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# Effect of laser radiation on the growth of *Rhodotorula mucilaginosa* isolated from rumin fluid of cows in the Nineveh, Iraq

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**Abstract**

**Background:** The fungi *Rhodotorula* species are widespread airborne contaminants and are thought to be natural occupants of human skin, lungs, urine, and feces. Therefore, *Rhodotorula mucilaginosa*, *Rhodotorula minuta*, and *Rhodotorula glutinis* are three of the most prevalent species.

**Aim:** This study aims to isolate *R. mucilaginosa* from the rumen fluid of cows in the province of Mosul and to determine how laser light irradiation affects the growth and morphological traits of these Fungi.

**Methods:** From the rumen fluid of AL-Restaki and AL-Karadi of cows, the *R. mucilaginosa* was isolated. Using the traditional approach and the ID-Yst card system Vitek 2. A semiconductor laser system with a power of 50 mW and a wavelength of 450 nm was used in the experiment to evaluate the light laser irradiation effects on the culture growth of *R. mucilaginosa* directly under two light irradiation conditions of 30 and 60 minutes.

**Results:** According to traditional methods and the ID-Yst card system Vitek 2, *R. mucilaginosa* predominated 7/30 (23.3%), and these strains effectively grow on medium sabouraued dextrose agar as evidenced by the carotenoid pigments that gave their colonies a salmon-pink to coral-red. Compared with a control group where no laser was used, the impact of light laser irradiation was assessed 24 hours after the irradiation using biomass (dry weight measuring yeast cell content in suspension) and microscopic analysis using Gram stain. Microscopic examinations showed the irregular shape of the cells linked to one another. The irradiated subculture of on Sabouraued dextrose agar and incubation at 37°C for 3 days demonstrated inhibited growth in 4/7 (57.1%) isolates. In addition, there was no discernible difference vertically at $p < 0.05$ between the control group and the *R. mucilaginosa* biomass concentration under light irradiation circumstances (30 and 60 minutes).

**Conclusion:** This study proved that *R. mucilaginosa* is found in the rumen fluid of cows. Also, the isolated *R. mucilaginosa* displayed sensitivity to laser irradiation lights, revealing the more significant topographical alterations of the cell structure that had happened, the irregular shape of the cells, and how they were connected as a result of evolution.

**Keywords:** Laser application, *Rhodotorula mucilaginosa*, Rumen fluid of cow.

## Introduction

The last few decades have seen a significant rise in fungal infections that require medical help. Although *Aspergillus* and *Candida* species are responsible for most of these infections, less frequent pathogens become responsible for many infections (Pfaller and Diekema, 2004). Several authors have referred to these fungi as "emerging pathogens." Several of them historically have been regarded as contaminants in the laboratories or as being low virulent. These emerging pathogens include a variety of dematiaceous and hyaline molds and yeasts other than *Candida* species (Pfaller and Diekema, 2004; De Almeid *et al*., 2008). *Rhodotorula* spp. are opportunistic pathogens that can cause invasive infections in the right circumstances. This understanding developed, and approximately a hundred *Rhodotorula* infections cases have been reported in the previous 40 years (Lanzafame *et al*., 2001; Perniola *et al*., 2006).

Species of the sporidiobolaceae family called *Rhodotorula* are colored basidiomycetous yeasts (Fell *et al*., 2000). That is, only 3 of the 37 species in this genus, including *Rhodotorula mucilaginosa* (previously *Rhodotorula rubra*), *Rhodotorula glutinis*, and *Rhodotorula minuta*, have been identified as human pathogens (Biswas *et al*., 2001; Miceli *et al*., 2011). Recently, three unique species that are not harmful to people were described *Rhodotorula silvestris*, *Rhodotorula straminea*, and *Rhodotorula rosulata* (Golubev *et al*., 2010). Most *Rhodotorula* species produce colonies on Sabouraud dextrose agar that are pink to coral in color, while some can also be orange or red due to the presence of carotenoid pigments (Fell *et al*., 2004; Wirth and Goldain, 2012). The colony shape is supple, smooth,

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wet, and occasionally mucoid. Also, in this context, *Rhodotorula* species are fungi that have quick growth rates, ease of growing on various media, and lack of dietary fastidiousness. Under a microscope, they appear as oval budding or round cells, and pseudohyphae and sometimes a faint capsule form around the cell. Rumen yeasts may not be crucial to rumen fermentation, but from a veterinary perspective, they may be significant as a source of infection (Zened *et al*., 2020). The three domains of life (bacteria, archaea, and eukaryotes), as well as viruses, are all represented in the very dense and diverse population of microbes found in the rumen (Zened *et al*., 2020). Bacteria dominate and perform the majority of the rumen's metabolic tasks ($10^{10}$–$10^{11}$ cells per g of ruminal material), Protozoa ($10^{6}$ cells per g of ruminal fluid), which make up 30%–50% of the rumen's microbial biomass, and fungi are examples of eukaryotes ($10^{5}$ zoospores per g of ruminal content) (Zened *et al*., 2020). *Rhodotorula mucilaginosa* has a probiotic effect and high nutritional value, The cell walls of *R. mucilaginosa* contain glucan and mannan, which can improve neutrophil and macrophage migration and phagocytosis, reduce intestinal inflammation, increase the resistance of animals, encourage the reproduction of helpful bacteria, and inhibit competitively the colonization of the harmful bacteria (Dalmo and Bogwald, 2008). Products made from *R. mucilaginosa* contain lots of carotenoids and zymochromes (Aksu and Eren, 2005). Previous research has demonstrated the advantages of carotenoids for animal and human health (Mannazzu *et al*., 2015). Carotenoids are known as super antioxidants because they regulate cell communication and gene expression, which helps them prevent cancer, block gene mutations, and fend off the impacts of environmental genotoxic chemicals (Dalmo and Bogwald, 2008; Bhagavathy and Sumathi, 2012). These perform various health-related tasks, such as boosting host defenses, exhibiting anti-oxidant and anti-tumor action, and decreasing blood pressure (Sharma and Ghoshal, 2020). Nevertheless, there are not many studies on using *R. mucilaginosa* in raising animals, and its safety hasn't been established yet (Sharma and Ghoshal, 2020). Even though some yeast species may survive in the rumen and enter there with animal food, they are frequently regarded as transient, nonfunctional microorganisms in this ecosystem. The primary rumen environment conditions include pH values between 5.3 and 7.1, a temperature between 38°C and 40°C, a concentration of volatile fatty acids between 39 and 190 mmol $l^{-1}$, and a humidity range between 82% and 90% (Vargas-Bello-Perez *et al*., 2016; Fernandes *et al*., 2019). By modifying the ruminal environment to make it more hospitable for microorganisms, yeast can operate as a growth booster. One of the yeast's crucial functions is oxygen scavenging, which lowers the amount of oxygen in the rumen and increases the survival of other anaerobic microorganisms (Fonty, 2006; Sirisan *et al*., 2013).

In several medical specialties, including dentistry, controlling infections brought on by bacteria and fungi is a significant difficulty. We urgently need to concentrate on finding new approaches to treat rapidly evolving medication resistance and recurrent candidiasis, which cannot be emphasized enough (Seyedmousavi *et al*., 2014). Photoantimicrobial therapy is superior to conventional therapies because it is safe, effective, and simple to use and because it has an activity spectrum that includes bacteria, fungi, viruses, and protozoa. Laser medicine development has produced several new therapy modalities capable of harming pathogenic organisms (Wainwright *et al*., 2017). Studies have shown that using lasers has antifungal and bactericidal effects (Seyedmousavi *et al*., 2014). Many organisms, including fungi and their secondary metabolites, can flourish because light is a crucial element (Kong *et al*., 2019). Only some lines of *R. mucilaginosa* produce capsules and pseudohyphae in the serum; most traces of *R. mucilaginosa* are in a position to make the biofilm in serum at 37°C in polystyrene microplates. However, the dimension of the biofilm after 3 days of incubation is extensively lower than in the contrast traces of *Candida albicans*(Pawl, 2010).

In this study, *R. mucilaginosa* was isolated from the ruminal fluid of cows in the province of Mosul, and the effects of light laser irradiation on the morphological traits and growth of *R. mucilaginosa* were assessed.

## Material and Methods

### *Animal experimental*

Thirteen 5-year-old AL-Restaki and AL-Karadi cows with an average weight of 600 kg were stabled in cubicles in the Cogley region, Mosul province, from December 2022 to February 2023.

### *Isolating, identifying, and characterizing R. mucilaginosa*

Rumen fluid samples from cows were collected 24 hours after food consumption and collected via a ruminal cannula in the ventral rumen sac in hermetically sealed sterile bottles for mycological analysis and immediately transported to the laboratory laser and photonics research center. Since all ruminal fluid samples were obtained from cow nutrition fed consisting of crushed wheat with bran, flour, and straw with a little yellow corn fed. *Rhodotorula mucilaginosa* isolated according to the methodology (Marrero *et al*., 2013; Alhasan *et al*., 2022). Isolation media include Sabouraud dextrose agar, supplemented with chloramphenicol (0.05 g/ml). Sabouraued dextrose broth was placed in each tube as 9 ml, and 1 ml of rumen fluid was added to the first tube and mixed well. Then 1 ml was added to a second tube from the first tube, and so on to obtain a tenfold serial dilution series ($10^{-1}$ to $10^{-7}$). 1 ml was inoculated on Sabouraud dextrose agar from each dilution ($10^{-3}$–$10^{-5}$) and then incubated in the plate at 37°C for 3 days until a colony of *R. mucilaginosa* was obtained. The

culture was examined macroscopically for colony character, growth rate, surface, reverse color, and colony uniformity. Gram staining was first utilized to examine positive fungus colonies microscopically (De Hoog and Guarro, 1996; Begum *et al*., 2020). Identification of *R. mucilaginosa* using the Vitek 2 system (BioMerieux, France) is a fully automated instrument for the identification of fungi.

***Laser application***

An experimental study used the influence of light intensity exposure on the direct vertical for culture of *R. mucilaginosa* by a semiconductor laser system (450 = nm wavelength and 50 mW power laser) under two duration of irradiation conditions vertically at a distance of 10 cm for 30 and 60 minutes (Fig. 1). Each culture irradiation was subculture on Sabouraued dextrose agar incubation at 37°C for 3 days.

***Dry weight of yeast***

Measuring yeast concentration in suspension before and after exposure to laser irradiation was determined as follows. Select colony, inoculate in 40 ml of Sabouraued dextrose broth incubate at 37°C for 2 days (triplicate three independent experiments). After that, centrifuged at 2,000 g for 15 minutes, the supernatant fraction was decanted, the residue was washed with ice-cold water, then centrifuged at 2,000 g for 15 minutes and translated to the final yeast pellet to the culture plate, drying overnight at 35°C. Weigh a new culture plate, record the weight (W1), weigh dry yeast pellet on the culture plate, record weight 2 (W2), and calculate the yeast dry weight W = W2−W1 (Noumia *et al*., 2015).

**Fig. 1.** Laser Irradiated on the direct vertically at a distance of 10 cm for the culture of *R. mucilaginosa* by a semiconductor laser system (450 = nm wavelength and 50 mW power laser).

***Statistical analysis***

The results obtained from this study were entered and statistically analyzed by the social science statistical package version 23 for Windows software. The data are reported as mean ± SEM and were analyzed statistically using the ANOVA test followed by the least significant difference (LSD) multiple comparison test, and values of $p < 0.05$ were considered significant (Field, 2005).

***Ethical approval***

A procedure authorized by the Ethical Council for Animal Research was employed to care for the cows used in this study (No = UM.VET.2022.035).

## Results

In this study, 7/30 (23.3%) isolated *R. mucilaginosa* from the fresh rumen fluid of cows. The results of the isolates of *R. mucilaginosa* exhibited by their colonies on SDA were able to produce carotenoid pigments conferring a salmon-pink to the coral-red color of colonies. Using Gram stain, *R. mucilaginosa* cells appeared Gram-positive buddy yeasts spheroidal to an oval-like without the rudimentary hyphae formation shown in Figure 2. The results obtained by the Vitek YST system for the identification of the total isolated

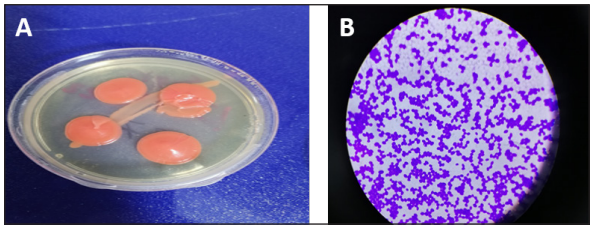


**Fig. 2.** *Rhodotorula mucilaginosa* growth on Sabouraued dextrose agar at 37°C for 3 days, (A) A salmon-pink to the coral-red color of colonies (B) Gram stains- 1,000× (oil immersion), *R. mucilaginosa* cell appeared Gram-positive buddy yeasts spheroidal to oval like.

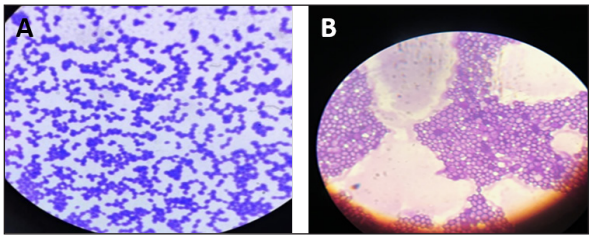


**Fig. 3.** (A) Gram staining of *R. mucilaginosa* before exposure to laser irradiation lights shows spheroidal to oval budding cells. (B) Gram staining of the same culture of *R. mucilaginosa* after exposure to laser irradiation lights, topographical changes of the cell structure occurred, irregular shape of cells and their connection with each other.

**Table 1.** Statistical analysis of the effect of laser on biomass growth *R. mucilaginosa* after exposure to laser irradiation lights 30 and 60 minutes.

| Groups | Time (mean ± SE) | | Mean ± SE |
|---|---|---|---|
| | 30 minutes | 60 minutes | |
| Culture without exposure to laser (before) control group | 0.438 ± 0.108$^{Aa}$ | 0.438 ± 0.108$^{Aa}$ | 0.438 ± 0.073$^{A}$ |
| Culture exposure to laser (after) | 0.600 ± 0.206$^{Aa}$ | 0.597 ± 0.090$^{Aa}$ | 0.628 ± 0.108$^{A}$ |
| Mean ± SE | 0.549 ± 0.116$^{Aa}$ | 0.517 ± 0.071$^{Aa}$ | 0.533 ± 0.067 |
| LSD ($p < 0.05$) | For groups = 0.282, for times = 0.282 interaction = 0.398 | | |

Similar capital letters denote no significant difference vertically at $p < 0.05$, and similar small letters indicate no significant difference horizontally.

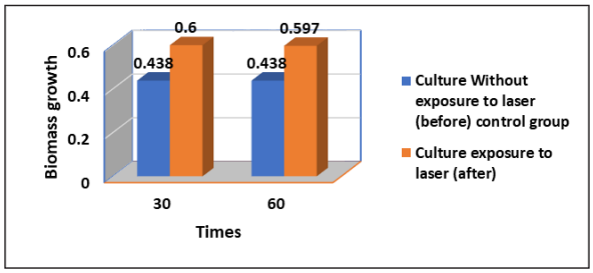


**Fig. 4.** Statistical analysis of biomass concentration of *fungi* under light irradiation conditions 30 and 60 minutes compared with the control group.

*R. mucilaginosa* revealed that the percentage was 7/30 (23.3%).

Examined irradiated culture revealed using Gram staining it was observed the more considerable topographical changes of the cell structure occurred, irregular shape of cells and their connection with each other (Fig. 3). The irradiated culture subculture on sabouraued dextrose agar and incubation at 37°C for 3 days revealed some inhibition growth in 4/7 (57.1%) isolates. The biomass concentration of *R. mucilaginosa* under light irradiation conditions 30 and 60 minutes compared control group showed no significant difference vertically at $p < 0.05$. The results indicated that strong light intensity could inhibit the fungus growth. According to the report from Sakaki *et al*. (2001) (Table 1, Fig. 4).

## Discussion

Yeasts are unicellular fungi that offer high-quality protein and vitamins for feeding rumen microbes and host animals (Paserakung *et al*., 2015). *Rhodotorula* species are widely distributed. It can be found in dairy products, lakes, the ocean, air, and soil. Moreover, it is a non-pathogenic colonizer of mammals, plants, and humans (like shrimps) (Capoor *et al*., 2014). Although there is a wealth of information regarding medical mycology in Iraq, it has never been the subject of any previous bibliographic study (Hussain *et al*., 2021). As a result, we chose to conduct our new research in Iraq because there are no studies on the isolation and identification of *R. mucilaginosa* from the rumen fluid of cows. Seven out of 30 of *R. mucilaginosa* (23.3%) isolates, the colonies it formed were orange-red, and the yield of carotenoids was very high, according to the findings of this study, which were determined by examining the colony's characteristics, each individual's morphology, by using the Vitek 2 system. Formerly regarded as contaminants, *Rhodotorula species* have gradually come to be understood as human diseases over the past two decades. *Rhodotorula* can cause severe and deadly invasive infections, although having less virulence than *Trichosporon* or *Candida* (Merkur and Hodge, 2002; Almeida *et al*., 2008). The proportion of each isolated *R. mucilaginosa* 7/30 (23.3%) in the current investigation was inconsistent with that reported in Brazil by Fernandes *et al*. (2019), who reported the isolation of one species of *Rhodotorula dairenensis* from the rumen of a cow. According to earlier research, feeds typically transfer yeasts into the rumen, and the ruminal microbiota's composition varies depending on the diet and location (Sundset *et al*., 2009; Almeida *et al*., 2012; Henderson *et al*., 2015). *Rhodotorula* is a widespread genus found in various environmental samples, including soil, water, milk, fruit juices, and air samples. This species can assimilate glucose, sucrose, and galactose (Guaman and Carvajal, 2009). These variations in proportions between studies may be due to the different numbers of isolates included in each investigation and the expertise of laboratory researchers. However, various circumstances, including feed sources, roughage to concentrate ratios (R:C), and ruminant species, could lead to the discovery of new forms of yeast (Jimoh *et al*., 2011; Marrero *et al*., 2013).

Given the experimental circumstances of the current work, *R. mucilaginosa* culture exposure to laser light irradiation caused substantial topographical modifications of the cell structure to occur in all isolates, as well as irregular cell shape and connections between individual cells. Four out of seven (57.1%) isolates showed some growth suppression after being subculture on sabouraued dextrose agar and exposed to radiation for 3 days (Kong *et al*., 2019), who concluded

that exposure to light might change the growth traits and metabolite compositions of *R. mucilaginosa,* which suggested that photo-regulatory factors may exist in non-photosynthetic fungi that can manufacture carotenoids. Moreover, controlling the light may be a practical technique to manage the biosynthesis and creation of medicinal components in the pigmented microorganism. The biomass concentration of *R. mucilaginosa* under light irradiation settings 30 and 60 minutes compared to the control group in the current study evolutions revealed no significant difference, which is inconsistent with the study (Kong *et al*., 2019), they reported that, in contrast to the dark control, irradiation with 1,700 l× might stimulate the development and uptake of glucose by *R. mucilaginosa*, while the experiment with 3,500 l× showed some inhibitory effects. Some studies on the impact of laser radiation on bacteria and fungi indicate biostimulants or proliferative outcomes, theorizing that these effects are connected to modifications produced by increased energy intake provided by the radiation in the bacteria's respiratory chain (Kawamoto *et al*., 2000). As a unique application in medical management, laser therapy is a potentially useful treatment for this. Due to the constantly developing usage of laser technology in numerous fields of medicine, laser therapy has become indispensable in medical science.

## Conclusion

This study proved that *R. mucilaginosa* is found in the rumen fluid of cows. Also, the isolated *R. mucilaginosa* displayed sensitivity to laser irradiation lights, revealing the more significant topographical alterations of the cell structure that had happened, the irregular shape of the cells, and how they were connected as a result of evolution. The *R. mucilaginosa* biomass concentration under conditions of light irradiation 30 and 60 minutes comparisons between the control group showed no discernible difference vertically at $p < 0.05$. Although the current study will bring some new material to our knowledge of medical mycology in Iraq, it must be regarded as tentative and in constant need of updating. Our findings imply a thorough investigation to determine the impact of laser irradiation on microorganisms and the mechanisms underlying such effect, which is important to research in the future.

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***Conflict of interest***

The authors declare that there is no conflict of interest.

***Authors' contribution***

Hawraa Faisal: Conception and design of the study, wrote the first draft of the manuscript and design figures. Mumtaz Mati and Karrar Ali: critically revised the manuscript, funding acquisition. Inaam Mohmood: Writing one of the topics and critically revising the manuscript.

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