isolate on NB was relatively considerable (2.2 g/l) compared to RFO (1.5 g/l) and EPM (0.5 g/l). For these reasons, the nutrient broth was chosen, as a suitable medium to produce biosurfactants by strain CO100, intended to purify and identify BS-CO100.

The crude BS-CO100, produced on NB medium, was initially examined by thin layer chromatography (TLC) analysis. Pink and blue-violet spots were revealed when spraying with ninhydrin and phosphomolybdic acid reagents, respectively. These findings highlighted the presence of amine and fatty acid groups, suggesting the lipopeptide nature of BS-CO100. Later, crude BS-CO100 was subject to fractionation, using a column of silica gel. Twenty-five fractions (1 ml) were assembled and every fraction was subsequently analyzed by TLC in order to collect the similar ones corresponding to their polarity. Eight big fractions were pooled, but only two fractions showing positive responses with ninhydrin and phosphomolibidic acid, and demonstrating the ability to decrease the ST of water to 27 mN/m, were retained and analyzed by ESI-MS.

Fig.4 illustrates the results of mass spectra obtained from the purified biosurfactants BS-CO100. It showed the presence of peaks at m/z values between 1029 and 1081 Da. In pursuant to mass numbers noticed in previous similar studies (Hentati et al., 2019; Jemil et al., 2017), this group of peaks could be attributed to lipopeptides, especially to lichenysin and iturin families. The mass spectrum reported in Fig. 4a, corresponding to the 1st fraction, revealed the presence of three peaks $[M + Na]^+$, at m/z 1029.7, 1043.7 and 1057.7 Da which differ from each other by m/z 14. They are assigned to lichenysin C13, lichenysin C14 and lichenysin C15, respectively. Besides, another peak at m/z 1079.7 was revealed. This peak is assigned to iturin A or mycosubtilin with a chain length of fatty acids of 15 carbon atoms (Jemil et al., 2017). The second fraction revealed the presence of 3 other peaks [M + Na]+, at m/z 1053.5, 1067.5 and 1081.6, which are corresponded to bacillomycin D C14, bacillomycin D C15 and bacillomycin D C16, respectively (Jemil et al., 2017).

Researches on biosurfactants synthesized by *Staphylococcus* genus are relatively rare compared to that of the genera *Bacillus* and *Pseudomonas. Staphylococcus hoemolyticus* strain 1E, isolated from an algerian soil contaminated with crude oil, was described as a lipopetide producer, using olive oil as a substrate (Eddouaouda et al., 2012). Similarly, we have recently shown the capacity of *Staphylococcus capitis* strain SH6, isolated from the phosphate processing plant of Sfax and degrading mercaptans and hydrocarbons, to synthesize biosurfactants belonged to lipopeptide group, using crude oil or used motor oil as carbon sources (Chebbi et al., 2018). A further research reported the production of glycopidic biosurfactants by *Staphylococcus saprophyticus* strain SBPS 15, isolated from marine sediments contaminated by hydrocarbons, in India (Mani et al., 2016). Lonappan et al. (2017) mentioned that biosurfactant production was recorded by many species, among which *Staphylococcus pasteuri* (ASBCFS11), isolated from waste water samples generated from an activated sludge of a plant treating effluent of a commercial flight kitchen, India.

372 *3.3.3. Tensioactive properties of BS-CO100*

373 Critical micelle concentration (CMC) is an interesting parameter to examine the
374 efficiency of any tensioactive agent. The CMC of BS-CO100 was evaluated using different
375 carbon sources (nutrient broth, residual frying oil and expired powder milk) (Fig. 5). As
376 indicated in Fig. 5a, the CMC of purified BS-CO100 produced from NB was 65 mg/l, with ST
377 value of 28 mN/m. This concentration was increased in the case of crude BS-CO100 produced
378 from NB, to reach a value of 275 mg/l, with ST of 30 mN/m (Fig. 5b). We can conclude that
379 the CMC is influenced by biosurfactant purity. The more the degree of purification increases,
380 the more the value of CMC decreases (Silva et al., 2010). Moreover, the CMCs of BS-CO100
381 produced on RFO and EPM were 500 and 750 mg/l, respectively (Fig. 5c, d). At these
382 concentrations, the corresponded ST were 30 and 31 mN/m, respectively. The carbon source
383 is an important factor influencing the structure of the biosurfactant and subsequently its
384 physicochemical properties and its biological activities, such as the CMC and the ability to
385 reduce surface tension (Singh et al., 2014). Indeed, the latter researchers, found that in the
386 presence of dextrose, sucrose and glycerol, used as carbon sources, strain AR2 of *Bacillus*
387 *amylofaciens* produced lipopeptides as a mixture of surfactin, iturine and fengycin.
388 Nevertheless, in the existence of lactose, sorbitol and maltose, only iturin was secreted. The
389 surfactant properties of these lipopeptides were also affected depending on the used substrate.
390 In fact, the CMC values were between 80 and 110 mg/l, with ST is around 30 and 37 mN/m
391 (Singh et al., 2014). Our findings revealed that BS-CO100 is an efficient biosurfactant, due to
392 its low CMC compared to some synthetic surfactants as citrikleen, sodium dodecyl sulfonate
393 and tetradecyltrimethyl ammonium bromide (TTAB) (Whang et al., 2008). We have reported
394 previously, that the CMCs of lipopeptides SH6BS1 and SH6BS2, produced by
395 *Staphylococcus capitis* strain SH6, on used motor oil and diesel oil, respectively, were around
396 800 mg/l with ST about 31.7 for SH6BS1 and 38.7 mN/m for SH6BS2 (Chebbi et al., 2018).
397 In addition, the lipopeptides from the isolate *Staphylococcus haemolyticus* 1E showed their

efficiency to decrease the ST to 25-26 mN/m, with a CMC value about 750 mg/l (Eddouaouda et al., 2012).

The stability of biosurfactant BS-CO100 against various pH, temperatures and salinities was investigated (Fig. 6). We noted that BS-CO100 maintained approximately the same ST from pH 4.3 to pH 12 (ST = 29±1 mN/m) and the same diameters of halos formed when applying oil displacement test (ODT = 8.5 cm) (Fig. 6a). A slight increase in ST accompanied by a decrease in the halos formed, was observed at pH = 2.4 (Fig. 6a). Moreover, the surfactant properties of BS-CO100 stayed constant at temperatures between 4 and 121 °C (ST = 29±0.4 mN/m and ODT = 8.5 cm) (Fig. 6b). In addition, the ST and the ODT showed that the activity of BS-CO100 was not influenced by salt concentrations varying from 0 to 300 g/l (ST = 30±0.7 mN/m and ODT = 8.5 cm) (Fig. 6c). These data, showing the great stability of biosurfactant BS-CO100 faced to the environmental parameters, were in line with previous studies. For instance, the lipopeptides produced by *Staphylococcus capitis* strain SH6 maintained their surfactant properties against a large spectrum of pH (2-12), temperature (-20-100 °C) and salinity (20-150 g/l) (Chebbi et al., 2018). Moreover, the lipopeptides isolated from *Staphylococcus haemolyticus* strain 1E exhibited great steadiness in a broad spectrum of pH (2-12), temperature (4-55 °C) and NaCl concentrations (0-300 g/l) (Eddouaouda et al., 2012). Nevertheless, another research pointed out that the activity of certain biosurfactants was affected by acidic conditions. Indeed, at pH values between 2 and 4, the lipopeptides synthesized by strain BS5 of *Bacillus subtilis* did not have surface activity (Abdel-Mawgoud et al., 2008). The latter researchers argued that this response could be related to the precipitation of certain biosurfactants at low pH, causing the low activity of these surfactants at acid pH (Khopade et al., 2012). The high steadiness of biosurfactant BS-CO100 against raised temperatures is in great demand in bioremediation operations (MEOR), as well as in the of application of biological surfactants in cosmetic, food and pharmaceutical products, where heating is applied to obtain the sterility (Khopade et al., 2012). The stability of

biosurfactants against the variation of different environmental parameters as pH, temperature and salinity, is a highly-sought-after criterion for various biotechnological applications, especially bioremediation field.

3.3.4. Cytotoxicity of BS-CO100

Nowadays, there is an immense concern regarding the toxicity and safety of biological surfactants, especially those intended for therapeutic or food purposes. In this context, cell viability of human HEK-293 cell line was evaluated after being treated with different concentrations of biosurfactant BS-CO100 (125-1000 µg/ml) for 24 h and 48 h, with regard to untreated cells (control). As illustrated in Fig. 7, the percentage of cell viability decreased slightly with an augmentation of lipopeptide concentrations and exposure times. In fact, after 24 h of incubation, the cell viability was decreased to 88.64%, at the maximum BS-CO100 concentration (1000 µg/ml). After 48 h of treatment, the cell viability was further reduced reaching 85.2%, in the presence of 1000 µg/ml of BS-CO100. No decrease on the percentage of cell viability was noticed for control (Fig.7). The lipopeptides BS-CO100 could be considered as non-toxic products, at concentrations range between 125 and 1000 µg/ml ($p \geq 0.05$), since the cell survival is over than 80%, according to ISO 10993-5, 2009 (ISO reports, 2009). A recent study by Jemil et al. (2020), stated that the lipopeptides from *Bacillus methylotrophicus* DCS1 have no cytotoxicity effect against HEK 293 cells at concentrations within 30 to 250 µg/ml, since the cell survival was above 50%. Moreover, the lipopeptides PE1 and PE2, synthesized by *Paenibacillus ehimensis* B7 exhibited no cytotoxicity effect towards HEK293 cells, at concentrations ranging from 1 to 128 µg/ml (cell viability > 95%) (Huang et al., 2013). Our findings encourage the use of biosurfactant BS-CO100 in cosmetic, pharmaceutical and food applications.

3.4. Application assays of BS-CO100

3.4.1. Enhanced recovery of hydrocarbons contaminants using biosurfactant BS-CO100

Hydrocarbons are persistent and hydrophobic compounds which tend to adsorb strongly on the organic matter of soil (Duran and Cravo-Laureau, 2016). The use of biological surfactants in the restoration of contaminated sites seems to be a promising strategy which promotes the bioavailability of hydrophobic products (Cazals et al., 2020). Therefore, hydrocarbons removal using biosurfactants produced by strain CO100, and certain synthetic surfactants, was carried out. As shown in Fig. 8, the remobilization effect of used motor oil by the crude biosurfactants produced by strain CO100 on RFO and EPP, was more marked than that of chemical surfactants (Tween 20, Tween 80, Triton X-100 and SDS) ($p < 0.05$) (Fig. 8). The hydrocarbon solubilization was more accentuated using cell-free cultures of CO100 on RFO and EPP (6.2 and 5.4 fold solubility, respectively), in comparison with crude biosurfactants (4.8 and 4.6, respectively) ($p < 0.05$) (Fig. 8). We reported previously, that lipopeptides Bios-Cnaph3, BS-FLU5, SH6-BS1 and SH6-BS2, were strongly effective in hydrocarbons recovery, compared to synthetic surfactants (Chebbi et al., 2018; Cheffi et al., 2020; Hentati et al., 2019). Biosurfactants, as amphiphilic molecules endowed by interfacial activity, have the ability to increase the solubilization of hydrophobic compounds by reducing surface tensions, which leads to increase the bioavailability and mobility of contaminants (Banat et al., 2014b). Interestingly, the application of cell-free culture of CO100 in the petroleum industry (cleaning-up oil spills, enhancing oil recovery, etc), could be an economical and profitable approach which presents an attractive productivity, since the extraction steps of biosurfactants are avoided (Silva et al., 2010).

3.4.2. Biofilm control

Biofilms are often considered as a source of problems in the medical field as well as in the industry or even the environment (Banat et al., 2014a). They are responsible for several nosocomial infections, an alteration of the organoleptic qualities of food products, and certain number of degradations (buildings, corrosion leading to the perforation of the hull of boats,

474 etc) (Wahl et al., 2012). The use of biological surfactants to resist these problems have
475 received particular attention in recent years, because of their biodegradable nature, the low
476 cytotoxicity and the anti-microbial and antibiotic activities (Banat et al., 2014a). In this
477 context, the application of BS-CO100 in the prevention (pre-treatment) and the elimination
478 (post-treatment) of biofilms formed by pathogenic microorganisms was carried out (Table 1).
479 Indeed, the pre-treatment test consists in evaluating the capacity of the biosurfactant, in
480 inhibiting the formation of biofilms of microorganisms, by adding this biological surfactant
481 before the formation of biofilms (anti-adhesive activity). On the other hand, the post-
482 treatment test consists in evaluating the capacity of the biosurfactant to destabilize and disturb
483 the biofilms already formed (antibiofilm activity). As indicated in table 1, BS-CO100
484 presented a concentration-dependent anti-adhesive and anti-biofilm effects. Indeed, we
485 noticed an augmentation of these biological activities, along with the increase of BS-CO100
486 concentration. For the pre-treatment evaluation, we highlighted that the greatest anti-adhesive
487 effect was noticed against *C. albicans*, *S. aureus* and *B. cereus* (74.9, 66.8 and 62.7%
488 respectively). Intermediate inhibition was obtained for *E. coli* and *S. typhimurium* (54.2 and
489 51% respectively), while the effect on *E. faecalis*, *P. aeruginosa* and *K. pneumoniae* was
490 lower (47.5, 45.3 and 36.5% respectively) (Table 1a). For the post-treatment test, the
491 maximum disruption was: 70.3% for *S. aureus*, 66.2% for *P. aeruginosa*, 61.4 for *B. cereus*,
492 58.4% for *C. albicans*, 58.3% for *E. faecalis*, 55.1% for *S. typhimurium*, 50.3 for *E. coli* and
493 47.2% for *K. pneumoniae* (Table 1b). Biosurfactants, endowed with an interfacial activity,
494 can anchor into the membrane of the cell wall, resulting in the increase of fluidity of the cell
495 membrane structure which leads to the leakage of intracellular components, and consequently,
496 to the modification of cellular hydrophobicity (Chebbi et al., 2017). Furthermore, these
497 surfactants are known by their antibiotic and anti-bacterial activities, thus inhibiting the
498 growth of Gram negative bacteria as positive (Rivardo et al., 2009). Comparing our findings
499 with similar previous researches, we can highlight that the lipopeptides BS-CO100 were

powerful agents that have significant anti-adhesion and anti-biofilm activities against pathogens. Banat et al. (2014a) reported that lipopeptides are among the most powerful biosurfactants having the potentiality to avoid and disrupt microbial biofilm. In fact, Coronel-León et al. (2015) showed a remarkable capacity of lichenysins synthesized by *Bacillus licheniformis* AL1.1 to disperse and prevent the biofilm formation by pathogenic microorganisms. Lipopeptides isolated from *Bacillus cereus* NK1 showed notable reduction in biofilm formation by *Pseudomonas aeruginosa* and *Staphylococcus epidermis* (Sriram et al., 2011). Another study conducted by Rivardo et al. (2009) reported that two lipopeptides produced by *Bacillus subtilis* V19T21 and *Bacillus licheniformis* V9T14 showed interesting specific anti-adhesion activity against two pathogenic strains *Escherichia coli* and *Staphylococcus aureus.*

As mentioned above, the formation of biofilm is a phenomenon that affects many sectors, and can cause several problems. The maritime and petroleum sectors are obviously not immune to this problem (Wahl et al., 2012). Biofilms occur on various surfaces as boat hulls, oil platforms, pipelines as well as aquaculture and harbour structures. They can disturb and damage scientific measuring devices (cameras or sensors). In the case of ships for instance, the accumulation of microbial biofouling below the waterline leads to a speed reduction of the boat and consequently an overconsumption of fuel, a high maintenance cost, as well as a reduction in the lifespan of ships (Salta et al., 2013). The beneficial properties of anti-adhesive and anti-biofilm activities make strain CO100 an efficient candidate to resolve these problems in marine and petroleum sector, as well as in other fields (medical, hygienic, food, etc).

4. Conclusion

A halotolerant marine bacterium CO100 of *Staphylococcus pasteuri* was isolated from hydrocarbons-polluted sediments collected from the fishing harbour of Sfax, Tunisia. This

strain exhibited a notable capacity to degrade crude oil and grow on PAHs (phenanthrene, fluoranthene and pyrene), used as the sole carbon and energy sources, under high salinity up to 100 g/l NaCl. Moreover, the isolate CO100 highlighted an interesting potential to synthesize tensioactive agents using low-value carbon sources (Residual frying oil and expired powder milk), at 100 g/l NaCl. The ESI-MS analysis of the purified BS-CO100 revealed that the latter belonged to lipopeptide family, more specifically, the lichenysin and iturin members. BS-CO100 showed interesting tensioactive properties: an important reduction of the surface tension; low CMCs and a high steadiness, faced to a wide spectrum of pH, temperature and salinity. Moreover, CO100 isolate was found to have no cytotoxic effect against human HEK293 cells, even at high concentrations (125-1000 µg/ml). The biosurfactant BS-CO100 was able to recover oil more effectively than a number of synthetic surfactants, either as crude form or cell-free broth. In addition, lipopeptides BS-CO100 exhibited interesting anti-biofilm and anti-adhesion activities, allowing the prevention and the disruption of the microbial biofilms. Taken together, these promising findings point out that *Staphylococcus* sp. strain CO100, as well as its biosurfactant BS-CO100, could be strongly used for the bioremediation of oil-contaminated environments, even under high salinity conditions. The provision of alternative substrates represents an important contribution to future biosurfactant production industries. The optimization of culture media and bioprocess conditions, using adequate experimental designs, is requested to achieve the highest biodegradation rates and biosurfactants yields, allowing then large-scale applications.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at xxxxx.

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Table

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Table 1.

Evaluation of the prevention (pre-treatment) and disruption (post-treatment) of microbial biofilm formation on the polystyrene surface by biosurfactant BS-CO100.

BS-CO100 (µg/ml)	*Escherichia coli*	*Klebsiella pneumoniae*	*Salmonella typhimurium*	*Pseudomonas aeruginosa*	*Bacillus cereus*	*Enterococcus faecalis*	*Staphylococcus aureus*	*Candida albicans*
Pre-treatment (Anti-adhesive activity)								
4	54.2 ± 0.3	36.5 ± 1.8	51 ± 1.3	45.3 ± 2.2	62.7 ± 2.1	47.5 ± 1.6	66.8 ± 0.4	74.9 ± 1.6
2	50 ± 1.0	32.7 ± 1.9	48.2 ± 0.8	41.8 ± 1.8	60.3 ± 1.4	46.8 ± 1.3	62.7 ± 1.1	73.2 ± 1.7
1	44.6 ± 1.2	30.6± 2.4	42 ± 0.6	37.4 ± 1.5	58.8 ± 1.3	40.9 ± 0.5	54.7 ± 1.3	70.9 ± 1.2
0.5	37.8 ± 1.3	26.2 ± 1.8	39.4 ± 1.5	32.22 ± 1.3	54.9 ± 0.8	37.8 ± 0.6	46.2 ± 1.6	67.2 ± 2.3
0.25	31.3 ± 0.6	22.2 ± 1.8	33.7 ± 1.8	30.8 ± 1.5	44.8 ± 1.2	35.5 ± 1.9	37.5 ± 1.8	62.9 ± 2.1
0.125	25.4 ± 1.4	18.2± 1.9	25.6 ± 1.7	27.56 ± 1.8	40.5 ± 1.8	32.6 ± 1.6	31.3 ± 1.3	54.8 ± 0.5
0.062	20 ± 2.4	16.1± 2.5	21.2 ± 0.3	21.74 ± 2.3	37.3 ± 0.4	30 ± 2.3	27.4 ± 0.8	48.9 ± 2.3
0.031	17.5 ± 1.7	11.5 ± 1.36	18.5 ± 2.4	16.5 ± 1.5	32.1 ± 1.2	27.4 ± 1.7	25.9 ± 2.2	40.8 ± 1.7
0.015	13.5 ± 2.1	7.5± 1.6	15 ± 1.2	10.6 ± 2.6	20.8 ± 3.3	26.1 ± 1.2	23.1 ± 1.7	27 ± 1.6
Control	0	0	0	0	0	0	0	0
Post-treatment (Anti-biofilm activity)								
4	50.3 ± 1.3	47.2 ± 1.1	55.1 ± 0.8	66.2 ± 1.1	61.4 ± 1.1	58.3 ± 1.2	70.3 ± 2.1	58.4 ± 1.5
2	50.1 ± 0.9	42.1 ± 1.6	44.5 ± 1.1	63.13± 2.1	54.7 ± 1.5	57.4 ± 0.9	69.2 ± 0.6	56.7 ± 2.0
1	45.7 ± 2.2	40.43 ± 2.5	43.4 ± 1.5	57.3 ± 1.1	50.8 ± 0.5	50.1 ± 1.5	66.8 ± 0.9	53.3 ± 1.3
0.5	44.3 ± 1.2	36.2 ± 1.7	41.7 ± 2.4	51.4 ± 1.6	50.1 ± 0.9	48.1 ± 1.3	60.3 ± 1.3	50.1 ± 1.5
0.25	33.2 ± 1.6	31.7 ± 1.2	40.8 ± 1.3	48.2 ± 0.3	44.6 ± 1.3	37.1 ± 1.7	54.2 ± 1.5	42.8 ± 1.1
0.125	30.1 ± 1.6	28.2 ± 0.8	35.9 ± 1.7	44.6 ± 2.4	40.9 ± 1.6	30.3 ± 1.1	47.7 ± 1.2	36.1 ± 0.9
0.062	20 ± 2.7	26.3 ± 1.8	26.9 ± 2.3	33.2 ± 1.9	35.3 ± 1.8	30 ± 1.7	38.1 ± 1.5	33.3 ± 0.6
0.031	17.5 ± 2.5	21.4 ± 2.0	21.7 ± 0.4	21.9 ± 1.7	33.1 ± 2.3	23.9 ± 2.9	33.6 ± 1.4	27.3 ± 1.5
0.015	13.5 ± 2.4	15.4 ± 2.3	19.4 ± 1.4	16.2 ± 1.42	23.2 ± 2.1	19.3 ± 2.2	30.1 ± 2.7	19.7 ± 0.8
Control	0	0	0	0	0	0	0	0

Figure captions

Fig. 1. Growth of strain CO100 on basal medium containing crude oil (1%, v/v) at 100 g/l NaCl, 37 °C and 180 rpm, monitored by measuring OD 600 nm (■) and by enumeration of bacterial cell counts (CFU/ml) (♦). Biotic control (●), Abiotic control (▲).

Fig. 2. GC-MS profiles of the aliphatic fraction of crude oil remaining in basal medium, with strain CO100 during 0, 10 and 20 days (Day 0, Day 10 and Day 20), and without CO100, as abiotic control (AC), after 20 days (AC Day 20), at 100 g/l NaCl, 37 °C and 180 rpm. C_{13} - C_{29} indicate *n*-alkanes with the number of carbon atoms from 13 to 29.

Fig. 3. Evaluation of growth (OD at 600 nm and log (CFU/ml)) (■), surface tension (●) and oil displacement test (▲) of strain CO100 growing on basal medium containing residual frying oil (1%, v/v) (a) and expired powder milk (20 g/l) (b), at 37 °C and 180 rpm.

Fig. 4. ESI-MS spectrum of molecular mass biosurfactant produced by strain CO100 of *Staphylococcus pasteuri*. (a) and (b) correspond to two fractions having different polarity obtained after TLC analysis.

Fig. 5. Critical micelle concentrations (CMCs) of purified BS-CO100 produced on nutrient broth NB (a) and crude BS-CO100 biosurfactants produced on nutrient broth NB (b), residual frying oil RFO (c) and expired powder milk EPM (d).

Fig. 6. Stability of BS-CO100 against various pH, temperatures and salinities.

Fig. 7. Percentage of cell viability of human HEK293 cell line after treatment by different concentrations of biosurfactant BS-CO100.

Fig. 8. The BS-CO100 remobilization potential using used motor oil-contaminated soil (20%, w/v) against Triton X-100, Tween 20, Tween 80 and SDS. (RFO: Residual Frying Oil; EPM: Expired Powder Milk). Values given represent the mean of three replicates ± standard

deviation. [a] $p < 0.05$: Cell-free broth CO100 (RFO) vs. other groups; [b] $p < 0.05$: Cell-free CO100 broth (EPM) vs. other groups; [c] $p < 0.05$: BS-CO100 (RFO) vs. other groups; [d] $p < 0.05$: BS-CO100 (EPM) vs. other groups.

Fig. 1.

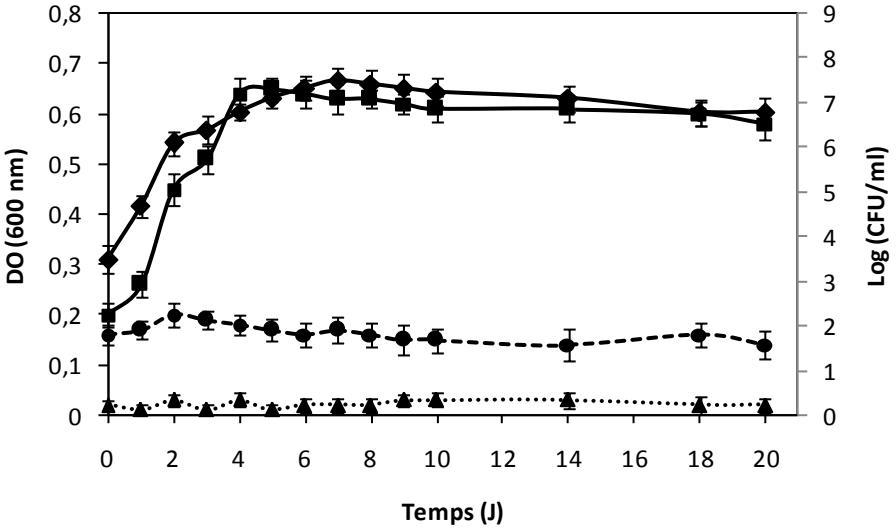

0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
DO (600 nm)
9
8
7
6
5
4
3
2
1
0
Log (CFU/ml)
0
2
4
6
8
10
12
14
16
18
20
Temps (J)

Fig. 2.

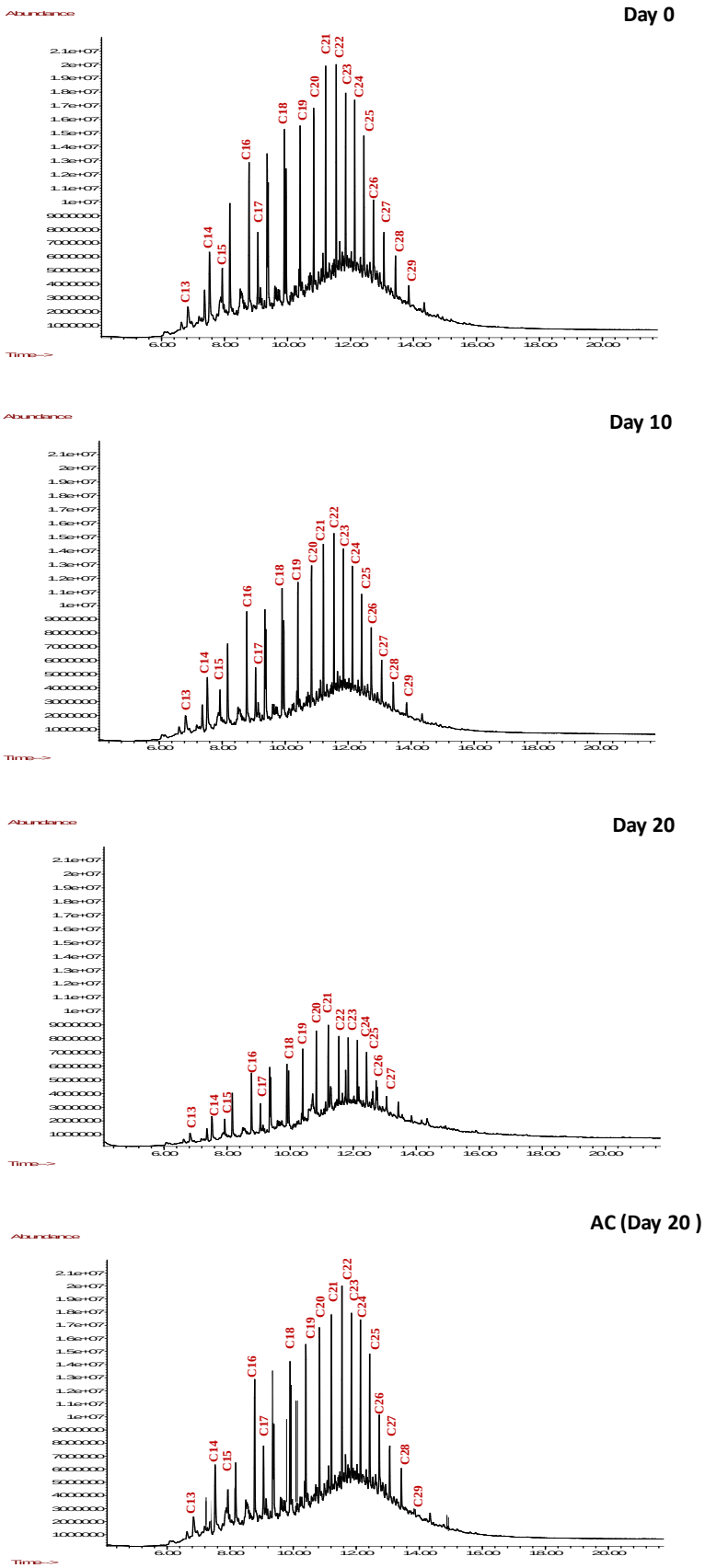

Day 0
Abundance
Time-->
C13
C14
C15
C16
C17
C18
C19
C20
C21
C22
C23
C24
C25
C26
C27
C28
C29
Day 10
Abundance
Time-->
Day 20
Abundance
Time-->
AC (Day 20)
Abundance
Time-->

Fig. 3.

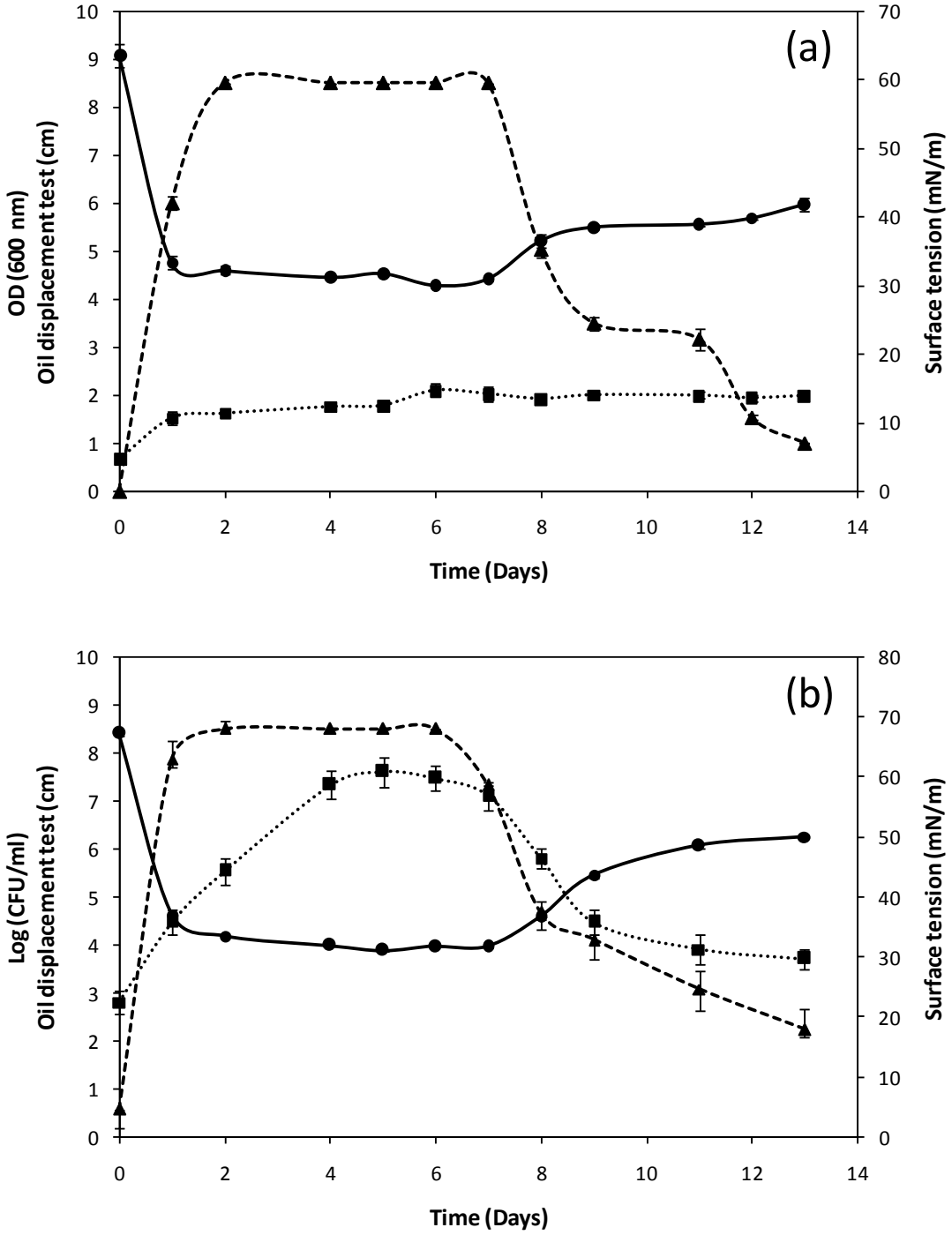

(a)
OD (600 nm)
Oil displacement test (cm)
Surface tension (mN/m)
Time (Days)
(b)
Log (CFU/ml)
Oil displacement test (cm)
Surface tension (mN/m)
Time (Days)

Fig. 4.

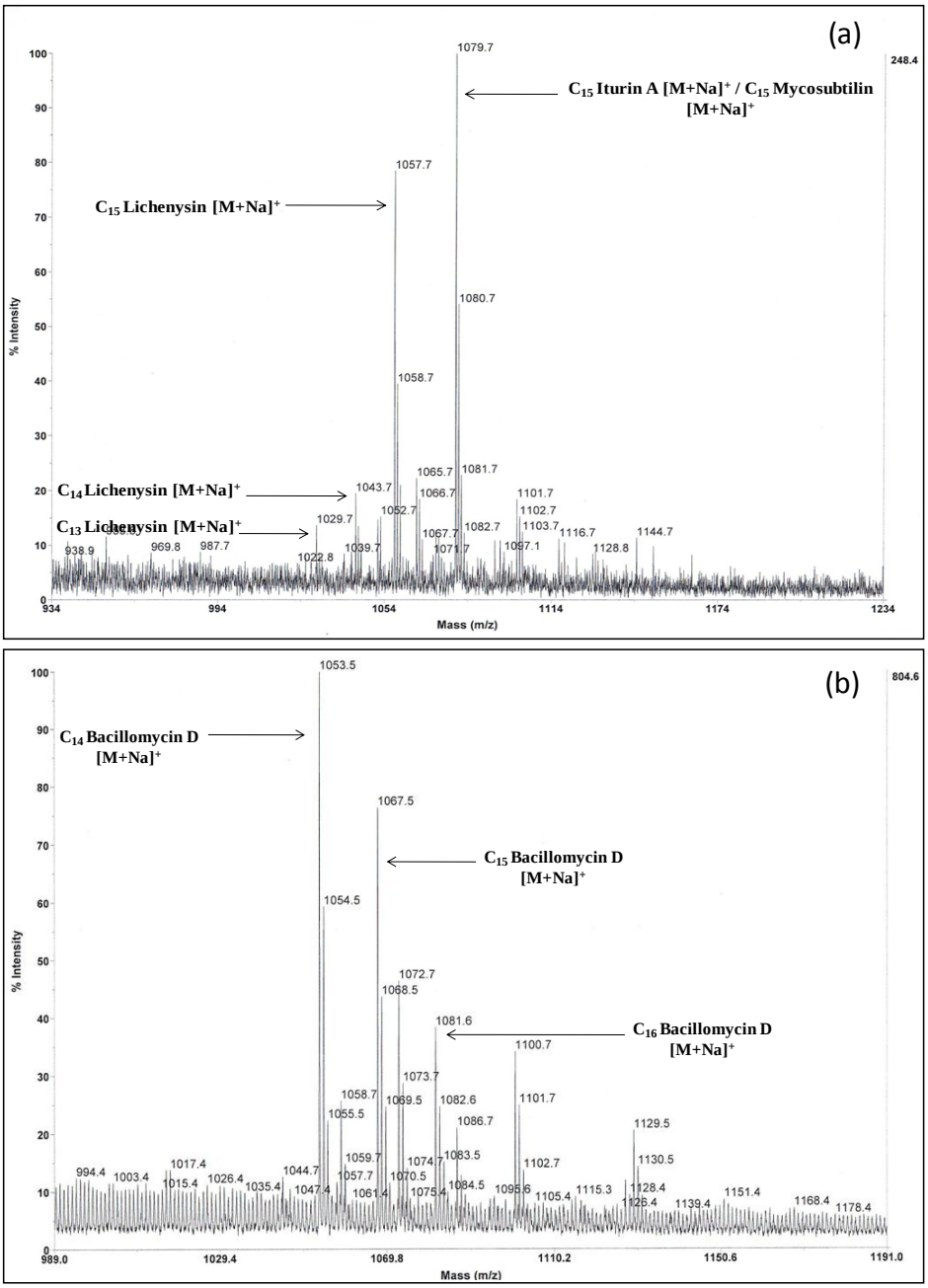

(a)
1079.7
248.4
C15 Iturin A [M+Na]+ / C15 Mycosubtilin [M+Na]+
1057.7
C15 Lichenysin [M+Na]+
1080.7
1058.7
1065.7
1081.7
C14 Lichenysin [M+Na]+
1043.7
1066.7
1101.7
1052.7
1102.7
C13 Lichenysin [M+Na]+
1029.7
1067.7
1082.7
1103.7
1116.7
1144.7
938.9
969.8
987.7
1022.8
1039.7
1071.7
1087.1
1128.8
% Intensity
Mass (m/z)
(b)
804.6
1053.5
C14 Bacillomycin D [M+Na]+
1067.5
C15 Bacillomycin D [M+Na]+
1054.5
1072.7
1068.5
1081.6
C16 Bacillomycin D [M+Na]+
1100.7
1073.7
1058.7
1069.5
1082.6
1101.7
1055.5
1086.7
1129.5
1059.7
1083.5
1130.5
1074.7
1102.7
994.4
1003.4
1017.4
1015.4
1026.4
1035.4
1044.7
1047.4
1057.7
1070.5
1061.4
1075.4
1084.5
1095.6
1105.4
1115.3
1128.4
1126.4
1139.4
1151.4
1168.4
1178.4
% Intensity
Mass (m/z)

Fig. 5.

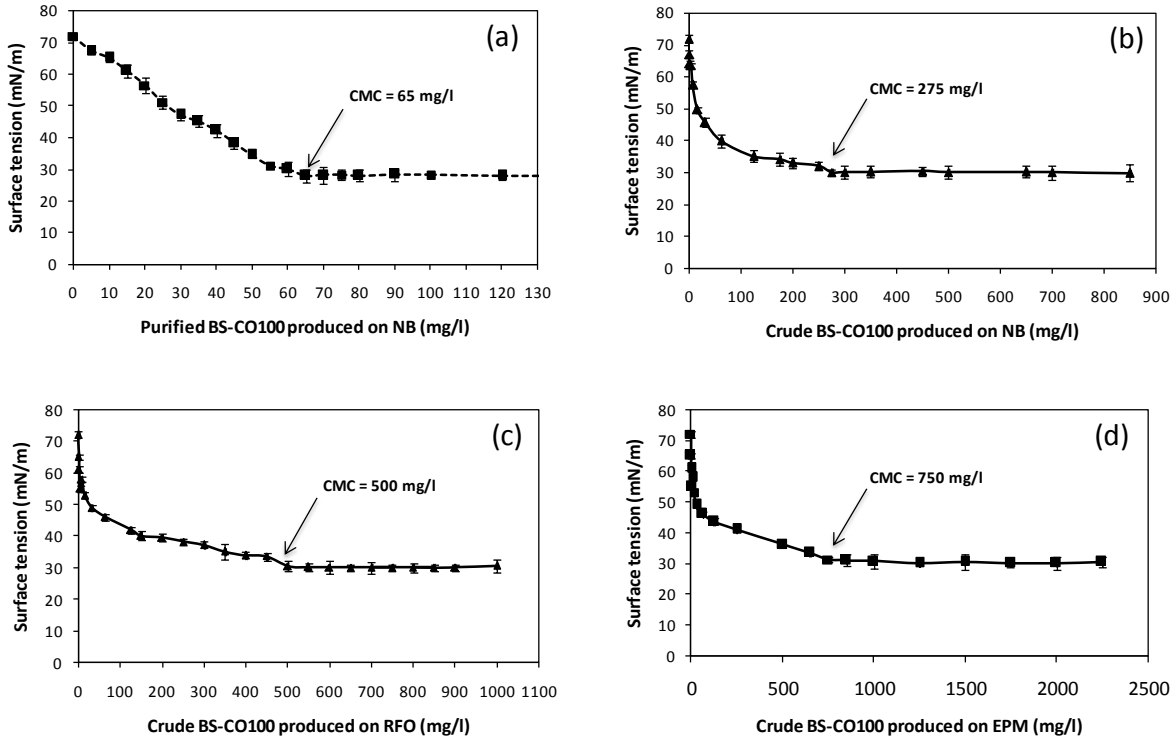

(a)
CMC = 65 mg/l
Surface tension (mN/m)
Purified BS-CO100 produced on NB (mg/l)
(b)
CMC = 275 mg/l
Surface tension (mN/m)
Crude BS-CO100 produced on NB (mg/l)
(c)
CMC = 500 mg/l
Surface tension (mN/m)
Crude BS-CO100 produced on RFO (mg/l)
(d)
CMC = 750 mg/l
Surface tension (mN/m)
Crude BS-CO100 produced on EPM (mg/l)

Fig.6.

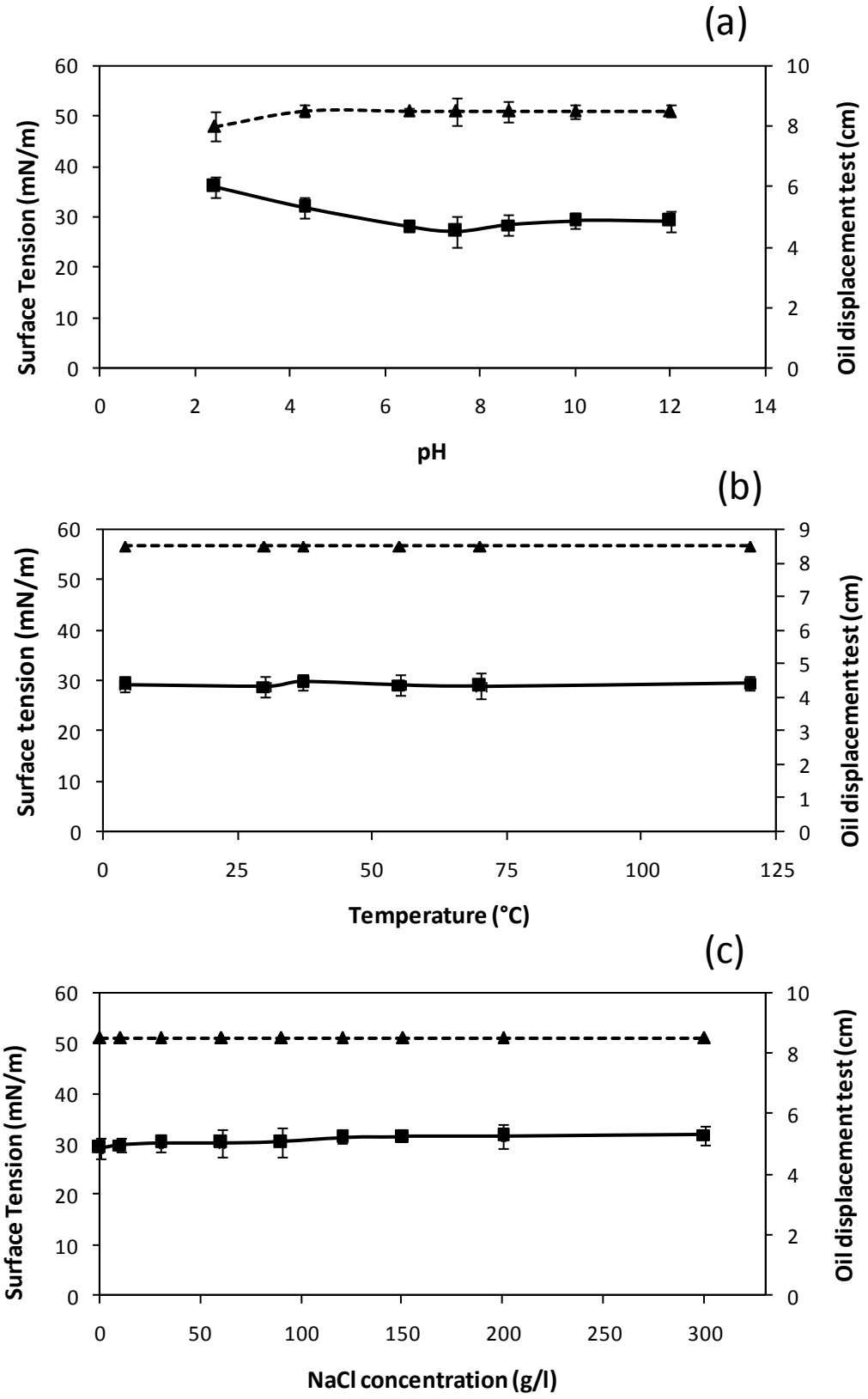

(a)
Surface Tension (mN/m)
Oil displacement test (cm)
pH
(b)
Surface tension (mN/m)
Oil displacement test (cm)
Temperature (°C)
(c)
Surface Tension (mN/m)
Oil displacement test (cm)
NaCl concentration (g/l)

Fig.7.

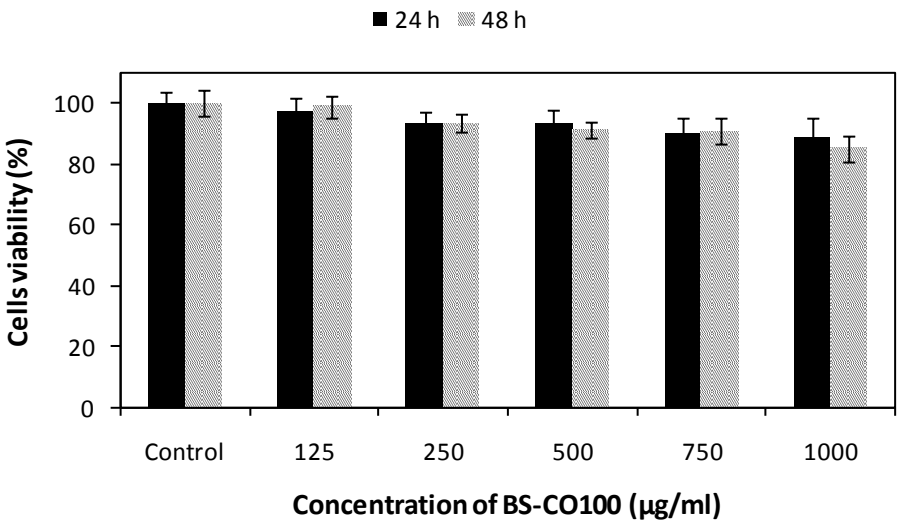
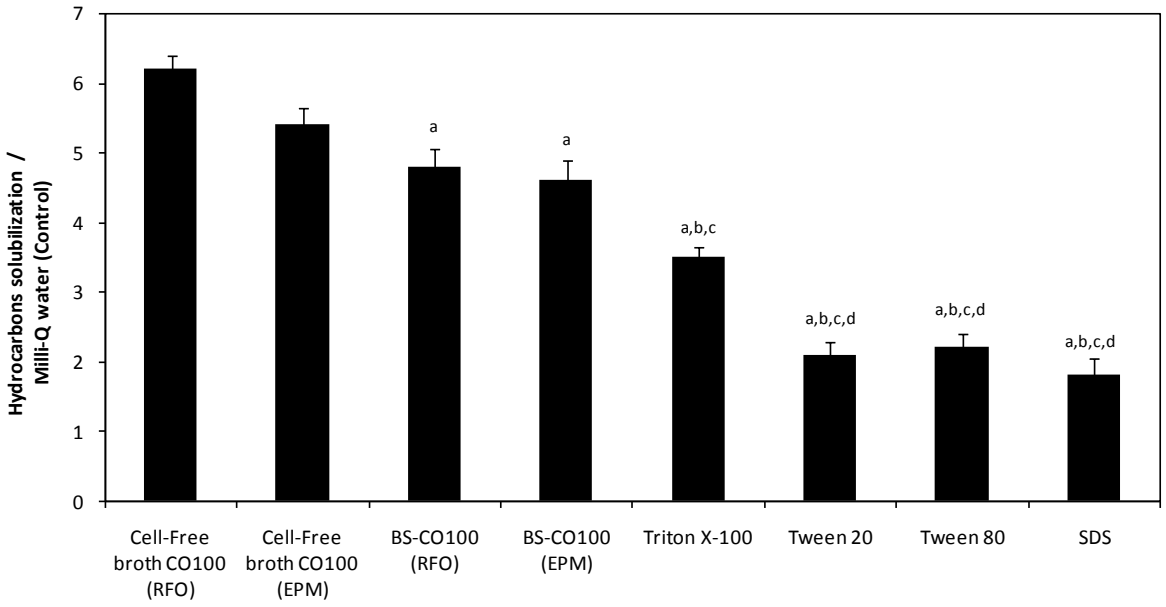

24 h
48 h
Cells viability (%)
100
80
60
40
20
0
Control
125
250
500
750
1000
Concentration of BS-CO100 (µg/ml)

Fig.8.


Hydrocarbons solubilization / Milli-Q water (Control)
7
6
5
4
3
2
1
0
a
a
a,b,c
a,b,c,d
a,b,c,d
a,b,c,d
Cell-Free broth CO100 (RFO)
Cell-Free broth CO100 (EPM)
BS-CO100 (RFO)
BS-CO100 (EPM)
Triton X-100
Tween 20
Tween 80
SDS