



Research Article

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The first report on the mycelial growth performance and antibacterial activity of Collybia reinakeana RGR-FE –NSC strain, a Philippine endemic edible mushroom

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ABSTRACT

A pioneering work on the mycelial growth performance and antibacterial activity of Collybia reinakeana RGR-FE –NSC strain, a Philippine endemic edible mushroom is hereby presented. Fruiting bodies of mushroom were rescued from the wild and pure cultures were produced following the standard laboratory protocol for the domestication of wild edible mushrooms. Mycelial growth performance was evaluated on four commercially available dehydrated culture media namely sabouraud dextrose agar, potato dextrose agar, mycological agar and malt extract agar. The antibacterial activity was also investigated against Staphylococcus aureus ATCC 29213. Results of our investigation revealed that sabouraud dextrose agar stimulated the mycelial growth of Collybia reinakeana RGR-FE –NSC strain. The mycelial growth on potato dextrose agar, mycological agar and malt extract agar yielded mycelial sectors. For the first time, the bactericidal activity of Collybia reinakeana RGR-FE –NSC strain is highlighted.

Key words: *Collybia reinakeana, mushroom nutraceuticals, mushroom mycelia*

INTRODUCTION

Collybia reinakeana P. Henn is a saprophytic edible mushroom in the Philippines that grows in clusters of fruiting bodies consisting of foot-long stalks with wide pileus. Its unusually big size of fruiting bodies aroused the curiosity of most Filipinos. It is a tropical species of leaf litter decomposing mushroom that naturally grows on decomposing piles of bamboo leaves, leaves of rain tree and rice straw usually from September to February when rainfall becomes occasional. Considered as endemic in the Philippines as there are no other reports in other parts of the world, several wild strains of this mushroom were sighted in the different parts of the Philippine archipelago particularly in Leyte, Romblon, Southern Tagalog, Central Luzon and Pangasinan. Its emergence in the different geographical areas in the Philippines implies that its occurrence is not only restricted in Laguna and Puncan, Carranglan, Nueva Ecija as previously reported [1] [2]. In 1997, Reyes and his research team [3] investigated the bio-physiological profile of Puncan, Carranglan strain which resulted in its successful domestication [4] [5]. *C. reinakeana* is considered as a nutraceutical mushroom. Aside from its appreciable amount of both essential as well as non - essential amino acids, *C. reinakeana* also contains standard and non – standard amino acids. This edible mushroom was reported to have anti-hypertensive properties due to its ability to inhibit the angiotensin converting enzymes [6].

RGR-FE –NSC strain is one of the newly domesticated strains of *C. reinakeana*. Its secondary mycelia were rescued from the fresh and healthy fruiting bodies that were found growing on decomposing piles of bamboo leaves. In this paper, the mycelial growth performance of RGR-FE –NSC strain including its anti-bacterial activity are hereby reported in our desire to establish the nutritional as well as its pharmacological attributes

MATERIALS AND METHODS

Rescue of secondary mycelia

Fruiting bodies of *C. reinakeana* RGR-FE –NSC strain were collected in Northern Luzon Island, Philippines. The secondary mycelia were rescued from the freshly collected healthy fruiting bodies (Figure 1) growing in the wild following the standard protocol for the isolation of mycelia [7] [8]. Pure cultures were maintained in potato dextrose agar (HiMedia Laboratories Pvt. Ltd., India) slants.



Figure 1. Wild fruiting bodies of *Collybia reinakeana* RGR-FE_NSC strain growing on piles of decomposing bamboo leaves

Evaluation of the mycelial growth on different commercially available culture media

The following dehydrated and commercially available culture media (HiMedia Laboratories Pvt. Ltd., India) were evaluated in terms of their ability to stimulate the growth of the secondary mycelia of *C reinakeana* RGR-FE –NSC strain: potato dextrose agar, mycological agar, malt extract agar and sabouraud dextrose agar.

Ten mm mycelial discs from a 7 day – old culture of *C reinakeana* RGR-FE –NSC strain were prepared using a sterile cork borer. Individual mycelial disc was inoculated at the center of previously plated culture media. The inoculated plated media were incubated at 28 – 30 °C until complete mycelial colonization.

Antibacterial assay

The ability of *C reinakeana* RGR-FE –NSC strain to inhibit the growth of the test bacterium, *Staphylococcus aureus* ATCC 29213 was assessed. Different dilutions (i.e. 40 ml sterile distilled water + 0.1 ml 12 hr bacterial suspension in nutrient broth, 60 ml sterile distilled water + 0.1 ml 12 hr bacterial suspension in nutrient broth, 80 ml sterile distilled water + 0.1 ml 12 hr bacterial suspension in nutrient broth, 100 ml sterile distilled water + 0.1 ml 12 hr bacterial suspension in nutrient broth, 120 ml sterile distilled water + 0.1 ml 12 hr bacterial suspension in nutrient broth) of the test bacterium were prepared. Using a sterile L – shaped metal rod, an aliquot (about 0.1 ml) of each dilution was spread on top of the previously plated Mhuiller Hinton Agar ((HiMedia Laboratories Pvt. Ltd., India). Subsequently, the 10 mm mycelial disc of the 7 day – old culture of *C reinakeana* RGR-FE –NSC strain was aseptically laid down at the center of the plated medium and incubated at 32°C. Zone of inhibition was initially recorded 12 hours after incubation. To determine the bactericidal activity, incubation period was extended beyond 12 hours and zone of inhibition was measured using a digital Vernier caliper. As a control set, paper disc containing

Ampicillin (10 µg/mL) was aseptically laid down on the surface of previously plated Mhuiller Hinton Agar with varying concentrations of bacterial suspension.

RESULTS AND DISCUSSION

The mycelial growth in the compost and casing layer as well as the ability of mycelia to produce fruiting bodies can be predicted based on the cultural characteristics of mushroom mycelia in solid media [9]. Thus, it is necessary to evaluate the mycelial growth performance of mushroom in appropriate culture media as a prelude to the successful cultivation of fruiting bodies. The mycelial growth performance of mushroom is dependent on the available nutrients in any propagating medium. In this investigation, *C reinakeana* RGR-FE –NSC strain grew best in sabouraud dextrose agar (Figure 2) with very dense mycelia. Sabouraud dextrose agar contains peptone and dextrose as nutritional ingredients. Its growth in the other culture media such as potato dextrose agar, mycological agar and malt extract agar produced mycelial sectors (Figure 3). Mycelial sector is a growth pattern which is not desirable in mushroom production. It is an indication of the degeneration of strain [10]. RGR-FE –NSC strain is a fast growing strain of *C reinakeana* that colonized the plated medium within 3 to 7 days. In previous report, Puncan, Carranglan strain of *C. reinakeana* grew best in malt extract agar [4]. This interesting observation suggests that different strains may exhibit varying performances when subjected to different sources of nutrients.

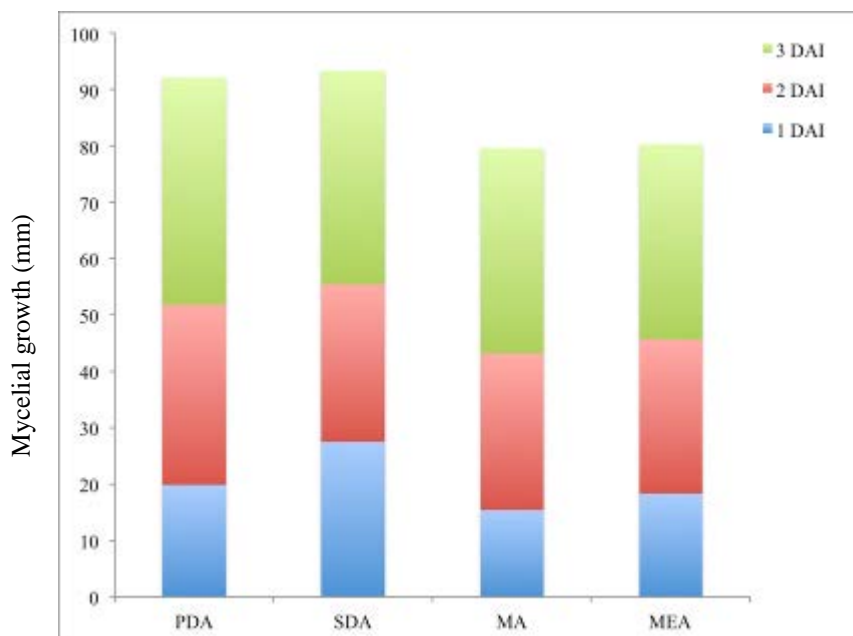


Figure 2. Mycelial growth of *Collybia reinakeana* RGR-FE-NSC strain cultured in different commercially available dehydrated culture media

Abbreviations: DAI – days after incubation, PDA – potato dextrose agar, SDA - sabouraud dextrose agar, MA – mycological agar and MEA – malt extract agar

In the course of its cultivation, the plated mycelial culture of *C reinakeana* RGR-FE –NSC strain produced a halo zone (Figure 4) which prompted us to evaluate its antibacterial activity. Results of our investigation revealed that *C reinakeana* RGR-FE –NSC strain exhibited antibacterial activity against *Staphylococcus aureus* 12 hours after incubation (Figure 5). This antibacterial activity persisted even beyond 125 hours after incubation (Table 1). This important observation suggests that the action of *C reinakeana* RGR-FE –NSC strain against *Staphylococcus aureus* is bactericidal rather than bacteriostatic. Even though mushrooms are known to exhibit antibacterial activities, the nature of their antibacterial activity was not elucidated. For instance, the acetone extract of *Ganoderma lucidum* inhibited the growth of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, *Bacillus subtilis*, *Salmonella typhi* and *Pseudomonas aeruginosa* [11]. Similarly, the phenolic extracts of *Lactarius deliciosus*, *Sarcodon imbricatus* and *Tricholoma portentosum* inhibited the growth of *Bacillus subtilis* and *B.*

ceruus [12]. In this investigation, we are reporting for the first time the bactericidal activity of *C reinakeana* RGR-FE –NSC strain.

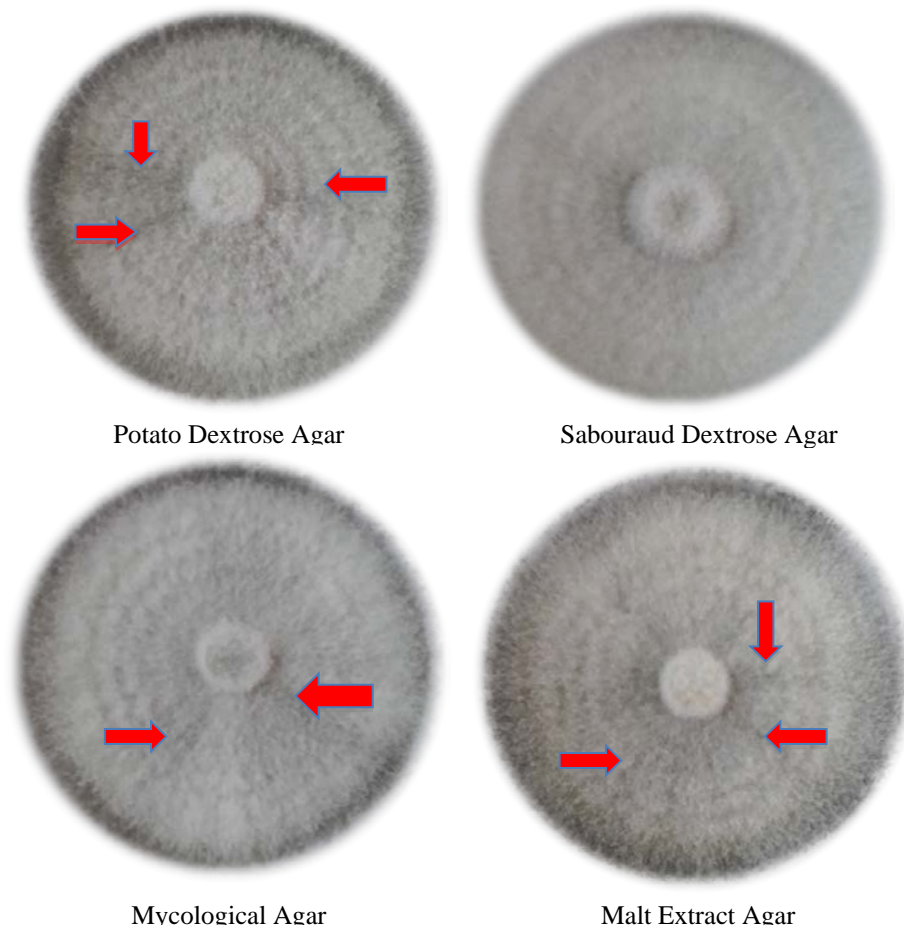


Figure 3. Cultural characteristics of *Collybia reinakeana* RGR-FE_NSC strain in different commercially available dehydrated culture media, 11 days after incubation at 30°C
Note: red arrow indicates mycelial sector

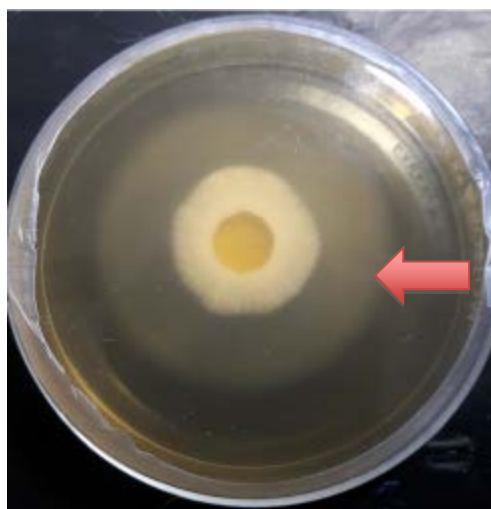


Figure 4. Natural formation of halo zone (red arrow) of *Collybia reinakeana* RGR-FE-NSC strain on saboraud dextrose agar, 24 hours after incubation at 30°C

Table 1. Antibacterial activity of *Collybia reinakeana* RGR-FE –NSC strain against *Stapylococcus aureus* ATCC 29213

Diluted Inoculum of <i>Stapylococcus aureus</i> ATCC 29213	Zone of Inhibition (mm) against <i>Stapylococcus aureus</i> ATCC 29213			
	Ampicillin (Control) 12 hours after incubation	<i>Collybia reinakeana</i> RGR-FE –NSC strain 12 hours after incubation	<i>Collybia reinakeana</i> RGR-FE –NSC strain 120 hours after incubation	<i>Collybia reinakeana</i> RGR-FE –NSC strain 240 hours after incubation
40 ml sterile distilled water + 0.1 ml 12 hr bacterial suspension in nutrient broth	20.31	14.0775	13.925	13.345
60 ml sterile distilled water + 0.1 ml 12 hr bacterial suspension in nutrient broth	18.63	13.07	13.885	13.405
80 ml sterile distilled water + 0.1 ml 12 hr bacterial suspension in nutrient broth	17.125	13.595	14.035	13.8375
100 ml sterile distilled water + 0.1 ml 12 hr bacterial suspension in nutrient broth	19.92	13.2775	13.44	13.035
120 ml sterile distilled water + 0.1 ml 12 hr bacterial suspension in nutrient broth	17.205	12.665	12.835	13.09

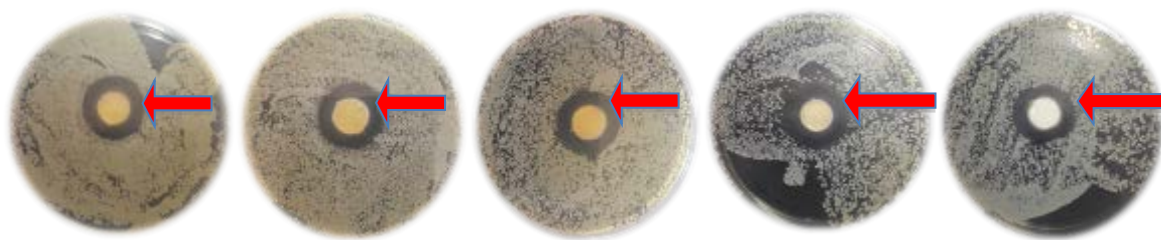


Figure 5. Zone of inhibition (red arrow) of *Collybia reinakeana* RGR-FE-NSC on *Staphylococcus aureus* - inoculated Mhuiller Hinton Agar plates, 12 hours after incubation at 32°C

Note: outer left plate (40 ml sterile distilled water + 0.1 ml 12 hr bacterial suspension in nutrient broth), inner left plate (60 ml sterile distilled water + 0.1 ml 12 hr bacterial suspension in nutrient broth), center plate (80 ml sterile distilled water + 0.1 ml 12 hr bacterial suspension in nutrient broth), inner right plate (100 ml sterile distilled water + 0.1 ml 12 hr bacterial suspension in nutrient broth), outer right plate (120 ml sterile distilled water + 0.1 ml 12 hr bacterial suspension in nutrient broth)

CONCLUSION

Secondary mycelia of *Collybia reinakeana* RGR-FE -NSC strain which exhibited bactericidal activity can be propagated luxuriantly without mycelial sectors in sabouraud dextrose agar.

ACKNOWLEDGMENT

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