ECM 2020

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University of MENTOURI Brothers. Constantine Algeria

Characterization and enzymatic profiling of a halotolerant *Penicillium* strain

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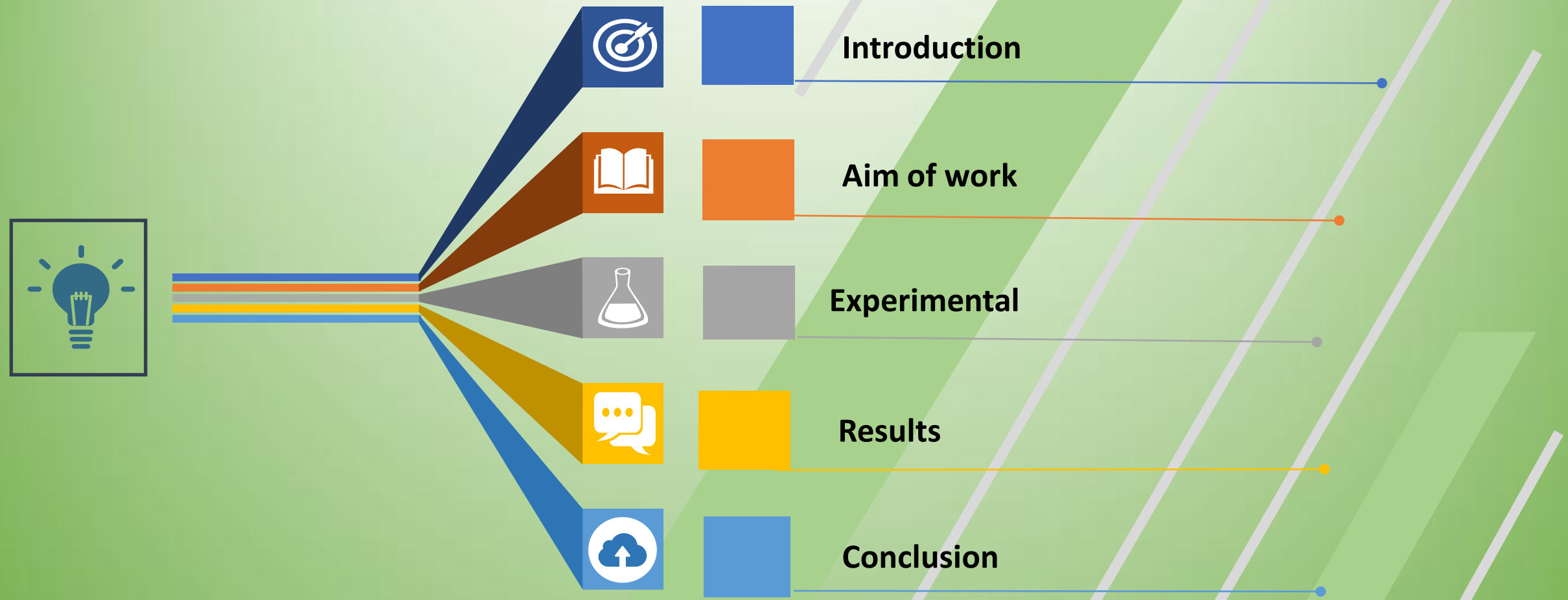
microorganisms

MDPI

Plan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INTRODUCTION

Introduction

Ability to grow and resist to saline conditions as well as other harsh conditions like ultraviolet radiations,extremes temperature and pH

Production of halotolerant metabolites with other resistances to alkaline pH, high temperature, ultraviol et radiation

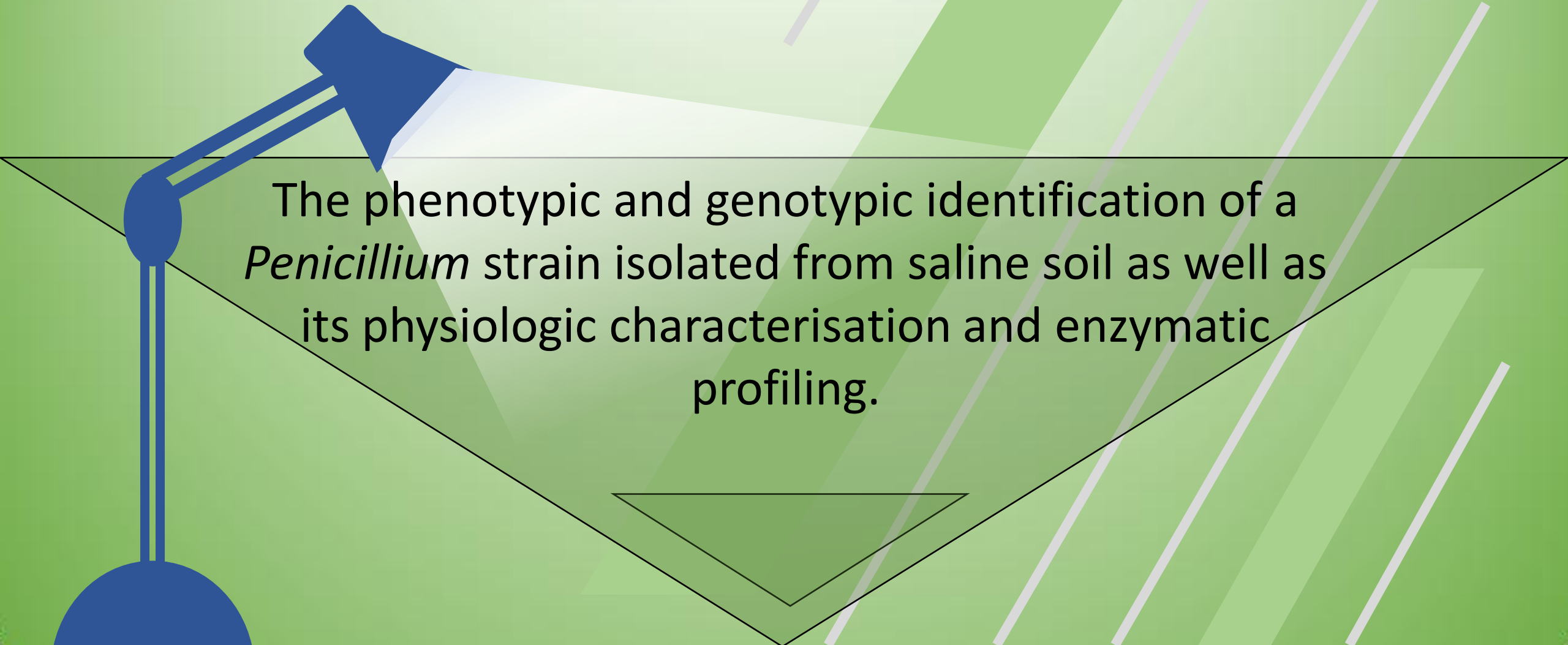
HALOPHILIC FUNGI

Organisms isolated from environments at salinities exceeding 10% and have the capability to grow *in vitro* at minimum 17% NaCl media

The most dominant genera in natural hypersaline environments are *Penicillium* species owing to their huge resistance's capacity to high concentrations of NaCl

AIM OF WORK

Aim of work



The phenotypic and genotypic identification of a *Penicillium* strain isolated from saline soil as well as its physiologic characterisation and enzymatic profiling.

EXPERIMENTAL

Experimental

Microorganism

Penicillium GS15 was isolated on PDA supplemented with 20% NaCl from saline soil of Ain-Ezzmoul's Sebkha (Oum-El-Bouagui Province. Algeria) and maintained on MEA slants at 4° C.

Experimental

Identification of *Penicillium spp*

<u>Macroscopic aspects</u> of Penicillium GS15 was evaluated By Pitt et Hocking (2009) method

The strain GS15 was inoculated for 7 days as 3-point cultures onto three different culture media at three different temperature 25, 37 and 5°C

- Czapek Yeast Agar (CYA)
- Malt Extract Agar (MEA)
- Glycerol 25% Nitrate (G25N)

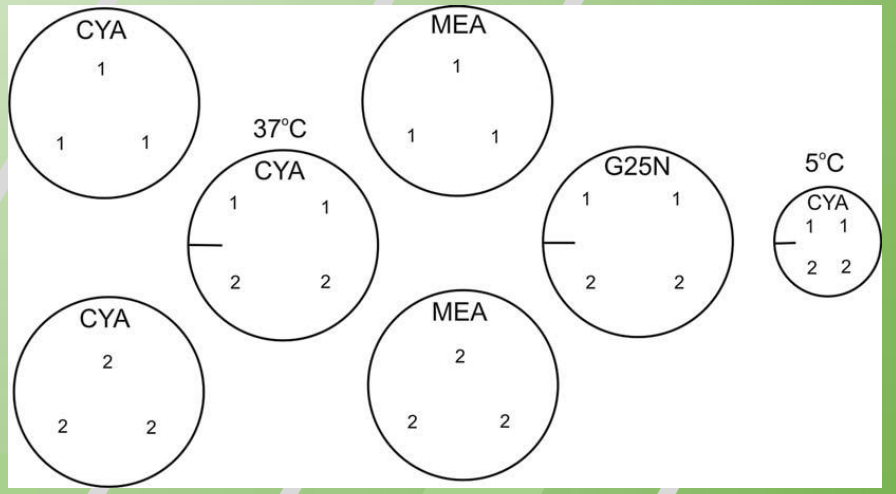


Fig. 1 Schema used for culturing fungal isolates

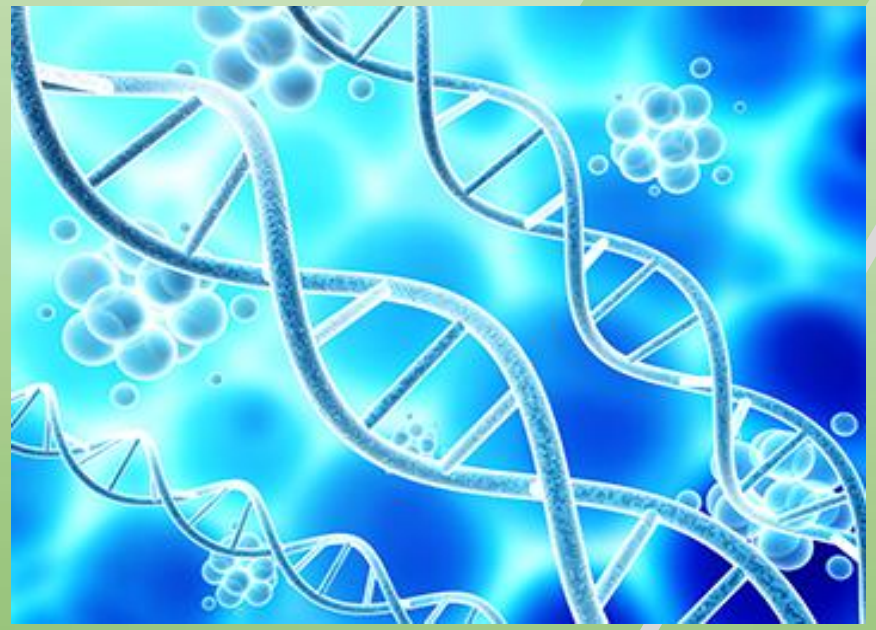
Identification of *Penicillium GS15*

Microscopic identification was carried out under x10, x60 and x100 objectives of optic microscope after coloration with blue cotton

Experimental

Identification of *Penicillium GS15*

Molecular identification was carried out with *β-tubilin* gene by the Laboratory of Systematic and Applied Mycology in the Catholic University of Louvain (ULC). Louvain, Belgium



Experimental

Physiologic features

Penicillium GS15 was cultivated at different conditions on Malt Extract Agar Medium for 7 days.

Temperature: The strain was cultivated at different temperature 5, 15, 25, 35 and 45° C, pH5

pH: The cultivation was carried out at 25°c with several pH values 5, 7, 9 and 11

Penicillium GS15

Aeration : The anaerobic conditions were assured with the use of anaerobic jar at 25°C, pH5

Light : Plates placed on dark (covered with Aluminium paper) or exposed to daylight at 25°C, pH 5

Experimental

Physiologic features

Penicillium GS15 was cultivated at different conditions on Malt Extract Broth Medium for 12 days at 25°C .

Penicillium GS15

Salinity: The selected fungus was incubated in presence of increasing concentrations of NaCl 0, 3, 6, 9, 12, 15, 18, and 21% then incubated under static conditions .

Agitation : the culture was carried out on 50ml of MEB with 0 and 180 rpm

Experimental

Enzymatic Activity Assays on Agar plates

Discs of mycelium were cut from edge of fresh colony of Penicillium GS15 then placing carefully upside down in the midpoint of the plate containing 2 % Agar and the corresponding substrate

Cellulase on 3 % cellulose

Pectinase on 2 % pectin

Amylase 1% soluble starch

Esterase on 2.5 % Tween 80

Penicillium GS15

Lactase on 1% lactose

Lipase on 0.1 % Tributyrin

Laccase on 0.5 % Guaiacol

Tannase on 0.5% of tannic acid

Proteases on 5 % casein, 5 % gelatine and 5 % albumin

RESULTS

Results

Identification of Penicillium GS15

The strain GS15 was identified phenotypically as *Penicillium sp* according to Pitt et Hocking (2009) besides Visagie et al. (2014) methods (Figure 1). Analysis of β-tubilin sequence with BLAST software and phylogenetic tree revealed the 100 % homology of the isolated strain with marine psychro-halophilic *Penicillium chrysogenum MF575001* (Corral et al., 2018)

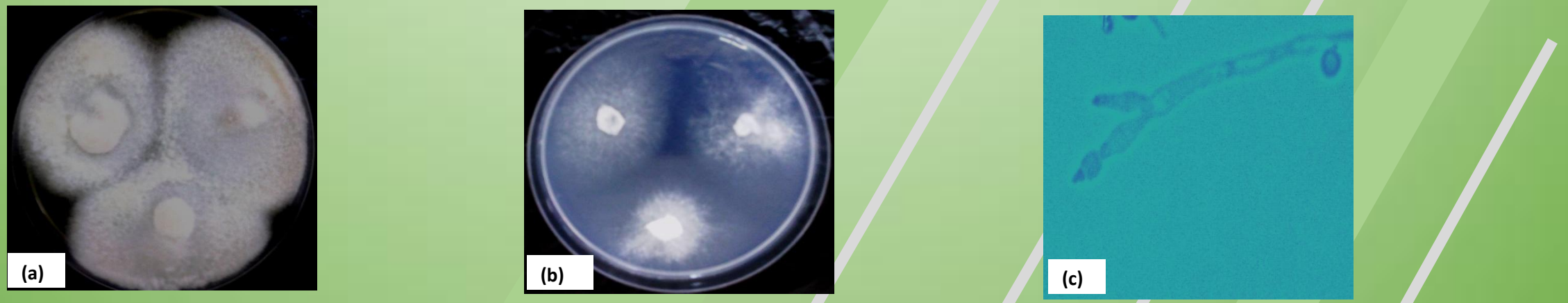
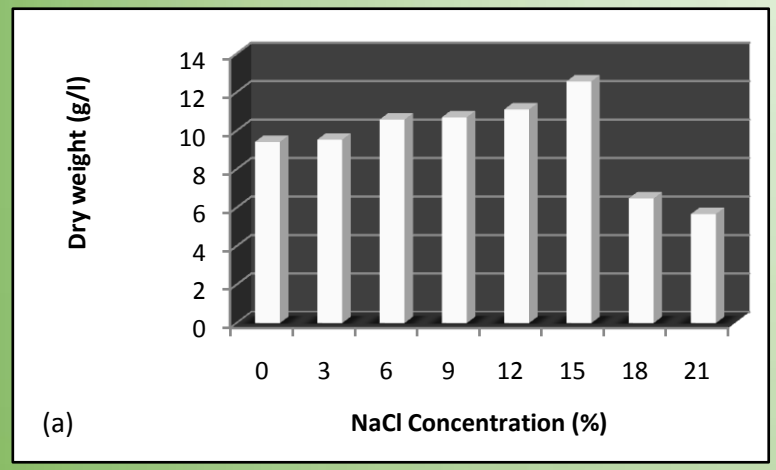
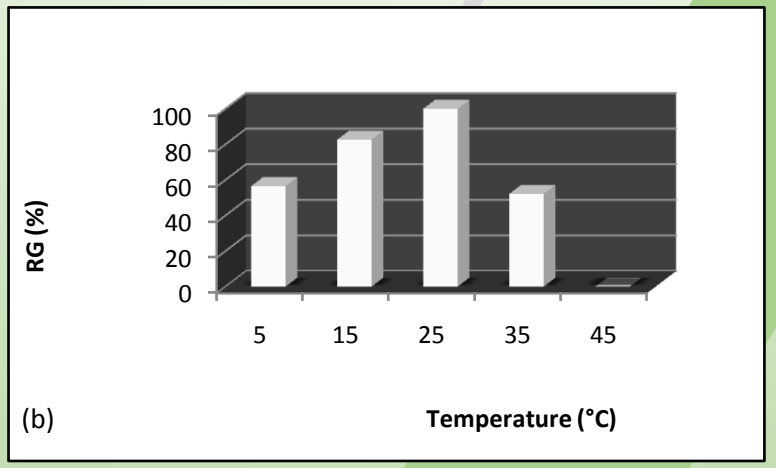


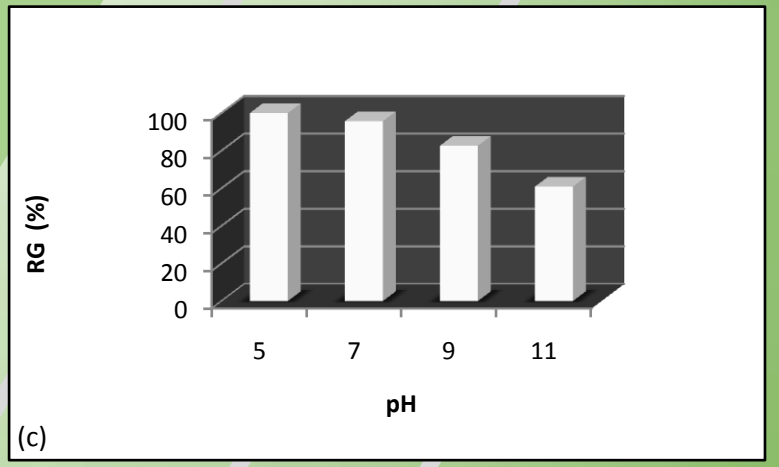
Figure 1: Different aspects of *Penicillium GS15*. (a): MEA, 25°C ; (b): CYA, 25°C; (c): microscope

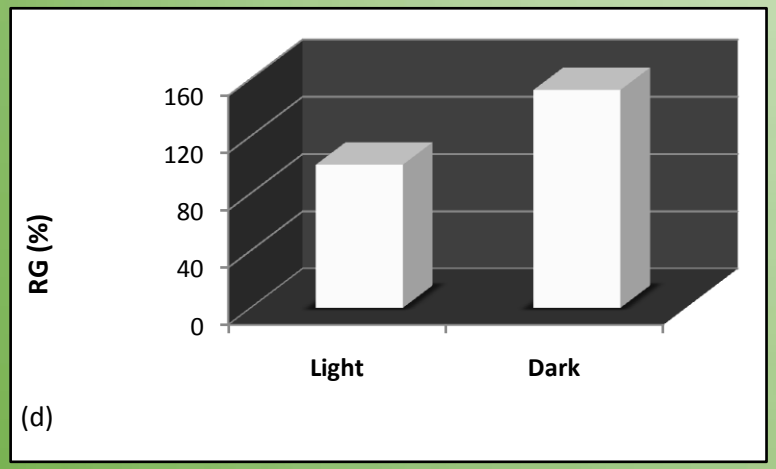
Results

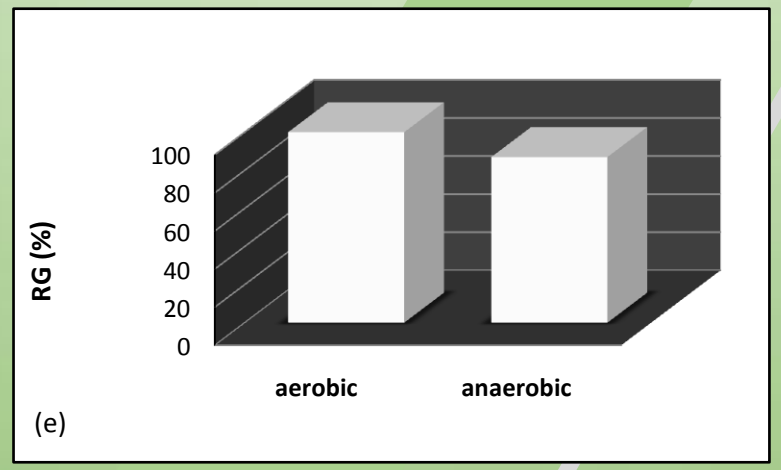
Physiologic features

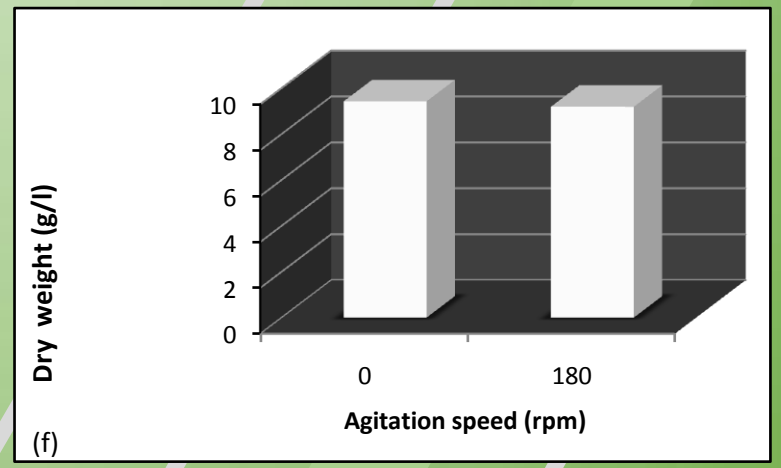












Effect of NaCl concentrations (**a**), temperature (**b**), pH (**c**), light (**d**), aeration (**e**) and agitation **(f)** on growth of *P.chrysogenum.* RG: Relative growth

Results

Enzymatic Activity Assays on Agar plates

Table 1: Enzymes activities of *P.chrysogenum* on solid media (G: Growth; H: Halo; RA: Relative Activity)

Enzymes	G (mm)	H (mm)	RA
Laccase	10	20	01
Tannase	27	35	1.30
Lactase	18	00	00
Amylase	20	20	01
Cellulase	25	00	00
Pectinase	24	00	00
Esterase	40	50	1.25
Lipase	12	18	1.5
Caseinase	35	49	1.4
Gelatinase	26	00	00
Albuminase	20	00	00

CONCLUSION

Conclusion

The highly halotolerant *P.chrysogenum MT891265* isolated in the present study appear to be remarkable resistant to some harsh conditions (wide ranges of pH, temperature and salinity) and to have simple requirement for growing (dark, air and lack of agitation)

The capability of extracellular enzymes's production such as laccase, tannase, amylase, esterase, lipase and caseinase

This strain an extremely desirable candidate in industrial processes such as agriculture, detergents, textile and environmental bioremediation of
lipolytic, proteolytic, amylolytic plus phenolic wastes.

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