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Penicillium spp. Strains as a Possible Weapon to Fight Microbial Infections

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INTRODUCTION:

Bacteria are becoming increasingly resistant to antibiotics, leading to untreatable infections and constituting a major public health hazard [1]. This problem is further increased by the reduction of effective antibiotics against resistant strains [2], turning the search for new compounds a vital priority.

We proposed using filamentous fungi, *Penicillium* spp. in particular, to source potential bioactive secondary metabolites with antimicrobial activity. *Penicillium* spp. have produced secondary metabolites such as Griseofulvin, mycophenolic acid and Penicillin G [3]. Culture conditions can be changed in order to optimise the production of secondary metabolites including: media, temperature, pH and light [4].

This study looks into the production of secondary metabolites from four *Penicillium* strains with potential activity against three model microorganisms: *Mycobacterium smegmatis* (model for tuberculosis and other mycobacteriosis), *Micrococcus luteus* (Gram-positive bacteria) and *Escherichia coli* (Gram-negative bacteria). Growth conditions were analysed to study the influence of different media on the antimicrobial activity of crude extracts, since solid media has been reported to lead to extracts with larger mass than the liquid equivalent [5]. Different extractions strategies were also analysed.

METHODOLOGY:

1. Pre-Screening of 10 *Penicillium* Strains → selection of 4 strains (P.1AB, P.2AB, P.3AB, P.4AB)

2. Chemical extractions with Ethyl Acetate, from strains grown in **I** - MEA, **II** - PDA, **III** – MEB, **IV** - MEB with 5% NaCl, **V** - MEB (H_2O) & **VI** - MEB with 5% NaCl (H_2O)

3. Concentration and preparation of crude extracts (evaporation of solvents, extracts dissolved in DMSO)

4. Antimicrobial Screening of all (24) crude extracts, by disk diffusion test against 3 model microorganisms

RESULTS:

During the initial screening (Fig.1), inhibition was seen on *E.coli* from all but one environmental strain, with minimal inhibition against *M. luteus* apart from strain P4.AB. Four strains were selected (Fig. 2).

Figure 1. Representative plate for initial screenings: plugs from grown strain P.4AB, on a lawn of *M. luteus.*

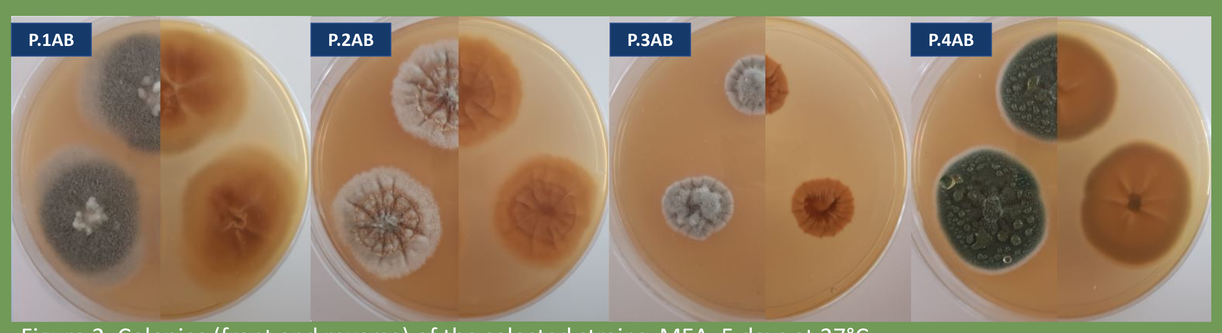

Figure 2. Colonies (front and reverse) of the selected strains. MEA, 5 days at 27°C.

Figure 3. Representative image of inhibition halos of crude extracts from the strain P.3AB grown on (A) PDA, (B) MEB, and (C) MEB 5%NaCl, against *M. luteus.*

Six crude extracts were produced for the 4 selected strains and their antimicrobial activity was tested (Fig. 3, Table 1).

Table 1. Growth inhibition by 24 crude extracts from four *Penicillium* strains (P.1AB, P2.AB, P.3AB and P.4AB). A - 15µl of extract, B - 5µl of extract, C - 15µl of DMSO, D - 5µl of DMSO. +++=strong inhibition (15-13mm diameter), ++=moderate inhibition (12-10mm diameter), +=weak inhibition (7-9mm diameter), -=no inhibition (Disk used =6.5mm).

	Penicillium strain:	P.1AB				P.2AB				P.3AB				P.4AB			
		E. coli															
Extract:		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
E1	MEA	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-
E2	PDA	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-
E3	MEB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E4	MEB 5% NaCl	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-
E5	H_20	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
E6	H_20 5% NaCl	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-
		M. luteus															
Extract:		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
E1	MEA	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
E2	PDA	-	-	-	-	-	-	-	-	++	+	-	-	-	-	-	-
E3	MEB	-	-	-	-	-	-	-	-	++	-	-	-	-	-	-	-
E4	MEB 5% NaCl	-	-	-	-	-	-	-	-	+++	+	-	-	+	-	-	-
E5	H_20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E6	H_20 5% NaCl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		M. smegmatis															
Extract:		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
E1	MEA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E2	PDA	+	+	-	-	-	-	-	-	++	+	-	-	-	-	-	-
E3	MEB	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
E4	MEB 5% NaCl	-	-	-	-	-	-	-	-	++	-	-	-	-	-	-	-
E5	H_20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E6	H_20 5% NaCl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

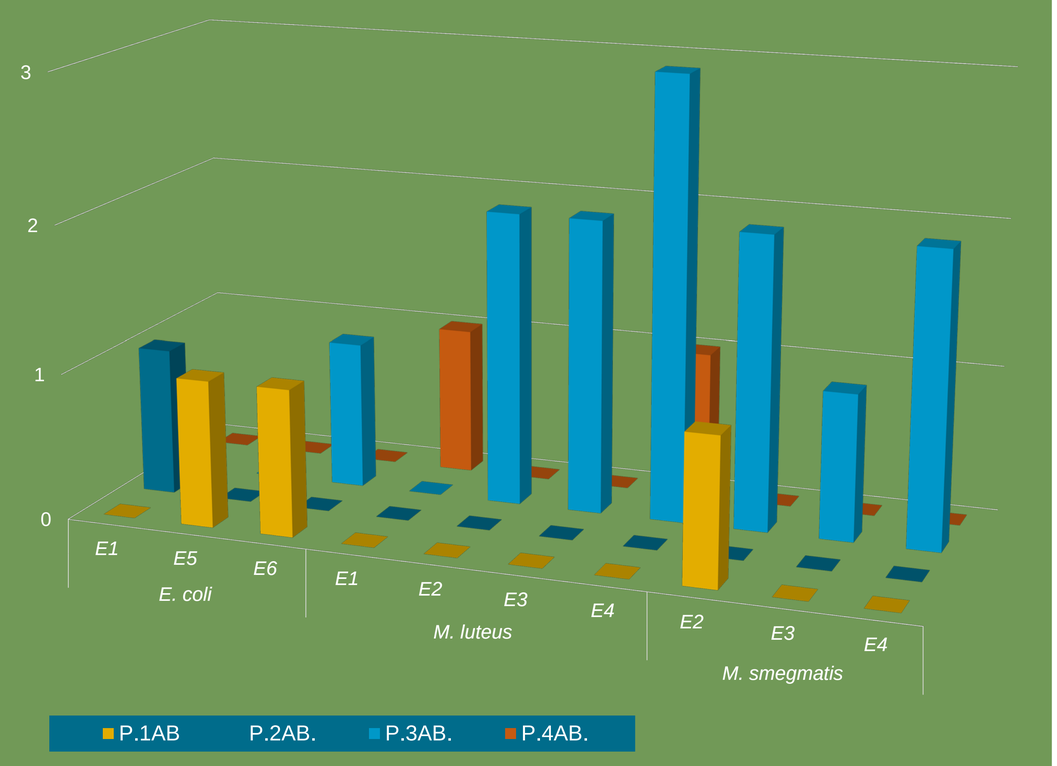

Figure 4. Observed growth inhibition of the produced crude extracts against *E. coli, M. luteus* and *M. smegmatis* (bar lengths: 3= strong inhibition (15-13mm diameter), 2= moderate inhibition (12-10mm diameter), 1= weak inhibition (7-9mm diameter).

Some of the extracts had higher inhibition of growth (Fig. 4).

DISCUSSION AND CONCLUSIONS:

- Initial screening of 10 *Penicillium* strains revealed that 4 inhibited the growth of bacteria.
- **MEB supplemented with 5% NaCl**, proved to be the preferred medium choice for extracts from the strain **P.3AB**, presenting higher growth inhibition against *M. luteus*. This strain showed potential antibacterial activity against all model microorganisms tested.
- Strains P.4AB, did not present as many active metabolites on its extracts, even though plugs from its colonies inhibited microbial growth.
- DMSO has recently been discovered to have the ability to protect *E. coli* from antibiotics [6], which could partially explain the lack of inhibition for some of the extracts.
- A different solvent, other than DMSO, should have been tested to avoid interferences with the microbial growth. This was chosen to guarantee solubility of all extracts.

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REFERENCES: [1] Lewis, K., 2013. Nat Rev Drug Discov, 12(5), pp.371-387. [2] Penesyan, A. *et al*, 2015. Molecules, 20(4), pp.5286-5298. [3] Boruta, T., 2018. *Bioengineered*, *9*(1), pp.12-16. [4] Brakhage, A.A., 2013 *Nat Rev Microbiol*, *11*(1), p.21-32. [5] VanderMolen, K.M. *et al*, 2013. *Amb Express*, *3*(1), pp. 1-7. [6] Brito, R. *et al*, 2017, *International Conference on Multidisciplinary Sciences, 3rd edition* (p. 4980).