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Research Article

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A New Record of the Mycoparasitic Habit of Collybia reinakeana RGR-FE- NSC Strain against Aspergillus flavus, Fusarium oxysporum and Cladosporium sphaerospermum

Renato G. Reyes[1*], Michael R. Umagat[1], Benjie L. Garcia[1], Ariel Joseph J. Barza[1], Ryo Sumi[2], Nobuo Mori[2], Noriko Miyazawa[3] and Fumio Eguchi[3]

[1]Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines.
[2]Nikken Sohonsha Corporation, Asahira, Fukujucho, Hashima, Gifu, Japan.
[3]Tokyo University of Agriculture, Sakuragaoka, Setagayaku, Tokyo, Japan.
**Email for correspondence: renato.reyes @ clsu .edu. ph*

ABSTRACT

The mechanism of the parasitic action of Collybia reinakeana RGR-FE-NSC strain was elucidated with our aim of establishing its mycoparasitic habit. Mycoparasitism was documented using Hitachi SU1510 scanning electron microscope. The ultrastructural examination of its fungal interaction with the three fungal epiphytes namely Aspergillus flavus, Fusarium oxysporum and Cladosporium sphaerospermum revealed its ability to exhibit mycoparasitic activity such as the thickening and coiling of its hyphae. Also, the twisting and dehydration of the hypha of the fungal epiphyte was observed as a result of the mycoparasitism of C. reinakeana.

***Key words**: Fungal Epiphytes, Mushroom, Mycoparasitism.*

INTRODUCTION

Mushrooms have been traditionally regarded as sources of culinary ingredients due to their strong aroma and nutrient contents. Depending on species, mushrooms are known to contain both essential as well as non - essential amino acids in addition to their mineral contents. In previous years, these important edible fungi have been reported to possess functional attributes against major lifestyle diseases such as hypertension, cancer and diabetes. Thus, mushrooms nowadays are considered as functional foods or nutraceuticals. Nutraceutical is a term coined to describe substances which are not traditionally recognized nutrients but which have positive physiological effects on the human body [1]. One of the nutraceutical mushrooms is *Collybia reinakeana* P. Henn. which exhibits antihypertensive properties in addition to its amino acid content [2]. It is a virtually unknown wild edible mushroom from the Philippines not until the rescue of its secondary mycelia was made successful in 1997 which lead to its first in vivo propagation [3] [4]. Recently, one of its strains, RGR – FE – NSC strain was reported to exhibit bactericidal activity against *Staphylococcus aureus* [5]. A number of mushrooms are known to express antibacterial activities [6] [7]. With the pioneering report on the antibacterial activities of *C. reinakeana*, our team became interested to investigate the other anti-microbial activities of *C. reinakeana* to include its anti-fungal activities.

In this paper, we are reporting for the first time the mycoparasitic habit of *C. reinakeana* RGR – FE – NSC strain against major fungal epiphytes as seen under the scanning electron microscope. Most of the reports on the antifungal activities of mushrooms were limited on the ability of their culture filtrates and extracts to inhibit the growth of

fungi [8] [9] [10]. However, in this paper, we are describing for the first time the ultrastructural mechanism of the antifungal activities of *C. reinakeana* RGR – FE – NSC strain.

MATERIALS AND METHODS

Fruiting bodies of *C. reinakeana* RGR – FE – NSC strain (Figure 1) were collected from the wild and the secondary mycelia were subsequently rescued following the standard protocol for isolation of mycelia [11]. Potato dextrose agar plate culture of *C. reinakeana* RGR – FE – NSC strain that was naturally contaminated with fungal epiphytes was used in this investigation. The mycoparasitic habit of a 14 day – old mycelial culture of *C. reinakeana* was observed by taking mycelial samples from the point of contact between (1) *C. reinakeana* and the fungal epiphytes which include (2) *Aspergillus flavus*, (3) *Fusarium oxysporum* and (4) *Cladosporium sphaerospermum* (Figure 2). The mechanism of mycoparasitism of *C. reinakeana* against these fungal epiphytes were observed ultrastructurally using Hitachi SU1510 scanning electron microscope.

Fig1. Cluster of fruiting bodies (left photo) and mycelia (right photo) of *C. reinakeana* RGR – FE – NSC strain

RESULT AND DISCUSSION

Fungi when confronted with biotic as well as abiotic components of the ecosystem exhibit different forms of mechanism in order to defend themselves against these external pressures. Spatial and nutrient competition, antibiosis by enzymes, production of secondary metabolites and mycoparasitism are some of the forms of defense of fungi when challenged by external pressures [12]. For instance, volatiles from *Cephalosporium roseo-griseum, Fusarium oxysporum* and*Trichoderma harzianum* inhibited the growth of *Pestalotia psidii* [13]. Different species of *Trichoderma* are the most popular fungal antagonists that attack fungal pathogens of crops. Their antagonistic mechanisms have been widely documented [14]. Mushrooms have also been reported to exhibit antifungal activities. For instance, the antifungal activities of Korean mushrooms against major plant pathogenic fungi such as *Botrytis cinerea*, *Colletotrichum gloeosporioides* and *Colletotrichum miyabeanus* was reported [9]. However, the previous work was only focused on the ability of the culture filtrates of mushrooms to inhibit the growth of the plant pathogenic fungi in vitro. In this investigation, the antifungal activities of *C. reinakeana* RGR – FE – NSC strain against fungal epiphytes such as *Aspergillus flavus*, *Fusarium oxysporum* and *Cladosporium sphaerospermum* are described. Though *C. reinakeana* RGR – FE – NSC strain did not exhibit inhibitory activity against these fungal epiphytes in vitro, mycoparasitism was however observed. In our investigation, mycoparasitism has been documented in different forms. As shown in Figure 3, the hyphae of *Aspergillus flavus* was encapsulated by the hyphae of *C. reinakeana*. In the process of attacking the hyphae of *Fusarium oxysporum*, hyphae of *C. reinakeana* became irregularly thickened and coiled. Similarly, the hyphae of *Cladosporium sphaerospermum* was parasitized by the ingress of the hyphae of *C. reinakeana* into the hyphae of the fungal epiphyte which resulted in the twisting and dehydration of the hyphae of the latter. Death of the hyphae of the fungal epiphyte is due to the external and

internal colonization of *C. reinakeana* into the host cell of the fungal epiphyte resulting in the plasmolysis of the hypha.

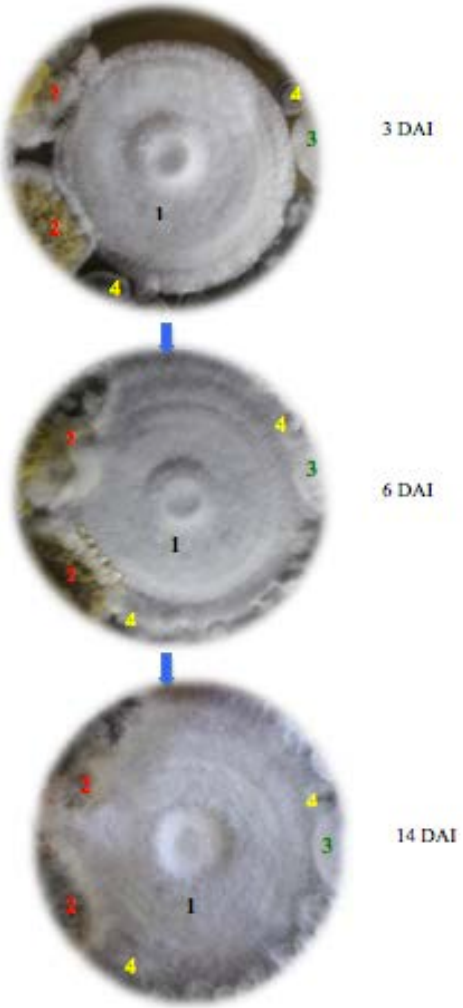

Fig 2. Progressive in vitro interaction of the mycelia of (1) *C. reinakeana* RGR-FE-NSC strain with the mycelia of (2) *Aspergillus flavus*, (3) *Fusarium oxysporum* and (4) *Cladosporium sphaerospermum*

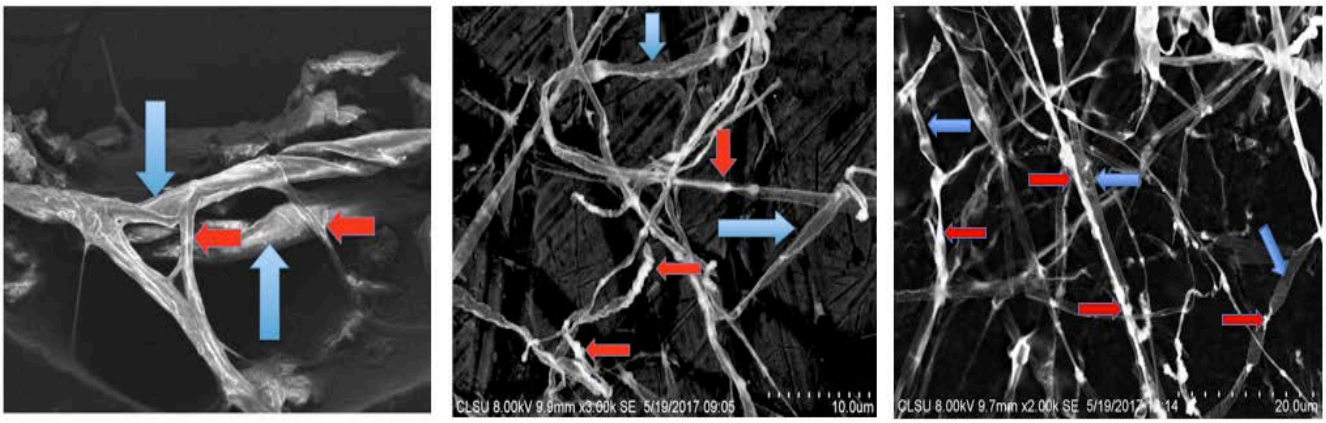

Fig 3. Mycoparasitic habit of the hyphae of *Collybia reinakeana* RGR-FE-NSC strain against *Aspergillus flavus* (left photo), *Fusarium oxysporum* (middle photo) and *Cladosporium sphaerospermum* (right photo)
Note: red arrow represents the hyphae of the fungal epiphytes and the blue arrow represents the hyphae of *Collybia reinakeana* RGR-FE-NSC strain

CONCLUSION

Collybia reinakeana RGR-FE-NSC strain is a mycoparasite of *Aspergillus flavus*, *Fusarium oxysporum* and *Cladosporium sphaerospermum.*

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