Utilization of Agro-industrial wastes as carbon source in solid-state fermentation processes for the production of value-added byproducts

Mahmoud W. Sadik1, Moustafa M. Zohair2,3,*, Ahmed A. El-Beih2, Eman R. Hamed2 and Mohamed Z. Sedik1

1Microbiology Department, Faculty of Agriculture, Cairo University, Egypt; 2Chemistry of Natural and Microbial Products Department, Pharmaceutical Industries Research Division, National Research Centre, Giza, 12311, Egypt; 3Nanomaterial Investigation Lab., Central Laboratories Network, National Research Centre, Dokki, Giza, 12622, Egypt

Abstract

Utilization of agro industrial wastes as solid support was evaluated in solid state fermentation (SSF) methods to produce value-added byproducts such as antioxidants and antimicrobial agents. Among these agricultural wastes added as a carbon source in SSF process are corn cobs, olive mill, wheat bran, rice straw, rice bran and sorghum. The biological activities of the extracts of ethyl acetate (EtOAc) of Aspergillus pseudocaelatus MG772677 and Trichoderma gamsii KX685665 strains were studied. The extract of ethyl acetate of A. pseudocaelatus cultured on medium containing sorghum as natural carbon source showed the highest inhibitory activity against tested microorganisms. The antioxidant activity results varied based on the used waste as a carbon source as well as the incubation period. The EtOAc extracts of A. pseudocaelatus and T. gamsii cultivated on different agricultural wastes media showed potent antioxidant activities of scavenging DPPH when compared to those cultivated on PDA media. The highest percentage of antioxidant activity (53.84%) for the EtOAc extract of T. gamsii was observed after 7 days of incubation period.

Keywords: Agro-industrial wastes Aspergillus pseudocaelatus MG772677, Trichoderma gamsii KX685665, Antimicrobial, Antioxidant and solid-state fermentation

1. Introduction

The manufacturing processing of agricultural raw materials generates a huge amount of wastes which are either burned or used for animal feeding. These wastes are usually rich in carbohydrates, proteins and minerals, and should be exploited as raw materials for other industries. Accumulation of agricultural wastes in huge quantities annually results not only in the environmental deterioration, but also in economic loss as these wastes can be utilized for production of high valuable products, for instance food, energy and chemicals producing (Singh, 2009). The carbon content, nutrient and moisture in these agricultural wastes offer favorable environment for the microbial growth, which opens up considerable potential for recycle in solid state fermentation (Mussatto et al., 2012).

The microbial species can exploit these collective wastes from agricultural industries, specially, fungi strains, which have the ability to produce hydrolytic enzymes such as chitinase and cellulose to ferment these wastes. The utilization of industrial agricultural waste in solid state fermentation is of particular importance because it is considered available and economic, as well as being an eco-friendly alternate for their discarding. The fermentation could be affected by some factors which could maximize the produced yields; these factors include the modifying of substrates size, moisture content, pH, temperature… etc. (Nigam and Pandey, 2009). In general, produced yield can be maximized by selecting an appropriate substrate or a combination of substrates with suitable conditions (Mussatto et al., 2012). The large scale production of secondary metabolites requires studying the effective factors which include abiotic and biotic factors (Shentu et al., 2013).

Fungi have been proven to be potent source for biologically active compounds with therapeutic potential (Hoeksma et al., 2019) and has produced a number of compounds of medical importance, including penicillin, Lovastatin and caspofungin (Goler 2007; Keating and Figgitt, 2003; Vandermolen et al., 2013). Aspergillus and Trichoderma species are a valuable source of commercial enzymes used in the recycling of cellulose waste (Reino and Guerrero, 2008).

New and better techniques for the recovery of agricultural waste have been developed due to industrial innovation and high technology. These techniques contribute to maintaining resources efficiency, sustainable production, consumption and reduction of negative environmental impacts (Duque-Acevedo et al., 2020). The aim of this research was to study the application of various industrial agricultural wastes as a carbon source or nutrient...
in SSF processes in order to produce antimicrobials and antioxidant agents.

2. Materials and Methods

2.1. Fungal strains

Two fungal strains, Aspergillus pseudocaelatus and Trichoderma gamsii, were recovered from rhizosphere area of medicinal plants (Aloe vera, Basil, Ocimum basilicum), and Peppermint (Menta piperita), planted in Sekem farm, Helioptolis University, Cairo, Egypt under organic farming regulations. The strains were maintained and stored on the surface of PDA slants at 4°C. They were identified on the basis of their morphological and microscopic characteristics as well as 18s rDNA (Zohair et al., 2018).

2.2. Solid State Fermentation

Corn cobs, olive mill pomace, rice bran, rice straw, sorghum and wheat bran wastes were obtained from the local fields in Giza governorate, Egypt, dried in oven at 55°C for 48 h and ground to 40-mesh (400 μm) were utilized as fermentation carbon sources. Three grams of the solid substrates were added to Erlenmeyer flasks (250 ml). The substrate moisture content was adjusted to 75% with the solution of minerals (KH₂PO₄; 2.0 g/L, CaCl₂; 0.3 g/L, MgSO₄; 0.3 g/L, FeSO₄; 0.11 g/L, ZnSO₄; 0.3 g/L). The pH of prepared solutions was adjusted to 6 then added to the solid substrate. The flasks contents were mixed well and autoclaved for 20 min at 121 °C at 1 atm and then incubated without inoculation, were used as a negative control, while flasks containing 100 ml of PDA medium inoculated with strains A. pseudocaelatus or T. gamsii strains were used as positive control.

2.3. Liquid culture and metabolite production

2.3.1. Extraction of crude extract

After different incubation period (7,11,15 and 19 days) cultivation medium and mycelial mats were soaked overnight in ethyl acetate (1:1, v/v), then were homogenized in ethyl acetate using an Ultra Sonic wave (J.P Selecta s.a). The extraction process was carried out at 25-28 °C up to 19 days. Flasks, incubated without inoculation, were used as a negative control, while flasks containing 100 ml of PDA medium inoculated with strains A. pseudocaelatus or T. gamsii strains were used as positive control.

2.3.2. Biological activity of the isolated fungal secondary metabolites

2.3.2.1. Antimicrobial activity

The antibacterial and antifungal activities were screened against different pathogenic strains through the agar disk diffusion methods (El-Sawy et al., 2015). Concentrations (1 mg/5 μl) of A. pseudocaelatus and T. gamsii ethyl acetate extracts were assessed against Gram positive bacteria such as Bacillus subtilis ATCC6633 and Staphylococcus aureus ATCC29213. It was also tested against Gram negative bacteria such as Escherichia coli ATCC25922, and Salmonella enterica ATCC25566. Also, it was tested against tested yeasts and fungal strains such as Fusarium solani NRC15 and Aspergillus niger NRC23, in addition to yeasts (Candida albicans, Candida. tropicalis). The concentration of tested fungi was 1× 10⁸ spores/ml. The concentration of the tested bacterial pathogens was 1× 10⁸ cfu/ml. The test was done within sterilized Petri dishes including 25 ml of sterilized PDA medium in case of fungi and in nutrient agar medium in case of bacteria. Thiophenicol and Treflucan antibiotics were used as positive controls for bacteria and fungi with concentration of 100 μg/disk. The Dimethyl sulfoxide antibiotic (DMSO) was used as a negative control. The prepared disks were loaded on Petri dishes containing the inoculated media then incubated for 24 hours at 30 °C for bacterial strains and 72 hours at 28 °C for fungal strains, respectively. The inhibition zones were measured.

2.3.2.2. In vitro determination of antioxidant activity

This assay was performed according to Hamed (2009) with some modifications. One mg of ethyl acetate fungal extract was dissolved in 1 ml of Dimethyl sulfoxide (DMSO) to prepare 1000 μg/ml stock solution. 2.2-Diphenyl-1-picrylhydrazyl (DPPH) (0.004 mg) was dissolved in 100 ml of methyl alcohol HPLC grade to make concentration 0.004% solution. Different concentrations (5-25 μg) of reference standard compounds such as Quercetin and Vitamin C were prepared. In a 96-well plate, 20 μl of stock solutions (samples—or standard) was added into each well then 180 μl of methanol solution of DPPH (0.004%) was added to complete the final concentration of evaluated samples 100 μg/ml. After 30 min. of incubation, the plate was scanned at λ = 540 nm using microplate reader. In case of blank, 20 μl of dissolving agent (DMSO) were added instead of 20 μl of samples. The assay was repeated twice to confirm the results. The activity of radical scavenging could be determined based on the given equation:

\[
\text{Scavenging Activity} (\%) = \left[ \frac{(A_{\text{blank}} - A_{\text{sample}})}{A_{\text{blank}}} \right] \times 100
\]

Where, A blank (Absorbance of mixture without sample “DPPH only”), A sample (Absorbance of test samples).

Samples that indicated 50% or higher antioxidant activity at 100 μg / ml concentration compared to control were considered active. The effect of scavenging on DPPH free radicals was estimated according to (Shimada et al., 1992).

2.4. Data Analysis

The analysis of variance (ANOVA) was evaluated using MSTATC software. The differences of significance between means were compared based on the least significance differences (LSD) test at 5% significant level.

3. Results and Discussion

3.1. Activity of the fungal extract as Antimicrobial agents

A. pseudocaelatus EtOAc extract showed a strong antimicrobial activity as shown in (Table 1). It presented a
strong inhibitory activity against pathogenic tested yeasts and fungal strains (Candida albicans, C. tropicalis, Fusarium solani, Rhizoctonia solani, Sclerotium rolfsii and Verticillium dahliae) with zones of inhibition 13.5, 15.5, 14, 15, 7 and 8 mm diameter, respectively. For bacterial tested strains, A. pseudocaelatus extract has a significant effect against B. subtilis ATCC 6633, E. coli ATCC 25922, S. aureus ATCC 6633 and S. enterica ATCC 25566 with inhibition zones of 20, 19.5, 18.5 and 14.5 mm, respectively.

### Table 1. Antimicrobial effect of A. pseudocaelatus extract.

<table>
<thead>
<tr>
<th>Pathogenic</th>
<th>A. pseudocaelatus extract</th>
<th>Positive control</th>
</tr>
</thead>
<tbody>
<tr>
<td>G+ve bacterial strains</td>
<td>B. subtilis 20±0.0</td>
<td>22.5±0.5</td>
</tr>
<tr>
<td></td>
<td>S. aureus 13.5±0.5</td>
<td>18.5±0.5</td>
</tr>
<tr>
<td>G-ve bacterial strains</td>
<td>E. coli 18.5±0.5</td>
<td>11.5±0.5</td>
</tr>
<tr>
<td></td>
<td>S. enterica 14.5±1.5</td>
<td>15±0.0</td>
</tr>
<tr>
<td>Yeast</td>
<td>C. albicans 13.5±0.5</td>
<td>7±0.0</td>
</tr>
<tr>
<td></td>
<td>C. tropicalis 15.5±0.5</td>
<td>7±0.0</td>
</tr>
<tr>
<td></td>
<td>F. solani 14±0.6</td>
<td>7±0.0</td>
</tr>
<tr>
<td></td>
<td>R. solani 15±0.0</td>
<td>7±0.0</td>
</tr>
<tr>
<td></td>
<td>S. rolfsii 7±0.0</td>
<td>7±0.0</td>
</tr>
<tr>
<td></td>
<td>V. dahliae 8±0.0</td>
<td>8.5±0.5</td>
</tr>
</tbody>
</table>

Notes: The inhibition zone measured as diameter expressed in (mm). (Values are mean ± S.D.)

Extract of T. gamsii fungus resulted in a moderate activity against all yeasts and fungal pathogenic tested pathogens strains (C. albicans, C. tropicalis, F. solani, R. solani, S. rolfsii and V. dahliae) with inhibition zones of 8, 7, 8, 7 and 7 mm diameter, respectively. In parallel, the extract of T. gamsii had a moderate effect against B. subtilis ATCC6633, S. aureus ATCC 25923, E. coli ATCC 25922 and S. enterica ATCC 25566 with diameter of inhibition zone 12, 13.5, 9 & 7 mm, respectively as shown in (Table 2).

### Table 2. Antimicrobial effect of T. gamsii extract.

<table>
<thead>
<tr>
<th>Pathogenic</th>
<th>T. gamsii extract</th>
<th>Positive control</th>
</tr>
</thead>
<tbody>
<tr>
<td>G+ve bacterial strains</td>
<td>B. subtilis 12±0.0</td>
<td>22.5±0.5</td>
</tr>
<tr>
<td></td>
<td>S. aureus 13.5±0.5</td>
<td>18.5±0.5</td>
</tr>
<tr>
<td>G-ve bacterial strains</td>
<td>E. coli 9±0.0</td>
<td>11.5±0.5</td>
</tr>
<tr>
<td></td>
<td>S. enterica 7±0.0</td>
<td>15±0.0</td>
</tr>
<tr>
<td>Yeast</td>
<td>C. albicans 8±0.0</td>
<td>7±0.0</td>
</tr>
<tr>
<td></td>
<td>C. tropicalis 7±1</td>
<td>7±0.0</td>
</tr>
<tr>
<td></td>
<td>F. solani 8±0.0</td>
<td>7±0.0</td>
</tr>
<tr>
<td></td>
<td>R. solani 7±0.0</td>
<td>7±0.0</td>
</tr>
<tr>
<td></td>
<td>S. rolfsii 7±0.0</td>
<td>7±0.0</td>
</tr>
<tr>
<td></td>
<td>V. dahliae 7±0.0</td>
<td>8.5±0.5</td>
</tr>
</tbody>
</table>

Notes: The inhibition zone measured as diameter expressed in (mm). (Values are mean ± S.D.)

Treflucan and Thiophenicol were applied with a concentration of 100 μg/disc as positive controls, DMSO was applied as negative control.

Saleh et al. (2011) found that some of Trichoderma spp crude extract had an antibacterial effect against some bacterial pathogens with zones of inhibition fluctuating from (10 to 28 mm). Also, Vinale et al. (2006) indicated that Trichoderma harzianum fungal strains (T22 & T39) secondary metabolites showed an inhibition activity against plant pathogens Pythium ultimum and R. solani. From the above results, the A. pseudocaelatus ethyl acetate extract was more effective than the T. gamsii extract. These results were in agreement with Zohair et al., (2018) who indicated that the biological activity of EtOAc extract of A. pseudocaelatus was more active than that of T. gamsii. It showed an effective inhibitory result against fungal test strains (C. albicans, C. tropicalis, F. solani, R. solani, S. rolfsii & V. dahliae).

Both fungi showed the capability of utilization of different agricultural industrial wastes used as carbon sources. As shown in Fig.1.

The most effective one as a medium was the agriculture waste, sorghum. The highest inhibition zone was obtained from A. pseudocaelatus cultivated on media containing sorghum as carbon sources after 15 days of incubation period. It exhibited antibacterial activity against both Gram-positive bacterial strains B. subtilis ATCC6633 and S. aureus ATCC25923 with inhibition zones of 26.0 and 22.5 mm diameter, respectively. Also, it exhibited antibacterial activity against Gram-negative bacterial strains E. coli ATCC25922 and S. enterica ATCC25566 with inhibition zones of 24.0 and 22.5 mm diameter, respectively as shown in (Fig. 2).

Many studies have suggested that sorghum and sorghum processing waste have a huge potential for the production of value added products due to its content of fermentable sugars (starch, sucrose, glucose and fructose),
and lignocelluloses feed stock. Makanjuola et al. (2019) reported that the high residual starch (up to 53% (w/w) in sorghum waste makes it rich medium for Aspergillus awamori growth for production of value added product. The anti-yeast activity was 22.5 and 13.0 mm with Candida albicans ATCC 10321 and Candida tropicalis ATCC750, respectively, while the antifungal activity was 21.0 and 20.0 mm against F. oxysporium and A. niger as presented in (Fig. 2 & 3).

Figure 2. Antimicrobial effect of ethyl acetate fungal extract of A. pseudocaelatus using solid state fermentation after 15 days of incubation period.

Pandey et al. (2001) reported production of Cyclosporin A from Tolypocladium inflatum using wheat bran and oxytetracycline antibiotic by S. rinsosus using corn cob in solid state fermentation. Ethyl acetate extract of A. pseudocaelatus on media containing sorghum as natural alternative carbon sources had the higher inhibitory activity against all tested microorganism than using PDA media. It showed antibacterial activity against both Gram-positive bacteria (B. subtilis ATCC6633 and S. aureus ATCC25923) and Gram-negative bacteria (E.coli ATCC25922 and S. enterica ATCC25566) with inhibition zones of 26.0, 22.5, 24.0 and 22.5 mm, respectively while the inhibition zone of EtOAc extract of A. pseudocaelatus growing on PDA media was 20, 19.5, 18.5 and 14.5 mm, against B. subtilis ATCC 6633, S. aureus ATCC 25923, E.coli ATCC 25922 and S. enteric 25566, respectively.

Also, the anti-pathogenic yeast activity was 22.5 and 13.0 mm with C. albicans ATCC 10321 and C. tropicalis ATCC 750, respectively using the sorghum as natural alternative carbon sources compared with 13.5 and 15.5mm diameter of ethyl acetate extract of A. pseudocaelatus fermented on PDA for 15 days of incubation.

3.2. In vitro antioxidant activity of ethyl acetate extract

The antioxidant capacity of the fungal isolates is shown in Table 3. The DPPH scavenging activity varied between the two fungal isolates ethyl acetate extracts. The T. gamsii extract showed a higher antioxidant activity than A. pseudocaelatus extract.

Table 3. Antioxidant potential of ethyl acetate (EtOAc) extract of A. pseudocaelatus and T. gamsii cultivated on PDA media

<table>
<thead>
<tr>
<th>Waste/ Incubation Period</th>
<th>7 days</th>
<th>11 days</th>
<th>14 days</th>
<th>19 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl acetate extract of A. pseudocaelatus</td>
<td>4.286</td>
<td>5.029</td>
<td>5.081</td>
<td>5.000</td>
</tr>
<tr>
<td>Ethyl acetate extract of T. gamsii</td>
<td>4.286</td>
<td>5.029</td>
<td>5.081</td>
<td>5.000</td>
</tr>
</tbody>
</table>

The antioxidant effects of the A. pseudocaelatus and T. gamsii extracts ranged from 3.681 to 31.33% for olive waste and corncobs after 11 and 19 days incubation times, respectively. Meanwhile it ranged 1.20 and 53.84 % of DPPH scavenging, respectively with A. pseudocaelatus and T. gamsii cultivated on olive mill and corncobs after 14 days and 7 days, respectively. The fungal extracts of A. pseudocaelatus and T. gamsii cultivated on PDA showed antioxidant activity of 1.92 and 14.78 %, respectively.

Awad et al. (2018) reported that the volatile compound extracted from Trichoderma viride mycelia showed antioxidant effects by 29.62%, 63.12% and 70.37% at concentrations of 10, 50 and 100 µg, respectively. Moreover, other constituents such as carbohydrates, proteins could have remarkable antioxidant effects ranging from 3.70 - 33.00% for proteins and 3.00 - 23.00% for carbohydrates at concentrations of 10 - 100 µg, respectively.

The ability of the used fungal strains to produce hydrolytic enzymes could offer a promising chance for

Figure 3. Antimicrobial effect of ethyl acetate fungal extract of T. gamsii using solid state fermentation after 15 days of incubation period.

The variation on the antioxidant activity can be attributed to the used waste as a carbon source and the incubation period. Both isolates showed differential utilization of the various carbon sources. The use of corn cobs waste as a carbon source enhanced the optimization of antioxidant activity percentage. The highest percentage of antioxidant activity observed was 53.84% for T. gamsii ethyl acetate extract after 7 days of incubation (Table 4).

Table 4. In vitro antioxidant activity (%) of A. pseudocaelatus and T. gamsii ethyl acetate extract cultivated on media containing different agricultural wastes and their different incubation periods (days).

<table>
<thead>
<tr>
<th>Waste/ Incubation Period</th>
<th>7 days</th>
<th>11 days</th>
<th>14 days</th>
<th>19 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl acetate extract of A. pseudocaelatus</td>
<td>4.286</td>
<td>5.029</td>
<td>5.081</td>
<td>5.000</td>
</tr>
<tr>
<td>Ethyl acetate extract of T. gamsii</td>
<td>4.286</td>
<td>5.029</td>
<td>5.081</td>
<td>5.000</td>
</tr>
</tbody>
</table>

© 2021 Jordan Journal of Biological Sciences. All rights reserved - Volume 14, Number 1
exploiting these strains in waste conversion. Zohair et al. (2018) reported that A. pseudocaelatus exhibited a high hydrolytic activity to utilize carboxymethylcellulose and a moderate hydrolytic capacity of chitin (62.14 and 34.29 %), respectively, while T. gamsii had a high activity for hydrolyzing of carboxymethyl cellulose (CMC) and chitin 70.00 and 36.25 %, respectively. The use of hydrolytic enzymes to degrade agricultural wastes to fermentable sugar is highly recommended due to the specific nature of the enzymes and their ability to work at mild process conditions. The saccharification of lignocellulosic material requires the use of several enzymes with complementary activities. The saccharification of lignocellulosic material is achieved by a series of reactions, involving the endoglucanase, which attacks regions in the interior of cellulose chains, the exoglucanases and cellobiohydrolases, which hydrolyze cellobiose units from the ends of cellulose chains and β-glucosidase, which converts cello-oligosaccharides and cellobiose into glucose (Stichnothe et al., 2016)

4. Conclusion

The ethyl acetate extract of A. pseudocaelatus cultured on medium containing sorghum as natural carbon sources showed higher inhibitory effect against the tested pathogenic microorganism than using PDA medium. In the same way, T. gamsii ethyl acetate extract of PDA medium showed the highest percentage of antioxidant activity 53.84% after 7 days of incubation period compared to 14.7 %. Exploiting of agro-industrial waste for the production of sustainable bio-resources and the conversion of these resources and wastes into valuable products should be initiated by implementing the biotechnological innovation perspective to establish economic sustainability

Acknowledgments

The authors would like to acknowledge National Research Centre (NRC), Egypt for financial support for the research project (No.11010103); "Production of some metabolic byproduct compounds which have biological activity from rhizospheric fungi."

Conflict of interest

The authors declare that there is no conflict of interest.

References


