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Microbial production of organic acids by endophytic fungi

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ABSTRACT

The microbial production of organic acids has been considered as a promising strategy to obtain building-block chemicals from renewable feedstocks such as lignocellulosic agro-industrial wastes. This approach is in accordance with the biorefinery concept where the production of biofuels is integrated to higher-value platform chemicals. However, finding a suitable microbial source and the optimum cultivation conditions remains a challenge for the implementation of such process. Endophytic microorganisms have been explored as a source of novel biochemical compounds for biotechnological applications, but the production of organic acids by endophytic fungi remains to be investigated. Here, the potential of using endophytic fungi for organic acids production has been evaluated by carrying out a screening of 35 fungal strains isolated from the leaves and branches of trees inhabiting two mangroves in the state of Sao Paulo, Brazil. The cultivation of a selected *Aspergillus awamori* 09 (4) strain under solid-state fermentation (SSF) using a mix of wheat bran and sugarcane bagasse (1:3) resulted in 135.5 mg/g of organic acids, which represents around 7-fold increase when compared to the use of sugarcane bagasse alone. These results indicate the potential of mangrove-associated endophytic fungi for organic acid production under SSF using agro-industrial wastes as feedstock, being compatible with the current bioeconomy demands.

1. Introduction

The microbial production of organic acids has been considered a promising strategy to obtain building-block chemicals from renewable feedstocks, being compatible with the biorefinery concept (Becker et al., 2015; Chen and Nielsen, 2016; Kamm and Kamm, 2004; Sauer et al., 2008). Such multi-product biorefinery concept comprises the efficient conversion of different renewable feedstocks into fuels, chemicals and novel materials, where the co-production of higher-value products such as organic acids are integrated to biofuels and contributes to the overall process economic feasibility (Cheali et al., 2015). Among the twelve sugar-based building-block chemicals identified by the US Department of Energy as the top value-added chemicals from biomass, nine are organic acids (Werpy and Petersen, 2004). Due to their functional groups, organic acids can be used as raw materials for the chemical industry, with applications in the production of biodegradable polymers, potentially replacing petroleum-based or synthetic chemicals (Sauer et al., 2008). Citric, oxalic, gluconic, fumaric, malic, and succinic acid are examples of organic acids with multiple industrial applications in the food, pharmaceutical, among others sectors (Ciriminna et al., 2017; Goldberg et al., 2006; Sauer et al., 2008; Yang et al., 2017). Furthermore, the production of organic acids in nature is related to the solubilization of soil minerals and release of nutrient ions to plants, thus suggesting a potential role of organic acids in the manufacture of biofertilizers as well (Klaic et al., 2017; Vassilev et al., 2015).

Although the microbial production of organic acids can be carried out by bacteria and yeasts, the fungi are well known for their production of high amounts of various organic acids (Max et al., 2010; Yang et al., 2017). In particular, the filamentous fungus *Aspergillus niger* has been considered as the workhorse for the industrial production of organic acids such as citric acid (Yang et al., 2017). Nevertheless, recent studies exploring the microbial biodiversity have demonstrated the potential of new sources of organic acids producers (Liaud, 2014). However, finding a potential strain and the optimized cultivation conditions to produce high yields of organic acids remains a critical challenge for the industrial implementation of this biotechnological process.

The endophytic fungi represent a potential source of microorganisms for organic acids production. Endophytic fungi inhabit the internal tissues of plants without causing any negative effects, being considered a potential source of novel biochemical compounds for biotechnological applications (Correa et al., 2014; Liu et al., 2017; Robl et al., 2013; Sebastianes et al., 2013, 2017). Comparative studies of the diversity of

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endophytic fungal isolated from the leaves and branches of trees inhabiting two mangroves in the state of São Paulo, Brazil, revealed a large reservoir of fungal diversity (Sebastianes et al., 2013, 2017). Therefore, it is of great interest to investigate the potential of endophytic fungi from such diversified communities for organic acids production.

Microbial cultivation processes for the industrial production of organic acids has been mostly carried out using submerged fermentation (Max et al., 2010). However, the cultivation of filamentous fungi under solid-state fermentation (SSF) has received increasing attention due to the inherent advantages of this cultivation system (Farinas, 2015; Soccol et al., 2006). In fact, several studies have described the use of SSF to produce organic acids, as recently reviewed by (Mondala, 2015). SSF is particularly advantageous for the cultivation of filamentous fungi, since it simulates the natural habitat of these microorganisms. From the environmental perspective, the benefit of SSF is related to the use of agro-industrial wastes as solid substrate, acting as sources of both carbon and energy (Cunha et al., 2017; Farinas et al., 2011; Farinas, 2015; Rodriguez-Zuniga et al., 2013).

Here, the collection of endophytic fungi isolated from the Brazilian mangrove tropical forests was assessed for the potential of organic acids production. For that, 35 fungal strains were initially screened using a plate assay and the selected strains were further evaluated for their production of organic acids by cultivation under SSF. The effect of the type of agro-industrial residue used as feedstock was also investigated by using a combination of sugarcane bagasse and wheat bran.

2. Materials and methods

2.1. Microorganism

The evaluation of the organic acids production was performed by screening 35 strains of endophytic fungi that had been previously isolated from two mangrove areas in the state of São Paulo, Brazil (Sebastianes et al., 2013). To preserve the collection of fungi, the fungal strains were grown on potato dextrose agar (PDA) medium and small samples were put in sterile distilled water and stored at room temperature. The fungal strains assessed belong to six different genera (*Aspergillus*, *Diaporthe*, *Fusarium*, *Hypocrea, Penicillium* and *Xylaria*).

2.2. Plate assay for screening of organic acid production

All 35 fungal strains were initially screened qualitatively for the production of organic acids using a plate assay based on pH change. For that, the endophytic fungi were inoculated in the center of Petri dishes containing Czapek-Dox agar medium (30.0 g/L sucrose, 0.01 g/L $FeSO_4{\cdot}7H_2O$, 0.5 g/L KCl, 1.0 g/L KH_2PO_4, 0.5 g/L $MgSO_4$, 2.0 g/L $NaNO_3$, 15.0 g/L agar), surfactant triton X-100 (1000 μL/L), and bromocresol green (0.1 g/L) as pH indicator. The initial pH of the medium was adjusted to 6. After inoculation, the plates were incubated at 30 °C for 4–7 days. After this period, the diameter of the yellow zone formed was measured and used as an indicative of the strain ability for organic acid production.

2.3. Solid-state fermentation

Production of organic acids was carried out by cultivation of the previously selected endophytic fungi strains under solid-state fermentation (SSF). The cultivations were carried out in 500 mL Erlenmeyer flasks containing 3 g of dry substrate (sugarcane bagasse with particle size of $1.0 \leq dp \leq 1.5$ mm and wheat bran), with the humidity adjusted to 75% (w/v) by addition of a nutrient medium adapted from (Kumar et al., 2003). The composition of the medium (% w/v) was as follows: 20% sucrose, 0.25% $(NH_4)_2SO_4$, 0.1% de KH_2PO_4, 0.025% de $MgSO_4{\cdot}7H_2O$, 0.004% de $CuSO_4$, pH 5.5. The moist substrate was sterilized at 121 °C for 60 min to provide proper cooking of the substrate and to increase its susceptibility to microbial attack. After autoclaving, 4% methanol was added to the medium. A concentration of 10^7 spores/g of dry substrate was inoculated and the cultivations were conducted under static conditions at 30 °C for up to 6 days. The organic acids were extracted by the addition of 1:15 (w/v) distilled water, with 200 rpm agitation at 30 °C for 30 min. The final acid extracts were vacuum-filtered, centrifuged at 10,000 rpm for 15 min at 4 °C, and used for the analytical assays. A control flask without inoculation was used for all the different cultivation conditions. For these control flasks, all the experimental steps were carried out similarly to the inoculated samples and the organic acids value measured in the control was subtracted from the corresponding sample. All cultivation experiments were carried out in triplicate, and the data were calculated as means ± standard deviations.

2.4. Determination of total reducing sugars

The inversion of sucrose was carried out by acid hydrolysis, where 1 mL of 2 N HCl was added to test tubes containing 1 mL of the diluted sample. The tubes were incubated at 100 °C for 5 min, followed by ice cooling for 5 min. After that, 1 mL of 2 N NaOH was added to neutralize the samples and the total reducing sugar concentrations were determined by the DNS method (Miller, 1959). All experiments were carried out in triplicate, and the data were calculated as means ± standard deviations.

2.5. Determination of organic acids

The quantification of total organic acids was carried out by measuring the volumetric titratable acidity using 1% phenolphthalein as indicator. For that, a sample containing 3 mL of the extract acid and 7 mL of distilled water was titrated with a 0.01 M NaOH solution. The quantitation of organic acids was performed by using citric acid as the equivalent factor in grams. The concentrations of gluconic, oxalic and citric acids were determined by HPLC (Waters Co system HPLC W515 pumps, W717 Injector and W486 UV reader), using an Aminex HPX-87H column (Bio-Rad), 5 mM sulfuric acid solution as the mobile phase (at a flow rate of 0.6 mL/min with isocratic pumping and the organic acid kit (Sigma-Aldrich) as standard. The injector and column temperature were 4 °C and 65 °C, respectively. The organic acids were detected at 210 nm. All experiments were carried out in triplicate, and the data were calculated as means ± standard deviations.

3. Results and discussion

3.1. Screening of fungal strains for organic acid production

Table 1 presents a list of 35 endophytic fungal strains screened for their potential of organic acids production. The fungal strains belong to six different genera (*Aspergillus*, *Diaporthe*, *Fusarium*, *Hypocrea, Penicillium* and *Xylaria*), which had been previously isolated from either branches or leaves of different plants collected from the mangroves of São Paulo state, Brazil (Sebastianes et al., 2013). Initially, each of these 35 fungal strains was assessed for organic acid production by using a plate assay with a pH indicator. The formation of a yellow zone around the inoculation was used as an indication of the formation of organic acids. For the strains that showed a positive result, the yellow zone diameter was measured and used for a qualitative classification (Table 1). Among the 35 strains evaluated, five presented a significant yellow zone in the plate screening assay. The four fungal strains of the genus *Aspergillus* showed the most significant yellow zones, followed by the *Penicillium* strain (Fig. 1). Therefore, these five strains (*A. awamori* 09(4), *A. awamori* 82(4), *A. awamori* 108(4), *A. niger* 56(3), *P. minioluteum* 24(4)) were selected to be further evaluated in terms of their organic acid production under solid-state fermentation (SSF) cultivation.

Table 1
List of 35 endophytic strains evaluated in the screening assay for organic acid production.

Taxonomic identification	Strain identification	Plant	Plant structure	Color change[a]
Aspergilus awamori	09 (4)	*Avicennia nítida*	Branch	+ + +
Aspergillus awamori	82 (4)	*Laguncularia racemosa*	Leaves	+ + +
Aspergillus awamori	108 (4)	*Avicennia nítida*	Leaves	+ +
Aspergillus niger	56 (3)	*Rhizophora mangle*	Leaves	+
***Diaporthe* sp.**	5.1 (1)	*Laguncularia racemosa*	Branch	–
***Diaporthe* sp.**	9 (3)	*Laguncularia racemosa*	Branch	–
***Diaporthe* sp.**	37 (4)	*Rhizophora mangle*	Branch	+
***Diaporthe* sp.**	67.1 (1)	*Avicennia nítida*	Branch	–
***Diaporthe* sp.**	94 (4)	*Avicennia nítida*	Branch	–
Diaporthe phaseolorum	51.5 (1)	*Rhizophora mangle*	Leaves	–
Diaporthe stwartti	10 (3)	*Laguncularia racemosa*	Branch	–
Diaporthe stewartti	12.2 (1)	*Laguncularia racemosa*	Branch	–
***Diaporthe* sp.**	57 (4)	*Laguncularia racemosa*	Branch	–
Diaporthe phaseolorum	97.4 (1)	*Avicennia nitida*	Leaves	–
***Fusarium* sp.**	21.2	*Laguncularia racemosa*	Leaves	+
Fusarium chlamydosporum	75 (3)	*Laguncularia racemosa*	Leaves	+
Fusarium sambucinum	3 (3)	*Laguncularia racemosa*	Branch	–
***Fusarium* sp.**	16.1	*Rhizophora mangle*	Branch	–
***Fusarium* sp.**	21.3	*Laguncularia racemosa*	Leaves	–
***Fusarium* sp.**	21.5	*Laguncularia racemosa*	Leaves	–
***Fusarium* sp.**	63.1	*Laguncularia racemosa*	Branch	–
Hypocrea koningii	36.3 (1)	*Avicennia nitida*	Branch	–
Hypocrea koningii	44 (4)	*Rhizophora mangle*	Branch	–
Hypocrea lixii	1.14	*Laguncularia racemosa*	Branch	–
Hypocrea lixii	1.16	*Rhizophora mangle*	Branch	–
Hypocrea lixii	12.6	*Laguncularia racemosa*	Leaves	–
Hypocrea lixii	47 (4)	*Rhizophora mangle*	Branch	–
Hypocrea lixii	48 (4)	*Rhizophora mangle*	Branch	–
Hypocrea lixii	68 (4)	*Laguncularia racemosa*	Branch	–
Penicillium minioluteum	24 (4)	*Rhizophora mangle*	Branch	+
***Penicillium* sp.**	60 (4)	*Laguncularia racemosa*	Branch	–
Xylaria enteroleuca	39.3 (1)	*Rhizophora mangle*	Leaves	–
Xylaria enteroleuca	53.2 (1)	*Avicennia nitida*	Leaves	–
Xylaria polymorpha	47.1 (1)	*Rhizophora mangle*	Leaves	–
Xylaria enteroleuca	106 (4)	*Avicennia nitida*	Branch	–

[a] (-) no halo formation; (+) halo diameter ≥ 5 mm; (+ +) halo diameter ≥ 25 mm; (+ + +) halo diameter ≥ 50 mm.

A screening study on the evaluation for organic acid production of 40 strains of *Ascomycota* and 26 strains of *Basidiomycota*, collected from around the world in different climatic conditions, revealed that the *Aspergillus* was also predominant in the group of fungi producing a high concentrations of a variety of organic acids (Liaud, 2014). The genus *Aspergillus* is widely used for the industrial production of bio-based products, including enzymes and organic acids (Yang et al., 2017). In fact, *A. niger* has been regarded as the workhorse microorganism for the industrial production of organic acids, being superior to other microorganisms for the commercial synthesis of citric acid because of its higher production yield (Show et al., 2015). Nevertheless, different species of *Aspergillus* such as *A. wenti*, *A. foetidus*, *A. aculeatus*, *A. awamori*, *A. fonsecaeus*, *A. phoenicis* and *A. carbonaries* have been found to produce significant amounts of citric and other organic acids (Max et al., 2010; Yang et al., 2017). Our findings on the initial screening of six different endophytic fungal genus revealed that *Aspergillus* was predominant in terms of potential for organic acid production.

3.2. Solid-state fermentation

The five strains previously selected in the plate screening assay were cultivated under SSF using sugarcane bagasse as substrate for 120 h (Fig. 2). Methanol was added to the SSF cultivation medium as it has been previously reported to increase membrane permeability and organic acid production (Prado et al., 2004). The organic acid production, the amount of sugar consumed as well as the pH were measured immediately after obtaining the extracts from each cultivation flask. Despite the possibility of less organic acid production due to slower growth of the different fungal strains, the aim of this step of the study was to selected potential strains that would present a productivity of organic acids considered reasonable for industrial applications.

The strain *A. awamo*ri 09(4) produced the highest amount of organic acid (15.2 mg/g of dry sugarcane bagasse), showing also an equivalent amount of reducing sugar consumed (Fig. 2). This indicates that a significant amount of the carbon source consumed was converted into organic acids. The medium with the lowest pH value (3.05) was also obtained by the *A. awamori* 09 (4) strain. The strain *A. awamori* 108 (4) produced a similar amount of organic acid to the *A. awamori* 09 (4), but with a higher sugar consumption. The lowest organic acid production was found for the *Penicillium* strain. Based on these results, the endophytic strain *A. awamori* 09 (4) was selected to be further evaluated in terms of their organic acid production under SSF cultivation carried out using different conditions.

The cultivation of *A. awamori* has been previously described for different applications, such as organic acids and enzymes (Castro et al., 2015; Chhokar et al., 2010; Gaind, 2017; Gottschalk et al., 2013). For instance, the cultivation of *A. awamori* (F18) under SSF has been used for the solubilization of rock phosphate by production of organic acids using orange peel as substrate (Gaind, 2017). The efficient production of xylanase, β-xylosidase, ferulic acid esterase and β-glucosidase enzymes by *A. awamori* 2B.361 U2/1 has been reported using submerged fermentation (Gottschalk et al., 2013). Moreover, the use of a cylindrical fixed-bed SSF bioreactor with forced aeration was investigated for the production of a pool of industrially relevant enzymes such as amylases, cellulases, xylanases and proteases by *A. awamori* IOC-3914 using babassu cake as raw material (Castro et al., 2015). These studies supports the additional investigation of the potential of the endophytic strain *A. awamori* 09 (4) for organic acid production under different cultivations conditions.

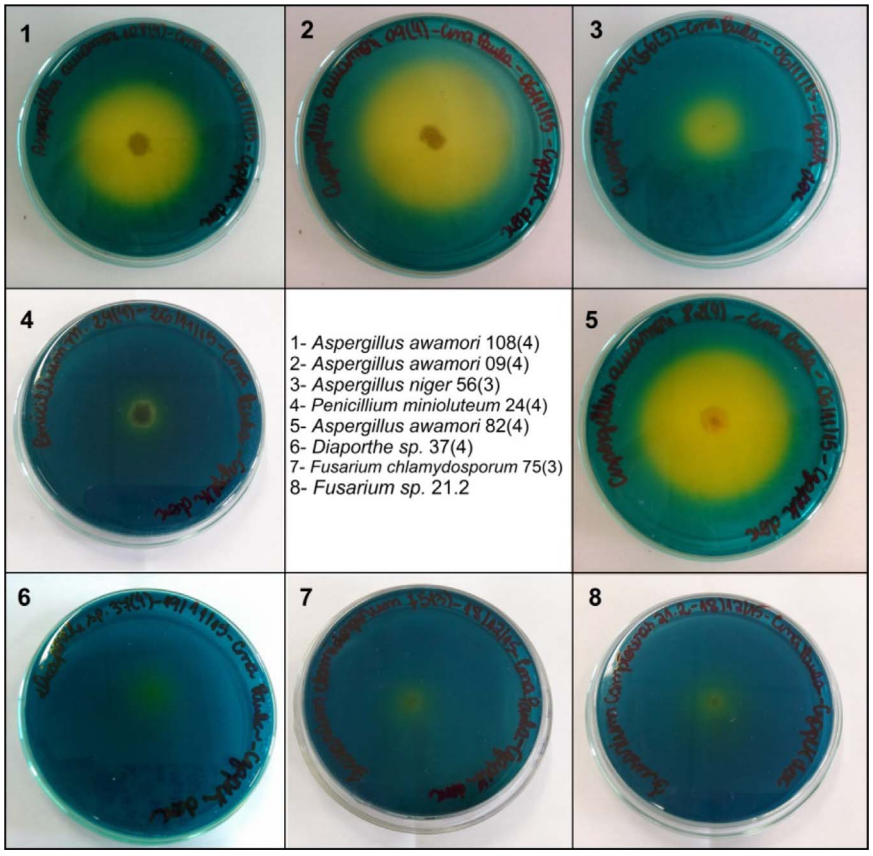

Fig. 1. Endophytic fungi strains of the genus *Aspergillus, Diaporthe, Fusarium* e *Penicillium* that showed a positive result for the formation of a yellow zone in the screening plate assay for organic acid production.

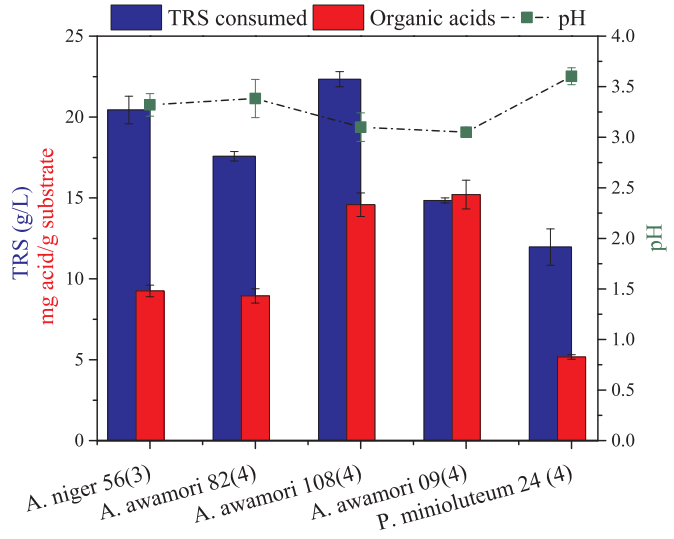

Fig. 2. Total organic acid, total sugar consumed and pH values measured after SSF cultivation of selected endophytic fungi strains for 120 h using sugarcane bagasse as substrate. Values represent the average of triplicates with the standard deviations.

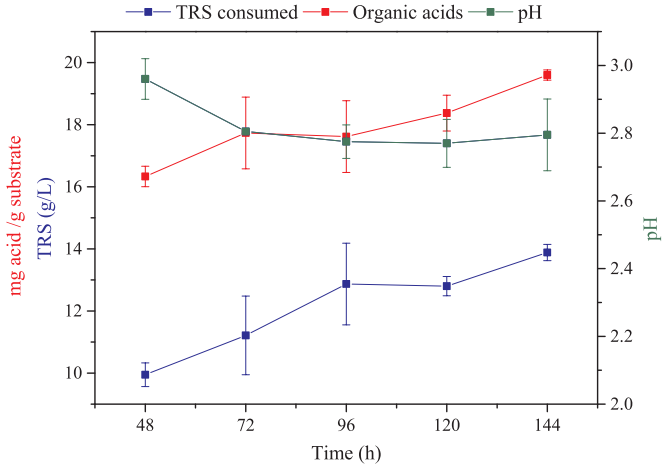

Fig. 3. Time profile of total organic acid, total sugar consumed and pH values during SSF cultivation of the endophytic strain *A. awamori* 09(4) for 144 h using sugarcane bagasse as the sole solid substrate.

3.3. Time profile of organic acid production

The time profile of the organic acid production by the strain *A.awamori* 09 (4) cultivated under SSF with sugarcane bagasse as substrate was evaluated for a total period of 144 h (Fig. 3). The highest production of organic acid was achieved after 144 h of cultivation (19.6 mg/g), leading to a medium with pH of 2.80. However, after 72 h of incubation the pH already had dropped to 2.80 and remained at this value along the whole cultivation period while the production of organic acids increased along the consumption of carbon source up to 144 h. This indicates that the acidification of the medium in the beginning of the cultivation may be also associated to the release of protons during the consumption of inorganic nitrogen sources (Max et al., 2010), which lower the pH and further contributes to the production of organic acids.

The time profile of organic acid production by the strain *A.awamori* 09 (4) is within the range reported in previous studies. For instance, the production of a variety of organic acids by strains of *Ascomycota* was observed after 6 days of fungi incubation, while the acidification of the medium started within 24 h and the final pH was already observed after 3 days of growth (Liaud, 2014). In another study, the maximum production of citric acid by *A. niger* cultivated under SSF using cassava

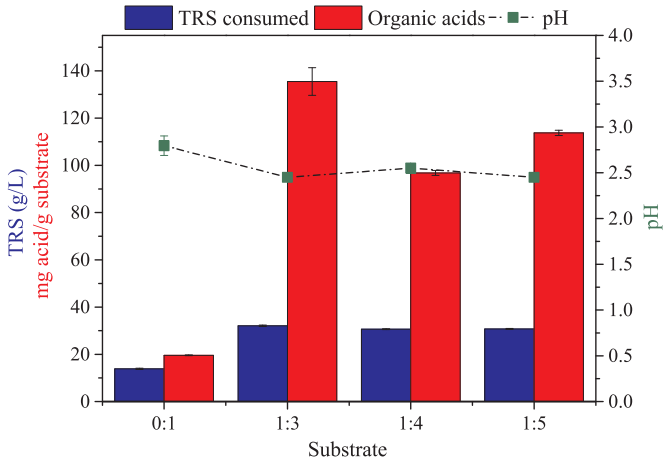

Fig. 4. Total organic acid, total sugar consumed and pH values measured after SSF cultivation of the endophytic strain *A. awamori* 09(4) for 144 h using the proportions of 0:1, 1:3, 1:4 and 1:5 of wheat bran to sugarcane bagasse as solid substrates.

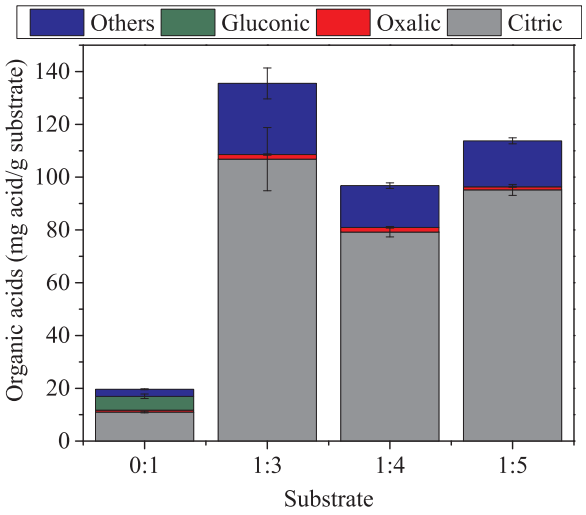

Fig. 5. Organic acids produced by cultivation of the endophytic strain *A. awamori* 09(4) for 144 h using the proportions of 0:1, 1:3, 1:4 and 1:5 of wheat bran to sugarcane bagasse as solid substrates.

bagasse was achieved after 120 h (Vandenberghe et al., 2000) while the maximum citric acid production by *A. niger* was observed after 9 days when using sugarcane bagasse for SSF cultivation (Khosravi-Darani and Zoghi, 2008).

3.4. Selection of the substrate of solid-state fermentation

The effect of the agro-industrial feedstock used as solid substrate for the production of organic acids by *A. awamori* 09 (4) was evaluated by carrying out SSF cultivations with different combinations of wheat bran and sugarcane bagasse, in the proportions of 1:3, 1:4 and 1:5 (wheat bran: sugarcane bagasse) for 144 h (Fig. 4). The SSF using the substrate proportion 1:3 of wheat bran to sugarcane bagasse resulted in 135.5 mg/g of organic acids, which represents around 7-fold increase when compared to the use of sugarcane bagasse alone. The pH value for this condition was 2.45, which is also the lowest pH obtained from the different cultivation conditions previously tested. This result indicates that the nutrients present in the substrate wheat bran further contributed to the production of organic acids.

Wheat bran is a good source of nitrogen and has been used in mixtures with sugarcane bagasse for the production of enzymes such as cellulases (Delabona et al., 2012; Rodriguez-Zuniga et al., 2011). A previous comparison study of sugarcane bagasse and wheat bran for citric acid production by *A. niger* under SSF revealed that bagasse was superior than wheat bran due to reduced agglomeration and better heat and mass transfer (Kumar et al., 2003). Here, we showed that the negative effect of the substrate agglomeration can be avoided by using a combination of these two agro-industrial feedstocks as solid substrate for SSF cultivations.

3.5. Characterization of the organic acids produced under SSF

The analysis of the acid organic being produced by *A. awamori* 09 (4) in each of the SSF cultivations using different combinations of wheat bran and sugarcane bagasse revealed that the organic acids produced were mostly citric acid, followed by oxalic acid and others not identified (Fig. 5). For the SSF cultivation using a proportion 1:3 of substrate, the concentration of citric acid was 106.8 mg/g.

Interestingly, there was a variation in the composition of the organic acids produced with the addition of wheat bran to the solid medium. For instance, gluconic acid was only detected when sugarcane bagasse was used alone. One possible explanation could be related to the differential aeration conditions obtained due to the higher medium porosity promoted by the sugarcane bagasse. A higher oxygen supply is reported to promote gluconic acid production because oxygen is a substrate of the glucose oxidase-catalase enzymatic complex which is required for gluconic acid formation (Ramachandran et al., 2006). The pH is also an important factor for gluconic acid formation, since a series of enzymes required for gluconic acid production are only activated at pH above 4 (Ramachandran et al., 2006). Therefore, the slightly higher pH observed using sugarcane bagasse alone may have contributed to the production of gluconic acid.

The production of organic acids obtained in the present study is very promising when compared to the literature. (Vandenberghe et al., 2000) reported citric acid values of 88 mg/g of dry cassava bagasse by *A. niger* cultivated under SSF for 120 h. Optimization of the SSF cultivation conditions of *A. niger* using sugarcane bagasse pretreated by urea resulted in citric acid production of 137.6 mg/g of dry sugarcane bagasse (Khosravi-Darani and Zoghi, 2008). Event though, these studies have been conducted using different experimental conditions, this comparison gives an indication of the magnitude of the values being reported in the literature and highlights the potential of using the endophytic strain *A. awamori* 09 (4) for the production of organic acid under SSF cultivations.

These results contribute to the utilization of lignocellulosic biomass as feedstock for organic acid production which is key to the development of future multi-products biorefineries and valorization of agro-industrial residues. Moreover, the development of efficient and economical production process can contribute to open new opportunities and markets for bio-based organic acids as stating materials for a sustainable chemical industry.

4. Conclusions

The potential of using endophytic fungi as a source of microorganisms for organic acids production has been evaluated here by carrying out an screening of 35 fungal strains isolated from the leaves and branches of trees inhabiting two mangroves in the state of Sao Paulo, Brazil. The cultivation of the selected *A. awamori* 09 (4) strain under SSF using a mix of wheat bran to sugarcane bagasse (1:3) resulted in 135.5 mg/g of organic acids, which represents around 7-fold increase when compared to the use of sugarcane bagasse alone. These results indicate the potential of exploring endophytic fungi strains for organic acid production using agro-industrial wastes as feedstock, thus being compatible with the current bioeconomy demands by providing a sustainable alternative to organic acid manufacture.

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References

Becker, J., Lange, A., Fabarius, J., Wittmann, C., 2015. Top value platform chemicals: bio-based production of organic acids. Curr. Opin. Biotechnol. 36, 168–175.

Castro, A.M., Castilho, L.R., Freire, D.M.G., 2015. Performance of a fixed-bed solid-state fermentation bioreactor with forced aeration for the production of hydrolases by Aspergillus awamori. Biochem. Eng. J. 93, 303–308.

Cheali, P., Posada, J.A., Gernaey, K.V., Sin, G., 2015. Upgrading of lignocellulosic biorefinery to value-added chemicals: sustainability and economics of bioethanol-derivatives. Biomass- Bioenergy 75, 282–300.

Chen, Y., Nielsen, J., 2016. Biobased organic acids production by metabolically engineered microorganisms. Curr. Opin. Biotechnol. 37, 165–172.

Chhokar, V., Sangwan, M., Beniwal, V., Nehra, K., Nehra, K.S., 2010. Effect of additives on the activity of Tannase from Aspergillus awamori MTCC9299. Appl. Biochem. Biotechnol. 160 (8), 2256–2264.

Ciriminna, R., Meneguzzo, F., Delisi, R., Pagliaro, M., 2017. Citric acid: emerging applications of key biotechnology industrial product. Chem. Cent. J. 11, 9.

Correa, R.C.G., Rhoden, S.A., Mota, T.R., Azevedo, J.L., Pamphile, J.A., de Souza, C.G.M., Polizeli, M., Bracht, A., Peralta, R.M., 2014. Endophytic fungi: expanding the arsenal of industrial enzyme producers. J. Ind. Microbiol. Biotechnol. 41 (10), 1467–1478.

Cunha, F.M., Badino, A.C., Farinas, C.S., 2017. Effect of a novel method for in-house cellulase production on 2G ethanol yields. Biocatal. Agric. Biotechnol. 9, 224–229.

Delabona, P., Pirota, R., Codima, C., Tremacoldi, C., Rodrigues, A., Farinas, C., 2012. Using Amazon forest fungi and agricultural residues as a strategy to produce cellulolytic enzymes. Biomass- Bioenergy 37, 243–250.

Farinas, C., Vitcosque, G., Fonseca, R., Neto, V., Couri, S., 2011. Modeling the effects of solid state fermentation operating conditions on endoglucanase production using an instrumented bioreactor. Ind. Crops Prod. 34 (1), 1186–1192.

Farinas, C.S., 2015. Developments in solid-state fermentation for the production of biomass-degrading enzymes for the bioenergy sector. Renew. Sustain. Energy Rev. 52, 179–188.

Gaind, S., 2017. Exploitation of orange peel for fungal solubilization of rock phosphate by solid state fermentation. Waste Biomass- Valoriz. 8 (4), 1351–1360.

Goldberg, I., Rokem, J.S., Pines, O., 2006. Organic acids: old metabolites, new themes. J. Chem. Technol. Biotechnol. 81 (10), 1601–1611.

Gottschalk, L.M.F., Paredes, R.D., Teixeira, R.S.S., da Silva, A.S., Bon, E.P.D., 2013. Efficient production of lignocellulolytic enzymes xylanase, beta-xylosidase, ferulic acid esterase and beta-glucosidase by the mutant strain Aspergillus awamori 2B.361 U2/1. Braz. J. Microbiol. 44 (2), 569–576.

Kamm, B., Kamm, M., 2004. Principles of biorefineries. Appl. Microbiol. Biotechnol. 64 (2), 137–145.

Khosravi-Darani, K., Zoghi, A., 2008. Comparison of pretreatment strategies of sugarcane baggase: experimental design for citric acid production. Bioresour. Technol. 99 (15), 6986–6993.

Klaic, R., Plotegher, F., Ribeiro, C., Zangirolami, T.C., Farinas, C.S., 2017. A novel combined mechanical-biological approach to improve rock phosphate solubilization. Int. J. Mineral. Process. 161, 50–58.

Kumar, D., Jain, V.K., Shanker, G., Srivastava, A., 2003. Citric acid production by solid state fermentation using sugarcane bagasse. Process Biochem. 38 (12), 1731–1738.

Liaud, N., 2014. Exploring fungal biodiversity: organic acid production by 66 strains of filamentous fungi.

Liu, J., Wang, X.C., Pu, H.M., Liu, S., Kan, J., Jin, C.H., 2017. Recent advances in endophytic exopolysaccharides: production, structural characterization, physiological role and biological activity. Carbohydr. Polym. 157, 1113–1124.

Max, B., Salgado, J.M., Rodriguez, N., Cortes, S., Converti, A., Dominguez, J.M., 2010. Biotechnological production of citric acid. Braz. J. Microbiol. 41 (4), 862–875.

Miller, G., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31 (3), 426–428.

Mondala, A.H., 2015. Direct fungal fermentation of lignocellulosic biomass into itaconic, fumaric, and malic acids: current and future prospects. J. Ind. Microbiol. Biotechnol. 42 (4), 487–506.

Prado, F.C., Vandenberghe, L.R.S., Lisboa, C., Paca, J., Pandey, A., Soccol, C.R., 2004. Relation between citric acid production and respiration rate of Aspergillus niger in solid-state fermentation. Eng. Life Sci. 4 (2), 179–186.

Ramachandran, S., Fontanille, P., Pandey, A., Larroche, C., 2006. Gluconic acid: properties, applications and microbial production. Food Technol. Biotechnol. 44 (2), 185–195.

Robl, D., Delabona, P.D., Mergel, C.M., Rojas, J.D., Costa, P.D., Pimentel, I.C., Vicente, V.A., Pradella, J.G.D., Padilla, G., 2013. The capability of endophytic fungi for production of hemicellulases and related enzymes. BMC Biotechnol. 13, 12.

Rodriguez-Zuniga, U.F., Couri, S., Neto, V.B., Crestana, S., Farinas, C.S., 2013. Integrated strategies to enhance cellulolytic enzyme production using an instrumented bioreactor for solid-state fermentation of sugarcane bagasse. Bioenergy Res. 6 (1), 142–152.

Rodriguez-Zuniga, U.F., Farinas, C.S., Neto, V.B., Couri, S., Crestana, S., 2011. Aspergillus niger production of cellulases by solid-state fermentation. Pesqui. Agropecu. Bras. 46 (8), 912–919.

Sauer, M., Porro, D., Mattanovich, D., Branduardi, P., 2008. Microbial production of organic acids: expanding the markets. Trends Biotechnol. 26 (2), 100–108.

Sebastianes, F.L.D., RomaO-Dumaresq, A.S., Lacava, P.T., Harakava, R., Azevedo, J.L., de Melo, I.S., Pizzirani-Kleiner, A.A., 2013. Species diversity of culturable endophytic fungi from Brazilian mangrove forests. Curr. Genet. 59 (3), 153–166.

Sebastianes, F.L.S., Azevedo, J.L., Lacava, P.T., 2017. Diversity and Biotechnological Potential of Endophytic Microorganisms Associated with Tropical Mangrove Forests. Springer Nature, Switzerland.

Show, P.L., Oladele, K.O., Siew, Q.Y., Zakry, F.A.A., Lan, J.C.W., Ling, T.C., 2015. Overview of citric acid production from Aspergillus niger. Front. Life Sci. 8 (3), 271–283.

Soccol, C.R., Vandenberghe, L.P.S., Rodrigues, C., Pandey, A., 2006. New perspectives for citric acid production and application. Food Technol. Biotechnol. 44 (2), 141–149.

Vandenberghe, L.P.S., Soccol, C.R., Pandey, A., Lebeault, J.M., 2000. Solid-state fermentation for the synthesis of citric acid by Aspergillus niger. Bioresour. Technol. 74 (2), 175–178.

Vassilev, N., Vassileva, M., Lopez, A., Martos, V., Reyes, A., Maksimovic, I., Eichler-Lobermann, B., Malusa, E., 2015. Unexploited potential of some biotechnological techniques for biofertilizer production and formulation. Appl. Microbiol. Biotechnol. 99 (12), 4983–4996.

Werpy, T., Petersen, G., 2004. Top added chemicals from biomass.

Yang, L., Lubeck, M., Lubeck, P.S., 2017. Aspergillus as a versatile cell factory for organic acid production. Fungal Biol. Rev. 31 (1), 33–49.