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Isolation and characterization of a microorganism that produces biosurfactants in corn steep water

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ABSTRACT

In this work a *Bacillus* strain with capacity to produce biosurfactants was isolated from commercial corn steep water (CSW). The identification of this bacterium was based on phenotypic characteristics. This is a Gram-positive *Bacillus*, macroscopically catalase-negative, that possesses high mobility and forms terminal endospore. The colonies formed by the isolated *Bacillus* strain are characterized by a creaming, dull and glistening phenotype with a small point-like elevation in the center. Regarding its antimicrobial activity, the isolated *Bacillus* strain was effective against *Candida albicans* and *Staphylococcus aureus* observing, in the presence of pathogenic strains, a phenotype dissociation, switching the colonies from a whitish to yellowish pigmentation. The isolated microorganism, contrarily to other *Bacillus* strains, has the ability to produce extracellular and cell-bound biosurfactants. The analyses revealed that both biosurfactant extracts are similar to the lipopeptide biosurfactant produced by *Bacillus subtilis*.

ARTICLE HISTORY

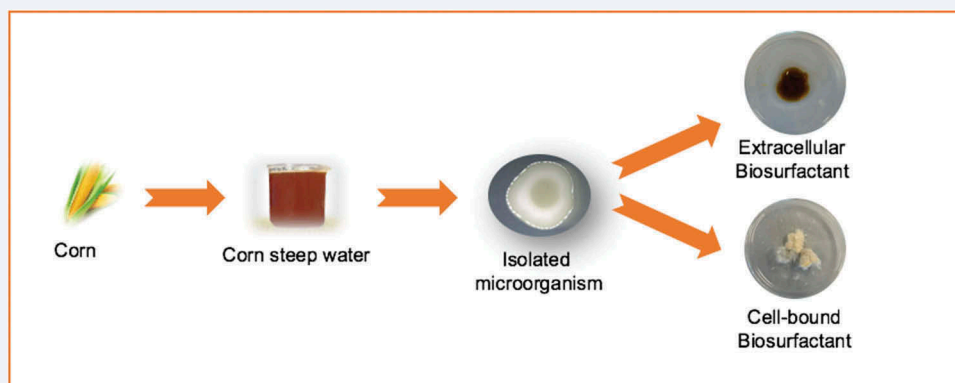
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Bacillus; phenotype; colonies; endospore; biosurfactant; corn

PALABRAS CLAVES

Bacillus; fenotipo; colonias; endosporas; biosurfactante; maíz



Aislamiento y caracterización del microorganismo responsable de la producción de biosurfactantes en licores de lavado de maíz

RESUMEN

En este trabajo, una cepa de *Bacillus* con capacidad para producir biosurfactantes fue aislada de los licores de lavado de maíz (Corn Steep Water (CSW)). La identificación de esta bacteria se basó en características fenotípicas. Esta es un *Bacillus* gram-positivo, catalasa-negativo que, a nivel macroscópico, posee alta movilidad y forma endosporas terminales. Las colonias formadas por la cepa aislada de *Bacillus* se caracterizan por un fenotipo cremoso, opaco y brillante, con una pequeña elevación en el centro. En cuanto a su actividad antimicrobiana, la cepa aislada de *Bacillus* fue eficaz contra *Candida albicans* y *Staphylococcus aureus* observando, en presencia de cepas patógenas, una disociación en su fenotipo, pasando las colonias de una pigmentación blanquecina a amarillenta. El microorganismo aislado, contrariamente a otras cepas de *Bacillus*, tiene la capacidad de producir biosurfactantes extracelulares y biosurfactantes ligados a su membrana. Los análisis revelaron que ambos extractos biosurfactantes tienen una composición similar al biosurfactante lipopéptido producido por *Bacillus subtilis*.

Introduction

Biosurfactants are biocompatible and biodegradable surface-active compounds produced by microorganisms. In contrast to most surfactants, which are obtained by chemical synthesis, biosurfactants present features such as good efficiency and low levels of toxicity and irritation in addition to the fact that they are environmentally friendly. They can be used to help in solubilisation and stabilization of emulsions and to enhance the sensory and rheological characteristics of mixtures including food formulations (Santos, Rufino, Luna, Santos, & Sarubbo, 2016).

On the other hand, it has been demonstrated that some biosurfactants have antimicrobial activities in addition to their surfactant capacity (Das, Mukherjee, & Sen, 2008). This characteristic can be an additional advantage for the incorporation of these secondary metabolites in different sectors including pharmaceutical, cosmetic and food industry (López-Prieto, Rodríguez-López, Rincón-Fontán, Moldes, & Cruz, 2019; Rodríguez-López, Rincón-Fontán, Vecino, Cruz, & Moldes, 2017; Ron & Rosenberg, 2001; Souza, Oliveira de Souza de Azevedo, Domínguez, Converti, & Pinheiro de Souza Oliveira, 2017; Vecino, Barbosa-Pereira, Devesa-Rey, Cruz, & Moldes, 2015a).

In recent years, agro-industrial wastes have gained attention as possible sources for the production and extraction of biosurfactants promoting their use as secondary raw materials (Rodríguez-López et al., 2017; Vecino, Barbosa-Pereira, Devesa-Rey, Cruz, & Moldes, 2014; Vecino et al., 2017). Therefore, Vecino et al. (2014) have proven that corn steep water (CSW), a fermented residual stream of corn-milling industry, is a direct source of biosurfactants due to the spontaneous growth of microorganisms producing biosurfactants. Nevertheless, these microorganisms have not been isolated at the moment.

In the corn wet milling industry, the steeping process of corn is carried out between 45°C and 52°C under acid conditions and in the presence of SO₂ (Hull, Yang, Venzke, Kulhavy, & Montgomery, 1996), therefore, it can be speculated that the existing microbial biomass in CSW possesses a special mechanism to support these extreme environmental conditions.

On the other hand, López-Prieto et al. (2019) showed that the biosurfactant extract obtained from CSW can be integrated in the dairy industry. These authors proved that the incorporation of this biosurfactant to a drinkable yogurt promoted the growth of *Lactobacillus casei*, considered a probiotic bacterium. Hence, it can be speculated that the biosurfactant extracted from CSW has prebiotic properties favoring the growth of probiotic bacteria in the intestine.

Regarding the composition of the biosurfactant extracted from CSW, it was characterized as a lipopeptide, composed by C16 and C18 fatty acids, comprising oleic, linoleic and stearic fatty acids which are linked to a small fraction of an organic nitrogen chain composed by amino acids (Rincón-Fontán, Rodríguez-López, Vecino, Cruz, & Moldes, 2017; Rodríguez-López, Vecino, Barbosa-Pereira, Moldes, & Cruz, 2016; Vecino, Rodríguez-López, Cruz, & Moldes, 2015b). Therefore, Rincón-Fontán et al. (2017) reported that this biosurfactant possesses a molecular weight similar to the lipopeptides produced by *B.subtilis*.

Although most of microbial biosurfactants are produced extracellularly, some microorganisms, including lactic acid bacteria like *Lactobacillus pentosus*, *Lactobacillus paracasei* or *Lactococcus lactis*, can produce cell-bound biosurfactants

(Rodrigues, Moldes, Teixeira, & Oliveira, 2005; Souza et al., 2017; Vecino, Barbosa-Pereira, Devesa-Rey, Cruz, & Moldes, 2015c; Vecino et al., 2017).

The aim of this work was to isolate and characterize morphologically the microorganism that produces lipopeptide biosurfactants in CSW and compare it with other commercial biosurfactants characterized as lipopeptides, like those produced by *B. subtilis*. The study also includes the evaluation of the antimicrobial capacity of the isolated microorganism against pathogenic bacteria and fungi.

Materials and methods

Isolation of the microorganism from Corn Steep Water (CSW)

For the isolation of the microorganism, 5 ml of CSW, provided by FeedStimulants (Reg. No. NL214247/Lot NL-2728DK 7) were inoculated in Tryptic Soy Agar (TSA) or Saboraud Dextrose Agar (SDA) medium, in 9 cm Petri dishes, previously autoclaved for 15 min at 121°C. After inoculation, plates were incubated at 37°C during 48–72 h. Following, a loop of the biomass cultivated in the plates was surface-streak and inoculated in the same culture media, under the previous conditions, in order to obtain isolated colonies.

Once the colonies were isolated, a Gram staining and a catalase test were carried out. Catalase test was performed by adding on the colonies a drop of 3% of hydrogen peroxide (H₂O₂) under sterile conditions as described in previous studies (Reiner, 2010).

In addition, isolated colonies were inoculated into 250 ml flasks containing 100 mL of Trypticase Soy Broth (TSB) or Saboraud Dextrose Broth (SDB) medium, previously autoclaved for 15 min at 121°C, in order to evaluate their capacity to produce biosurfactants. The incubation in both media was carried out for 48 h at 37°C and 150 rpm.

Microscopically characterization of the microorganism isolated from CSW

For the visual analysis, Petri dishes of the isolated microorganism in TSA and SDA media were directly evaluated under a Stereoscopic Zoom Microscope Nikon SMZ1500 (Nikon Instruments Europe B.V., Amsterdam, Netherlands) with an Infinity X camera capturing pictures of the isolated colony at the central and side points. Moreover, each colony from TSA and SDA media was re-suspended in sterile physiological water and visualized on a slide covered by a coverslip by scanning electron microscopy (SEM) under a Nikon Eclipse E800 microscope (Nikon Instruments Europe B.V., Amsterdam, Netherlands) with a DS-5 camera.

Antimicrobial activity of the microorganism isolated from CSW

The antimicrobial activity of the microorganism isolated from CSW was assessed by performing an antagonist experiment against three different pathogens obtained from the Spanish Type Culture Collection (CECT) (Valencia, Spain). The pathogenic strains selected were *Candida albicans* CECT-1392 (ATCC-2091), cultivated in Malt Agar (at 26°C for 48 h), *Aspergillus niger* CECT-2574 (ATCC-16404), cultivated in Potato Dextrose Agar (PDA) (at 24°C for 48 h) and *Staphylococcus*

aureus CECT-239 (ATCC-6538) which was cultivated in Nutrient Agar (NA) (at 37°C for 24 h), following the indications of CECT.

The antimicrobial experiments were carried out in 9 cm Petri dishes containing TSA or SDA medium autoclaved for 15 min at 121°C. Each pathogen was placed in the middle of the plate and colonies of the isolated microorganism from CSW were placed in the four corners of a square around the pathogens. Once the inoculation was conducted, the plates were incubated for 48–72 h at 37°C. The experiment was carried out in sterile conditions and all plates were performed by duplicate.

Extraction of extracellular and cell-bound biosurfactants

1 mL of the isolated microorganism, growth in TSB or SDB, was re-inoculated again in 100 mL of TSB or SDB medium at 37°C and 150 rpm, in 250 mL Erlenmeyer flasks, for 48 h. Following, the biomasses of fermented media were separated by centrifugation (during 15 min, at 5°C and 5000 rpm), and the supernatants were mixed with chloroform at 56°C for 1 h, in order to extract the extracellular biosurfactants produced in the fermented media, following the methodology described in previous studies (Vecino et al., 2015a). The ratio between fermented medium and chloroform was 1:2 v/v. Next, fermented media were separated from the organic phase into separatory funnels and the biosurfactant extracts were recovered and extracted from the organic phase by vacuum distillation in a rotatory evaporator. Afterwards, the extracts were lyophilized using a lyophilizer Telstar LyoQuest (Spain).

On the other hand, in order to test the production of cell-bound biosurfactants, the biomasses separated by centrifugation from the different fermented media were washed twice with demineralized water and re-suspended in 50 ml of phosphate buffer saline (PBS) (10 mM $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ and 150 mM NaCl with pH adjusted to 7.0) as described in previous studies (Vecino et al., 2015c). Subsequently, the microbial biomasses were removed by centrifugation (during 15 min, at 5°C and 5000 rpm) and the PBS extracts, containing the biosurfactants were filtered with a 0.22 μm membrane, PVDF Stericup® 150 ml Durapore® (EMD Millipore Corporation, Billerica, MA 01821 USA).

Afterwards, the PBS extracts containing the cell-bound biosurfactant were dialyzed against demineralized water at 4°C in a Cellu-Sep® membrane (molecular weight cut-off 6000–8000 Dalton; Membrane Filtration Products, Inc., USA) for 48 h, and finally the biosurfactants were lyophilized using a lyophilizer Telstar LyoQuest (Spain).

As it is shown in Figure 1, four different biosurfactants extracts were obtained from two different media (TSB and SDB), using two different extraction methods, to obtain both extracellular and cell-bound biosurfactants. These extracts were named as follows: BS₁, as the extracellular biosurfactant produced in TSB medium; BS₂, as the cell-bound biosurfactant produced in TSB medium; BS₃, as the extracellular biosurfactant produced in SDB medium and BS₄ as the cell-bound biosurfactant produced in SDB medium.

Evaluation of the surfactant capacity

The surface tension (ST) of extracellular biosurfactants contained in TSB and SDB media and ST of cell-bound biosurfactants contained in PBS was measured using a Krüss K20 EasyDyne tensiometer with a 1.9 cm platinum Willhemly

plate at room temperature. All determinations were carried out in triplicate in order to increase the accuracy of the measurements.

Elemental analysis of the biosurfactants extracted from CSW

An elemental analyzer (Fisons Carlo Erba EA-1108 CHNS-O, LabX, Midland, ON, Canada) was used for the determination of C, H, N and S in the biosurfactant extracts obtained from the isolated microorganism after cultivation in TSB or SDB medium.

FTIR characterization of biosurfactants extracted from CSW

1 mg of the biosurfactant extracts obtained from the isolated microorganism after the cultivation in TSB or SDB medium, were ground and pressed with 10 mg potassium bromide (7500 kg for 30 s) to obtain translucent pellets. Infrared absorption spectra of the extracellular and cell-bound biosurfactant extracts were recorded on a Nicolet 6700 FTIR system (Thermo Scientific) with a spectral resolution of 4 cm^{-1} and wavenumber accuracy between 400 and 4000 cm^{-1} . As background reference, a potassium bromide pellet was used in the evaluation of all measurements consisted in 32 scans. For comparative purposes, FTIR spectrum of commercial fengycin (Lot-SLBS1147V, Sigma Aldrich, Ca, MO, USA), a lipopeptide biosurfactant produced by *B. subtilis*, was included in the study.

Results and discussion

Isolation and microscopic characterization of biosurfactants produced by the isolated microorganism from CSW

In previous studies it has been demonstrated the presence of biosurfactants in CSW (Rodríguez-López et al., 2016; Vecino et al., 2014, 2015a). This industrial stream is characterized by a high viscosity (it contains 50% of solids), low concentration of sugars and high concentration of lactic acid, which has been employed by many researchers as nutritional supplement in the production of microbial metabolites (Guerra-Rodríguez & Vázquez, 2013).

Figure 2 shows a microscopy view of the strain isolated from CSW, which was characterized as a Gram-positive and macroscopically catalase-negative *Bacillus* strain. During the microscopical observation, it was noticed that this *Bacillus* possesses high mobility. In Figure 2 it can be observed the vegetative cells as well as the endospore formation of the isolated *Bacillus* strain, characterized by a terminal endospore.

Endospore formation is a distinctive feature of *Bacillus* spp. as it has been shown also in previous studies (Nitschke & Pastore, 2006; Rooney, Price, Ehrhardt, Swezey, & Bannan, 2009; Sella, Vandenberghe, & Soccol, 2014; Tan & Ramamurthi, 2014). Therefore, Sella et al. (2014) have reported that the life cycle of spore-forming *Bacillus* is divided in three different stages: vegetative growth, sporulation and germination. In turn, sporulation stage can be sub-divided in seven phases (Sella et al., 2014): *I*) first the nuclear material is disposed axially into filaments; *II*) the completion of DNA segregation and the invagination of the plasmatic membrane in an asymmetric position occurs forming

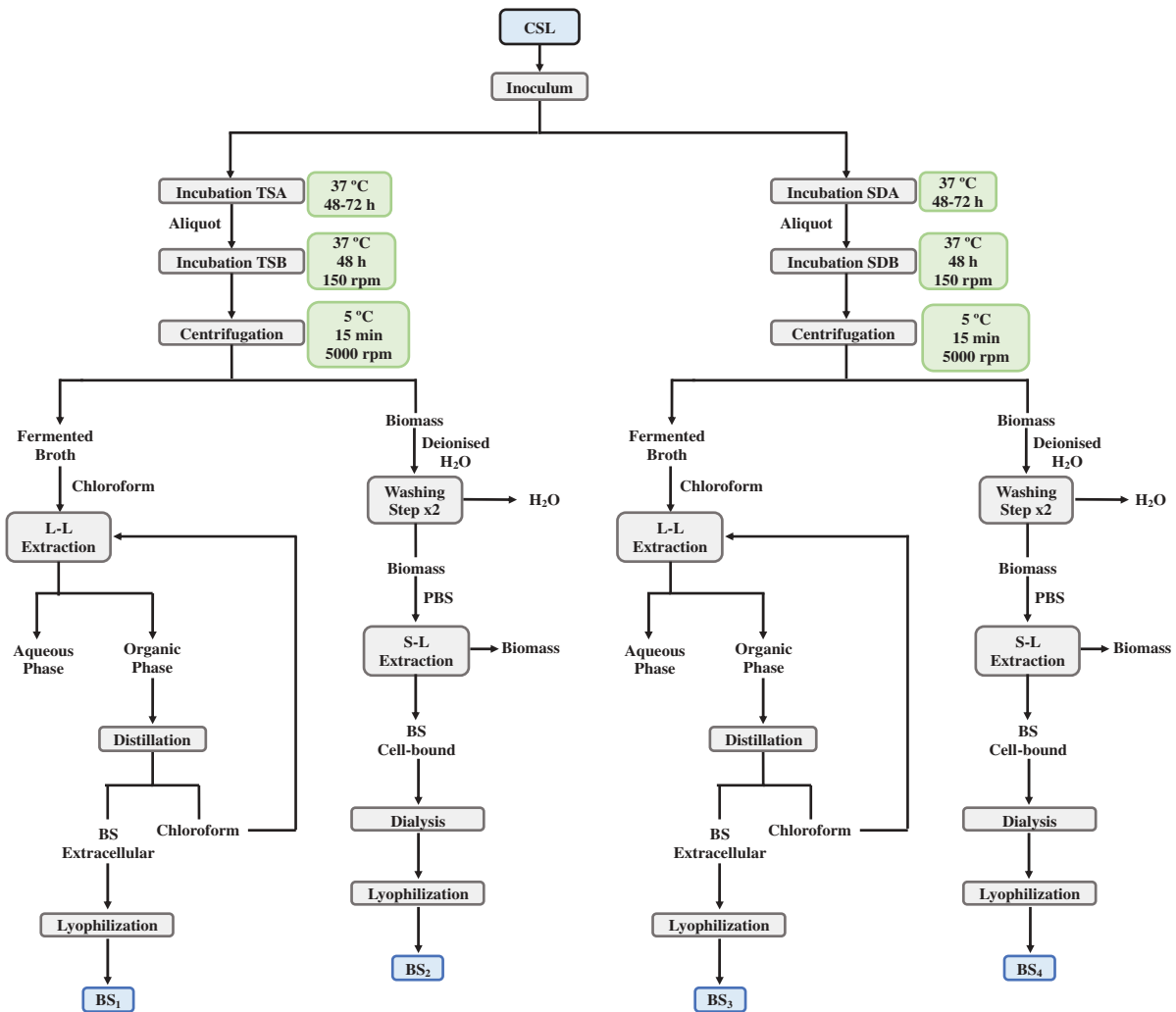


Figure 1. Flowchart of the methodology used in the isolation of the microorganism from CSW and in the extraction of biosurfactants. BS₁: extracellular biosurfactant extract produced in TSB medium; BS₂: cell-bound biosurfactant extract produced in TSB medium; BS₃: extracellular biosurfactant extract produced in SDB medium and BS₄: cell-bound biosurfactant extract produced in SDB medium.

Figura 1. Diagrama de flujo de la metodología utilizada para el aislamiento del microorganismo de CSW y la extracción de biotensoactivos. BS₁: extracto de biotensoactivo extracelular producido en el medio TSB; BS₂: extracto de biotensoactivo unido a células producido en el medio TSB; BS₃: extracto de biotensoactivo extracelular producido en el medio SDB y BS₄: extracto de biotensoactivo unido a células producido en el medio SDB.

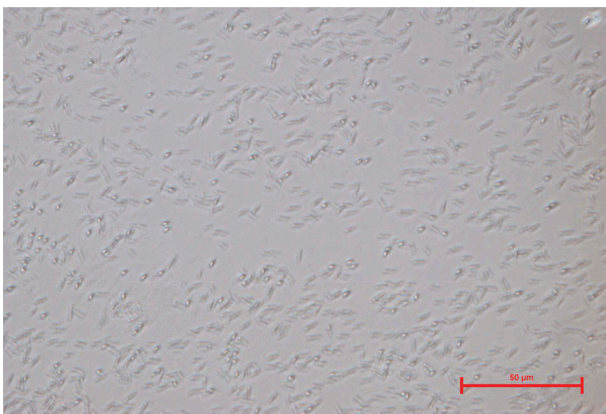


Figure 2. Images of the microbial strain isolated from CSW, obtained with a scanning electron microscopy (SEM).

Figura 2. Imágenes de la cepa microbiana aislada de CSW, obtenidas usando un microscopio electrónico de barrido (SEM).

a septum; *III*) the septum begins to curve, and the smaller forespore becomes entirely contained within the mother cell; *IV*) the mother cell mediates the development of the fore-spore into the spore; *V*) the spore coat is synthesized; *VI*) the mature

spores become resistant to heat and organic solvents and *VII*) lysis of the spore occurs.

Usually, endospores are formed at periods of time of nutritional stress, allowing the organism to persist in the environment until the conditions become favorable (Sella et al., 2014), like those conditions produced during the steeping process of corn. The process of endospore formation has profound morphological and physiological consequences, for instance, a radical post-replicative remodeling of two progeny cells can result in a symmetrical division by binary fission and, in some cases, this binary fission can be accompanied eventually by cessation of metabolic activity in one daughter cell (the spore) and death by lysis of the other (the 'mother cell').

On the other hand, Figure 3 shows pictures of colonies produced by the isolated *Bacillus* at different stages. These colonies are characterized by a soft, creaming, dull and glistening phenotype defined by a small point-like elevation in the center with a whitish gray color. No changes in the colony phenotype were noticed between the colonies growth in TSA or SDA (see Figure 3 (a–c)). Moreover, Figure 3b also shows the process of binary fission for the microorganism isolated from CSW and Figure 3d shows a colony of the isolated *Bacillus* in SDA producing ramifications simulating a tree-like formation. This dissociation of

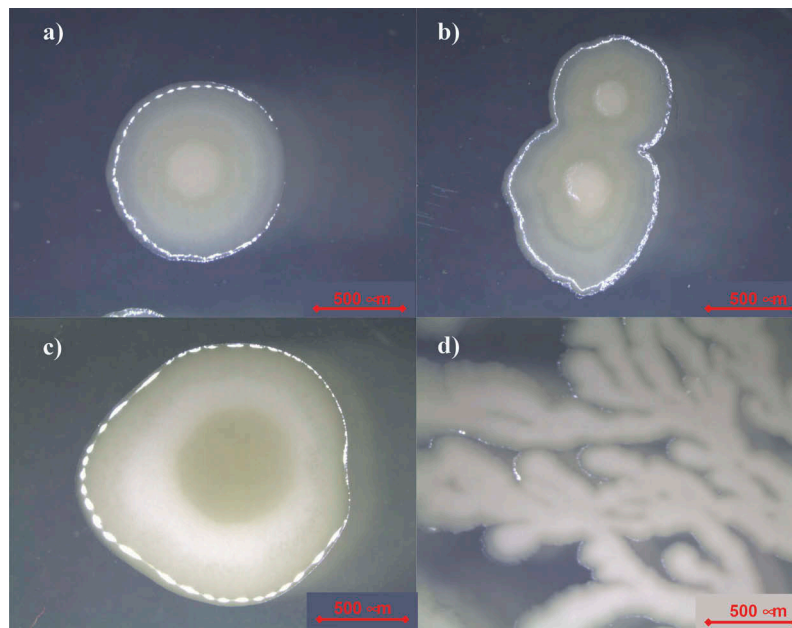


Figure 3. Microscopical images of the microbial strain isolated from CSW obtained with a Stereoscopic Zoom Microscope. (Colonies are shown in sporulation stage). **a)** Isolated colony on SDA **b)** Division of the isolated colony in two progeny cells **c)** Isolated colony on TSA **d)** Colony tree-like growth.

Figura 3. Imágenes microscópicas de la cepa microbiana aislada de CSW obtenida con un microscopio estereoscópico de zoom. (Las colonias se muestran en su etapa de esporulación.) **a)** Colonia aislada en SDA; **b)** División de la colonia aislada en dos células de proyección; **c)** Colonia aislada en TSA; **d)** Crecimiento en forma de árbol de colonia.

phenotype could be characteristic of the strain under some state of stress or reduction of the space able to grow, resulting in a cotton-like colony growth as it has been reported previously in *B. mycooides* (Di Franco, Beccari, Santini, Pisaneschi, & Tecce, 2002). The media used to grow the isolated microorganism from CSW only contained 2.5 g/l of dextrose as carbon source, thus, it can be speculated, that after several days, the reduced concentration of sugars in the medium acts as a trigger inducing changes in the colony phenotype.

Characterization of the biosurfactants produced by the isolated strain from CSW

In order to test the characteristics of the biosurfactants produced by the isolated microorganism from CSW, this was grown in TSB and SDB media and, after fermentation it was evaluated the capacity of this microorganism to produce extracellular and cell-bound biosurfactants. It was observed that this microorganism has the capacity to produce both extracellular and cell-bound biosurfactants, which represents a distinction from other *Bacillus* strains that only produce extracellular biosurfactants.

Table 1 shows the physico-chemical characteristics of the biosurfactants extracted from the *Bacillus* isolated from CSW. The biosurfactants extracts showed differences in the surface tension capacity. Hence, cell-bound biosurfactants reduced in lower extend the surface tension of water (up to 52.3–52.7 mN/m) than extracellular biosurfactants (up to 43.2–49.1 mN/m) depending on the fermentation media. Regarding their appearance, extracellular biosurfactants (BS₁, BS₃) were oily and viscous (see Figure 4a); whereas cell-bound biosurfactants (BS₂, BS₄) had a white powder appearance (see Figure 4b). The different aspect between the extracellular and cell-bound biosurfactants could be linked to the differences in their compositions (Table 1). Hence, it was observed that extracellular biosurfactants (BS₁ and BS₃) content higher amount of C than the cell-bound biosurfactants (BS₂ and BS₄). Regarding the N, H and S content, these were similar among all the biosurfactants extracts produced by the isolated *Bacillus* strain from CSW, except for the BS₃ biosurfactant extract that presents a lower N content and higher H percentage. Therefore, it can be speculated that BS₃ is composed by a higher amount of fatty acids. The percentage of N in the extracts can be correlated with the peptide content multiplying by a factor of 6.25 (Mariotti, Tomé, & Miranda, 2008).

Table 1. Physico-chemical characteristics of biosurfactants extracted from the isolated microorganism from CSW. ST (Surface Tension). BS₁: extracellular biosurfactant extract produced in TSB medium; BS₂: cell-bound biosurfactant extract produced in TSB medium; BS₃: extracellular biosurfactant extract produced in SDB medium and BS₄: cell-bound biosurfactant extract produced in SDB medium.

Tabla 1. Características fisicoquímicas de los biotensioactivos extraídos del microorganismo aislado de CSW. ST (Tensión superficial). BS₁: extracto de biotensioactivo extracelular producido en el medio TSB; BS₂: extracto de biotensioactivo unido a células producido en el medio TSB; BS₃: extracto de biotensioactivo extracelular producido en el medio SDB; y BS₄: extracto de biotensioactivo unido a células producido en el medio SDB.

Appearance	BS ₁	BS ₂	BS ₃	BS ₄
	Oily	White powder	Oily	White powder
ST (mN/m)	49.13 ± 1.55	52.33 ± 3.01	43.25 ± 1.03	52.67 ± 2.25
%N	7.98 ± 0.35	6.77 ± 0.06	4.86 ± 0.12	7.59 ± 0.11
%C	32.05 ± 1.91	24.33 ± 0.23	40.20 ± 0.93	24.75 ± 0.44
%H	4.82 ± 0.21	3.73 ± 0.11	6.93 ± 0.19	3.97 ± 0.07
%S	<0.30	<0.30	<0.30	<0.30

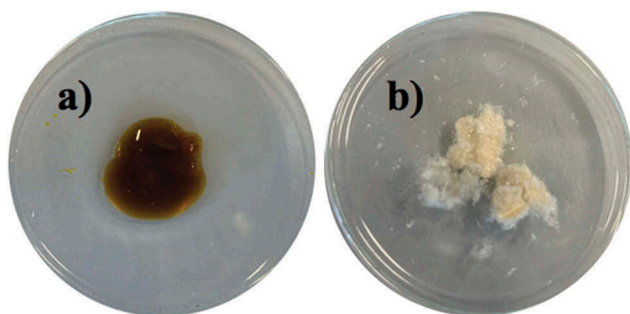


Figure 4. Biosurfactant extracts from the microorganism isolated from CSW. **a)** Extracellular biosurfactant **b)** Cell-bound biosurfactant.

Figura 4. Extractos de biotensoactivo obtenidos del microorganismo aislado de CSW. **a)** Biotensoactivo extracelular; **b)** Biotensoactivo unido a células.

In previous works it has been proved that differences in the composition of fermentation media can provoke changes in the composition, as well as in the surface tension capacity of the biosurfactants produced by a specific microorganism (Vecino et al., 2017), what would explain the differences in the composition found in the biosurfactant (BS₃) produced by the isolated *Bacillus* strain on SDB medium in comparison with the biosurfactant (BS₁) extracted from fermented TSB medium. These differences were less remarked when are compared with the cell-bound biosurfactants.

Comparing both extracellular and cell-bound biosurfactants, it was noticed that cell-bound biosurfactants extracts from the isolated *Bacillus* strain, reduced in lower extend the surface tension of water in comparison with the extracellular biosurfactants. This condition also was noticed by other authors, observing in general that cell-bound biosurfactants produced a lower reduction of surface tension than extracellular biosurfactants (Rodríguez-López, Rincón-Fontán, Vecino, Cruz, & Moldes, 2019; Vecino et al., 2017).

Additionally, Fourier-transform infrared spectroscopy (FTIR) was used to determine the possible similarities of the biosurfactants extracted from the isolated *Bacillus* strain, in comparison with a commercial biosurfactant, fengycin, which is a lipopeptide biosurfactant produced by *B.subtilis* that showed in previous works a higher similarity with the biosurfactant

extracted from CSW (Rincón-Fontán et al., 2017). In this case, it can be observed that the FTIR of fengycin gave a similar response than the biosurfactants extracts obtained from the isolated *Bacillus* strain (Figure 5), with a strong band between 3400 and 3200 cm⁻¹ as the result of N-H and O-H stretching indicating the amine and hydroxyl groups. Moreover, the peaks observed between 3000 and 2800 cm⁻¹ indicate the presence of aliphatic chains, C-H, and peaks between 1650 and 1500 cm⁻¹ represent CO-N bonds and the stretching mode of the C-N bonds respectively; whereas the band about 1400 cm⁻¹ indicates the presence of C = O bonds.

Antagonist effect of the microorganism isolated from CSW on pathogenic microorganisms

The possible antagonistic effect of the strain isolated from CSW was assessed against three pathogenic strains; *C.albicans*, *A.niger* and *S.aureus* on both TSA and SDA media. After incubation, a visual evaluation was carried out to study the ability of the isolated microorganism to inhibit the growth of the pathogens previously mentioned as it is showed in the images included in Figure 6. In order to compare the capacity of inhibition, Petri dishes controls of the three pathogens in TSA and SDA media were also incubated and photographed. It can be observed in the images included in Figure 6 that in TSA media, the growth of the strain isolated from CSW was not affected by the pathogenic strains used in the experiment. Therefore, it correlates with previous works where *Bacillus* bacteria shown to have inhibitory abilities against food-borne pathogens (Moore, Globa, Barbaree, Vodyanoy, & Sorokulova, 2013), probably due to the production of secondary metabolites such as the biosurfactants like those isolated in the current work. Likewise, in SDA medium, the isolated *Bacillus* strain was able to grow in the presence of *C.albicans* and *S.aureus* though it was not the case of *A.niger*, where the pathogen covered the isolated *Bacillus* strain. Benoit et al. (2015) studied the interaction between *B.subtilis* and *A.niger* resulting in an attachment of the bacteria on the hyphae of the fungus showing an alteration of both metabolisms, which could suggest why the pathogen covered the isolated strain from CSW completely.

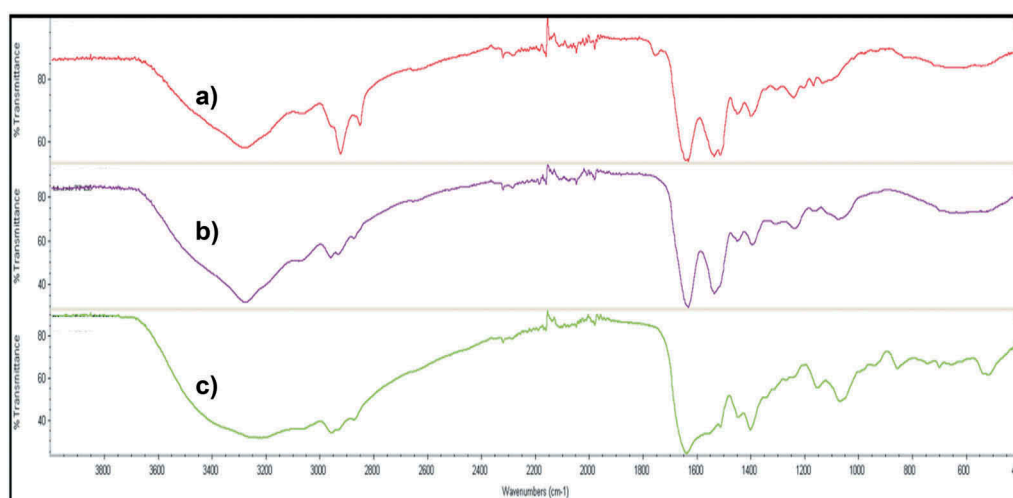


Figure 5. FTIR comparison of spectra of **a)** Fengycin **b)** Cell-bound biosurfactant extracted from the strain isolated from CSW in TSB medium and **c)** Extracellular biosurfactant extracted from the strain isolated from CSW in TSB medium.

Figura 5. Comparación FTIR de los espectros de **a)** Fengicina **b)** Biotensoactivo unido a células extraído de la cepa aislada de CSW en medio TSB y **c)** Biotensoactivo extracelular extraído de la cepa aislada de CSW en el medio TSB.

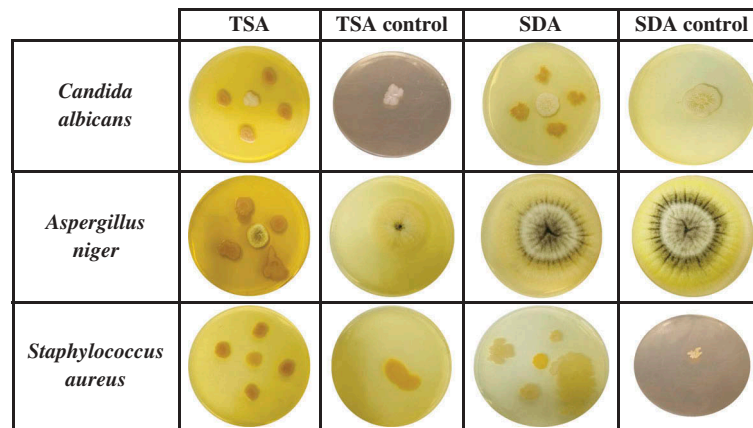


Figure 6. Antagonist effect of *Bacillus* strain, isolated from CSW, against *Candida albicans*, *Aspergillus niger* and *Staphylococcus aureus*.

Figura 6. Efecto antagonista de la cepa de *Bacillus*, aislada de CSW, contra *Candida albicans*, *Aspergillus niger* y *Staphylococcus aureus*.

On the other hand, when the isolated colonies are in contact with pathogenic microorganisms (see images of Figure 6), it produces a yellowish pigmentation. It can be speculated that these pigments could be also a responsive action of the strain to the presence of other microorganisms. Therefore, various authors have observed in several *Bacillus* spp such as *B.subtilis* (Nakamura, 1989), *B.atrophaeus* (Moeller, Horneck, Facius, & Stackebrandt, 2005) and *B.nakamurai* (Dunlap et al., 2016) that environmental stresses produced by the presence of pathogenic microorganisms or the presence of ultraviolet (UV) radiation, can induce dissociation of *Bacillus* phenotype resulting in the production of pigments. Khaneja et al. (2010) also claimed that this photoprotective pigments are related with the presence of melanins and carotenoids, which have been also suggested to play a role as virulence factors in some pathogens like *S.aureus* (Liu et al., 2005).

Conclusions

In this study, the microorganism responsible for the production of biosurfactants in CSW, a residual stream from the corn-milling industry, was isolated and microscopically characterized, observing that is a *Bacillus* strain with phenotypic and metabolic characteristics, similar to other *Bacillus* strains like *B.subtilis*, although with the peculiarity that it is macroscopically catalase negative and it has the ability to produce both extracellular and cell-bound biosurfactants. The *Bacillus* isolated from CSW showed to have ability to produce terminal endospores as well as to exhibit a pigmentation trait that switches from whitish to yellowish in the presence of pathogenic microorganisms. It can be speculated that the presence of endospores allowed to this microorganism to resist the extreme conditions of the corn steeping process and the high viscosity of CSW, which is concentrated up to 50% of solids. Regarding the biosurfactant produced by the isolated *Bacillus* strain, this possesses a similar composition to other commercial biosurfactants, like fengycin, corroborating that the biosurfactant contained in CSW should be a lipopeptide. Finally, the antimicrobial ability of the isolated *Bacillus* strain was evaluated against pathogenic bacteria and fungus showing a good antimicrobial activity against *C.albicans* and *S.aureus*.

Disclosure statement

No potential conflict of interest was reported by the authors.

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