Creation of a fungal library and screening of antimicrobial and anticancer activity

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Introduction

According to the World Health Organization, cancer and infectious diseases are two of the most concerning diseases. Cancer kills 10 million people every year and the emergence of resistance to antitumoral drugs is an important medical challenge. At the same time, antimicrobial resistance (AMR) is also a serious threat to human and environmental health. Besides mortality, AMR burdens healthcare services and dampens medical procedures such as surgeries, cancer treatments and other invasive procedures [2]. The development of new drug therapies to fight drug resistance is essential to contest the rising of resistant bacteria and reduction of the effectiveness of antitumoral drugs. Microorganisms have been a major source for natural compounds throughout the years. Fungi, renowned for their ability to produce an array of broad and diverse secondary metabolites, offer a rich resource for drug discovery [3].

The main objective of this work was to build a diverse fungal library and to evaluate the antimicrobial and anticancer potential of their secondary metabolites extracts.

Methods

– Fungal isolates were obtained from dried chestnut, chestnut flour and sunflower seeds. 5.8S rRNA gene and the two internal transcribed spacers of the rDNA region – ITS – was amplified and sequenced to obtain fungal identification.
– Crude ethyl acetate extracts were obtained after 15 days fermentations through liquid-liquid extraction techniques.
– Antimicrobial screening was performed by 24-hour exposure of *Saccharomyces cerevisiae*, *Candida glabrata*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* to fungal extracts at a concentration of 100 μg/mL.
– Prostate adenocarcinoma cells (PC3) and prostate epithelial cells (HPepiC) were exposed to fungal extracts at the same concentration. MTT assay was used to evaluate cell viability after 24 hours.

Results

1. The obtained fungal library has 175 isolates from 13 different genera

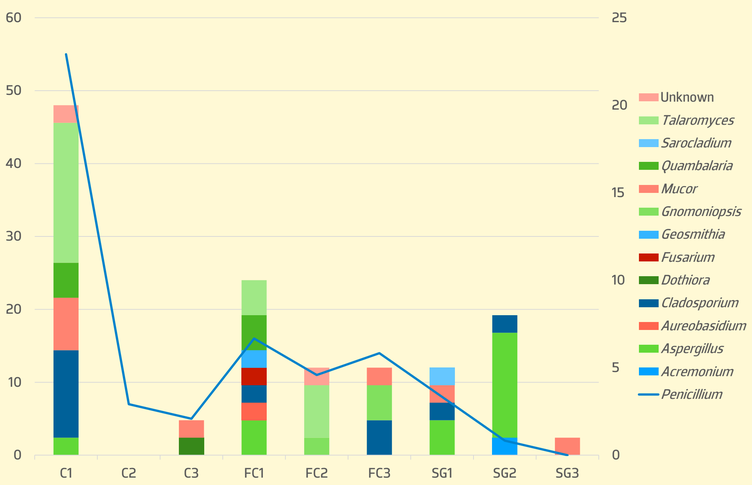

Figure 1.1- Fungal Isolates sampling distribuition. C – Dried chestnut; FC – Chestnut Flour; SG – Sunflower Seed.

2. Fungal extracts show similar antimicrobial activity as cloranfenicol

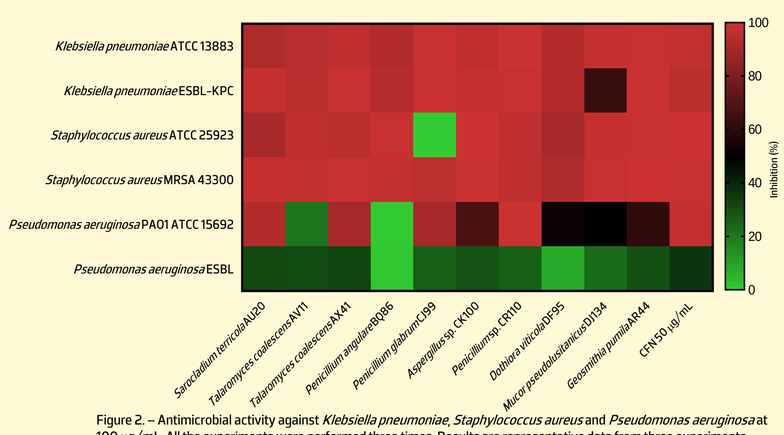

Figure 2. – Antimicrobial activity against *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* at 100 μg/mL. All the experiments were performed three times. Results are representative data from three experiments.

3. Isolates 106, 124 and 91 show stronger activity against *Candida glabrata*

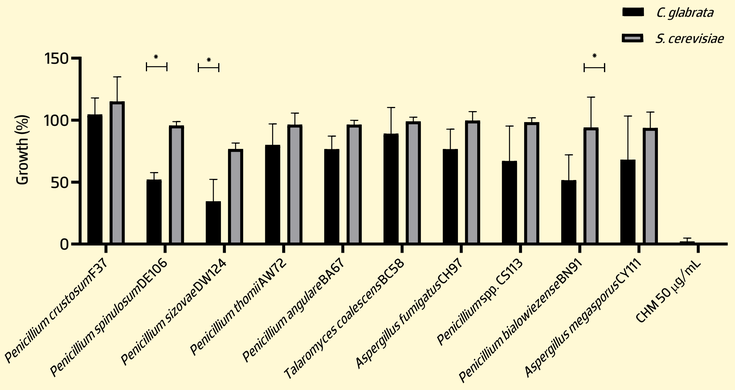

Figure 3.- Antifungal activity against yeast species. All the experiments were performed three times. Data show means and standard deviation. Statistical significances were determined by two-way ANOVA followed by Sidak's multiple comparison test (* $p < 0.1$; ** $p>0.01$).

4. Isolates 75, 76, 86, 95 and 98 show stronger citotoxic activity against tumoural cells

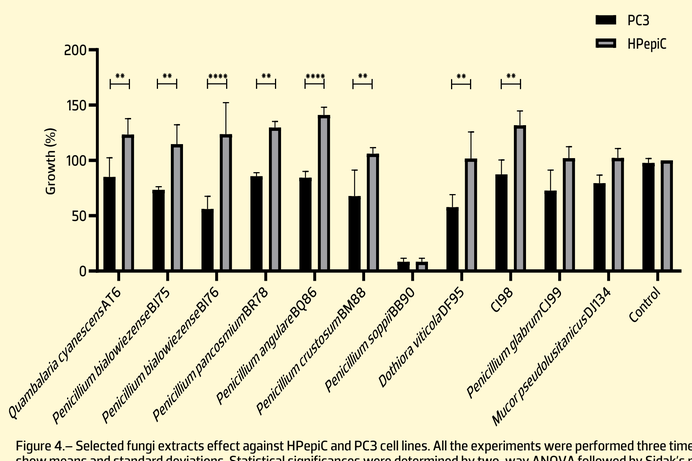

Figure 4.– Selected fungi extracts effect against HPepiC and PC3 cell lines. All the experiments were performed three times. Data show means and standard deviations. Statistical significances were determined by two-way ANOVA followed by Sidak's multiple comparison test (* $p < 0.1$; ** $p<0.01$; *** $p<0.001$; **** $p< 0.0001$).

5. Molecular identification and colony morphology

Isolate ID	Closest Hit Type Strain	Coverage	Identity
AU20	*Sarocladium terricola* CBS:243.59	93%	99.63%
AV11	*Talaromyces coalescens* CBS:103.83	100%	100%
AX41	*Talaromyces coalescens* CBS:103.83	100%	100%
BN91	*Penicillium bialowiezense* CBS 227.28	100%	100%
BQ86	*Penicillium angulare* NRRL 28157	94%	100%
CJ99	*Penicillium glabrum* CBS 125543	100%	100%
CK100	*Aspergillus* spp.	100%	100%
CR110	*Penicillium* spp.	100%	100%
DE106	*Penicillium spinulosum* CBS:344.59	100%	100%
DF95	*Dothiora viticola* FMR 13040	100%	98.92%
DJ134	*Mucor pseudolusitanicus* CBS 540.78	100%	98.92%
AT6	*Quambalaria cyanescens* CBS 357.73	100%	90%
BJ75	*Penicillium bialowiezense* CBS 227.28	100%	100%
BI76	*Penicillium bialowiezense* CBS 227.28	100%	100%
BQ86	*Penicillium angulare* NRRL 28157	94%	100%
BM88	*Penicillium crustosum* FRR 1669	100%	100%
BB90	*Penicillium soppii* NRRL 2023	100%	99.06%
CI98	-	-	-
CJ99	*Penicillium glabrum* CBS 125543	100%	100%

Table 1.– Molecular identification of the most promising selected candidates. ITS region was amplified and sequenced for all isolates. The resulting sequences were compared with those deposited in National Centre for Biotechnology (NCBI) Genbank database, using their Standard Nucletiotide BLAST search, limited to sequences from type material.

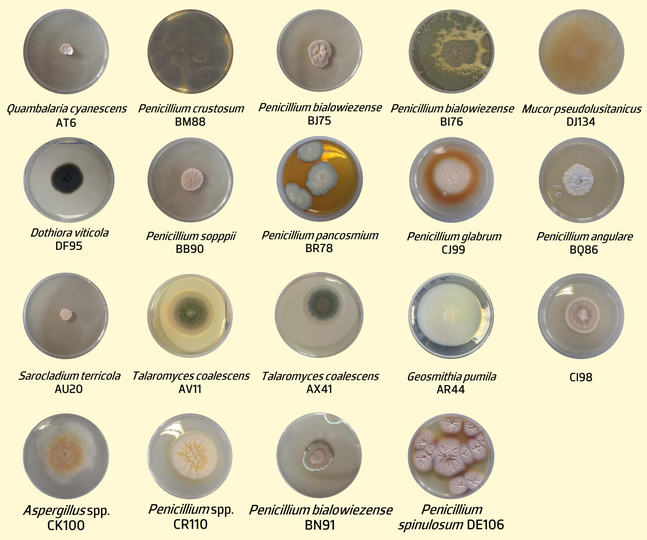

Figure 5. - Fungal isolates growth on DG18 medium plates.

Conclusion

Fungi isolation from dried chestnuts, chestnut flour and sunflower seeds, allowed us to obtain a significant and diverse fungal collection with 175 fungal isolates, from 13 different genera, being *Penicillium* (69% of isolates) the most common.

Our results so far show several extracts with antimicrobial and/or anticancer activity, some without decreasing cell viability of non-tumoral cells, showing their potential as therapeutic drugs without possible secondary effects. Cross referring our results with existing literature allowed us to select fungal isolates to continue our studies, molecular characterization with other barcoding genes, chemical characterization of their extracts and the studies with different extract concentrations will be performed to continue our search for bioactive compounds.

Acknowledgments

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