

Effect of *Saccharomyces cerevisiae* Supplemented with Silver Nanoparticles on *Propionibacterium acnes* In Vitro

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Abstract

Background: Acne is a chronic inflammatory disease of the pilo-sebaceous unit of the follicles in the skin that most commonly affects people throughout their adolescence or teenage years. Around 85% of youngsters globally are affected by this skin condition, according to research. Acne, in contrast, is not only a teenager's problem; in many cases, it lasts far into adulthood. **Objective:** The aim of this study was to investigate how *Saccharomyces cerevisiae* supplemented with silver nanoparticles impacted *Propionibacterium acnes* in Mosul City. **Materials and Methods:** Specimens were obtained from 65 acne patients who attended Mosul's private dermatological clinics with signs of inflamed and discharged pus. The specimens inoculate on blood agar, MacConkey agar, nutrient agar, Muller Hinton agar, and nutrient broth. Then, the effect of *S. cerevisiae* with silver nanoparticles on *P. acnes* was determined. **Results:** The results showed different inhibition with various concentrations of yeast extract against *P. acnes* in the medium, with the 50% concentration showing the highest inhibition diameter at a rate of 18 mm in the bacteria under study, while the inhibition diameter was at an average rate of 18 mm. For each bacterium, the highest rate of inhibition was at a concentration of 40% and a diameter of 15 mm, whereas the lowest rate of inhibition was at a concentration of 30% and a diameter of 12 mm. **Conclusions:** Silver nanoparticles, when coupled with *S. cerevisiae*, can be a very effective antibacterial against to *P. acnes*.

Keywords: Acne, *Propionibacterium*, *Saccharomyces cerevisiae*, silver nanoparticles

INTRODUCTION

Acne is a skin condition that occurs when dead skin cells (keratinocytes) and sebum clog hair follicles. The comedo is the primary lesion of the acne which is either represented by closed comedo (whiteheads) or opened comedo (blackheads) appear on the skin. Acne may affect people of all ages, although it is more common among teenagers. Acne may be effectively treated, even if it persists for a long time.^[1] Blisters and bumps take a long time to heal, and just as one begins to diminish, a new one arises. Acne is caused by a variety of factors, yet the most frequent ones of which are listed as follows: face, forehead, chest, upper back, and shoulders, where the bulk of the sebaceous glands are found, (i) excessive oil secretion (sebum), (ii) blocked hair follicles with oils and dead skin cells, and (iii) acne are more prevalent (sebum). The hair follicles are connected to the sebaceous glands.^[2,3]

Propionibacterium acnes is a gram-positive anaerobic bacterium that has been linked to acne, a prevalent skin ailment among teenagers and young adults. Other disorders, including as blepharitis, can be caused by it. Endophthalmitis, in contrast, is a common complication, particularly following ophthalmic surgery at the eye level.^[4]

These bacteria are considered part of the typical skin bacterial flora present on the surface of healthy human skin in the context of a helpful and complimentary connection; hence they belong to the group of bacteria coexisting with humans. It is also present in the digestive tracts of numerous animals, including humans. *Propionibacterium*

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acnes is a bacterium that lives deep within the pores and sweat glands, well below the skin's surface. It feeds on sebum, cellular detritus, and metabolic wastes from the skin tissues around it.^[5]

The yeast *Saccharomyces cerevisiae* has the ability to stop harmful fungus and bacteria, and can be utilized in the biological control of plant pathogenic fungi as well as in the preservation of food and feed. They've also been utilized as anti-fungal materials or in human therapy, in addition to their medicinal and therapeutic uses.^[6]

Size of silver nanoparticles (AgNps) ranges from one nanometer to one hundred nanometers. Despite being referred to as "Due to a substantial number of silver atoms on the surface, it contains a large fraction of silver oxide. Nanoparticles come in a variety of forms. The most common silver nanoparticles are spherical, although diamond and thin plates are also prevalent." Engineering, biology, physics, chemistry, and biological sciences are all covered by this technology, which is multidisciplinary.^[7]

This study was aimed to study the effect of *S. cerevisiae* with AgNp on *P. acnes*.

MATERIALS AND METHODS

Sample collection

This research was conducted between September 2021 and March 2022. Samples were taken from 65 acne patients who visited Mosul's private dermatological clinics. Patients ranged in age from 12 to 32 years old. The data sheet was filled up entirely using information obtained directly from the patients. Acne pus that was irritated and discharged was used to collect samples. For this aim, bacteriological loops or disposable sterile cotton swabs, as well as transport medium, were utilized.

These swabs or specimens, were immediately sent to the Department of Medical Lab. techniques in the technical institutes of Mosul in Northern Technical University and processed utilizing bacterial cultures, fungal cultures, and isolation and identification of bacteria and fungi. For initial isolation of anaerobes, the samples were inoculated in thioglycolate broth and incubated anaerobically at 37°C overnight.

Blood agar, MacConkey agar, nutrient agar, Muller Hinton agar, and nutrient broth were used to inoculate the specimens, which should be freshly prepared or utilized within 2 weeks after preparation. Pure colonies were isolated using the streaking technique.

Bacterial suspension preparation

Prepared by taking volume of pure colonies and culture them in test tubes containing thioglycolate broth medium with mixing test tubes well to ensure the spread of bacteria

in the nutrient medium, then the degree of growth in the liquid medium was measured with a tube MacFarland standard by making many dilutions up to a concentration (1.5×10), and incubated at 37°C for 2–3 days as shown in Figure 1.

Preparation of yeast extraction

Preparation of yeast extract (*S. cerevisiae*) by dissolving the specified weight of dry yeast type (Pakmaya) in a liter of warm water, adding to it the same weight of sugar 1:1 and leaving for 12 h for the yeast to grow and multiply. In this way, the concentrations used were prepared according to the specified proportions [Figure 2].

Syntheses of silver nanoparticles

AgNps used in the study were obtained from the American company (SkySpring Nanomaterials, Houston, TX, USA). They were added at concentrations of 1%, 2%, 3% and 4% to the medium containing acne-causing bacteria and made a hole in it, then incubated in the incubator at 37°C for 24 h, where the effectiveness of the concentration of the nanomaterial was determined by measuring the area of inhibition around each grove.

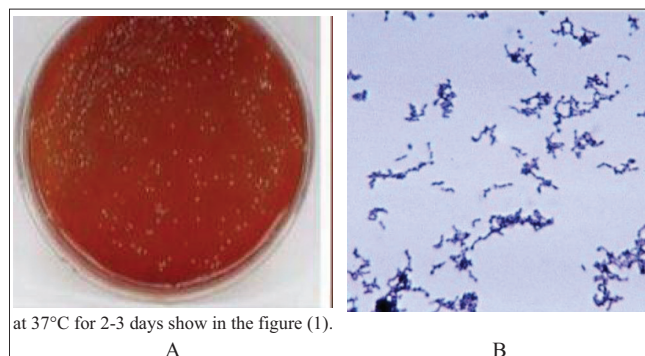


Figure 1: (A and B) bacterial cell which cause acne



Figure 2: Growth *Saccharomyces cerevisiae* on corn male agar

The effect of *Saccharomyces cerevisiae* on the growth of bacterial isolates

The method of digging diffusion was used diffusion well agar to test the sensitivity of bacteria to yeasts, based on what is mentioned in the and as described briefly: inoculate the surface of the blood agar with a sterile swab of bacteria culture containing 1.5×10 cells/mL, then leave the dishes to dry at room temperature for 15 min. Then, drilled 5 mm diameter holes in the medium using a sterile cork borer (Cork pore). Graduated concentrations of yeast were prepared using distilled water in the following proportion (20%, 40%, 80%). An amount of 1.0, 0.2, and 0.4 mL of the above concentrations was added to each hole in sequence and a control hole was made by adding distilled water. The dishes were incubated at 37°C for 24 h. The activity of each concentration of *S. cerevisiae* was determined by measuring the area of inhibition around each hole.

Mixing silver nanoparticles with yeast

Dilutions of yeast were prepared in volume proportions according to using a two-step mixing method. The silver nanoparticles were weighed using a balance (1mg.0.1mg=d (BL210S Sartorius). 0.1 g of silver nanoparticles were mixed manually with yeast, at an amount of 500 g under vacuum and nitrogen gas conditions using the vacuum glove box to prevent any interaction of silver nanoparticles with unwanted particles from the air.

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number 40008 on August 31, 2021 to get this approval.

RESULTS

The results revealed that acne-causing bacteria (*P. acnes*) colonies assumed on many different shades, such as white, yellow, or gray, and that their colonies grew in size over time, with their young colonies being smaller than their older colonies [Figures 1 and 2]. The bacteria were gram-positive and appeared in a variety of shapes, including spherical, rod-shaped, and club-shaped. Their cells were single, paired, grouped, or in the form of short chains, and they were frequently arranged in the shape of a letter Y or a letter V, or in the shape of Chinese characters.

In biochemical tests, the results showed that catalase, wet gelatin, nitrate reduction, and indole were positive in *P. acnes*. The oxidase and urease tests showed out negative, as did the blood type gamma test.

The results of Muller Hinton's yeast extract added to the culture medium by diffusion method in the pits and with various concentrations showed different inhibition

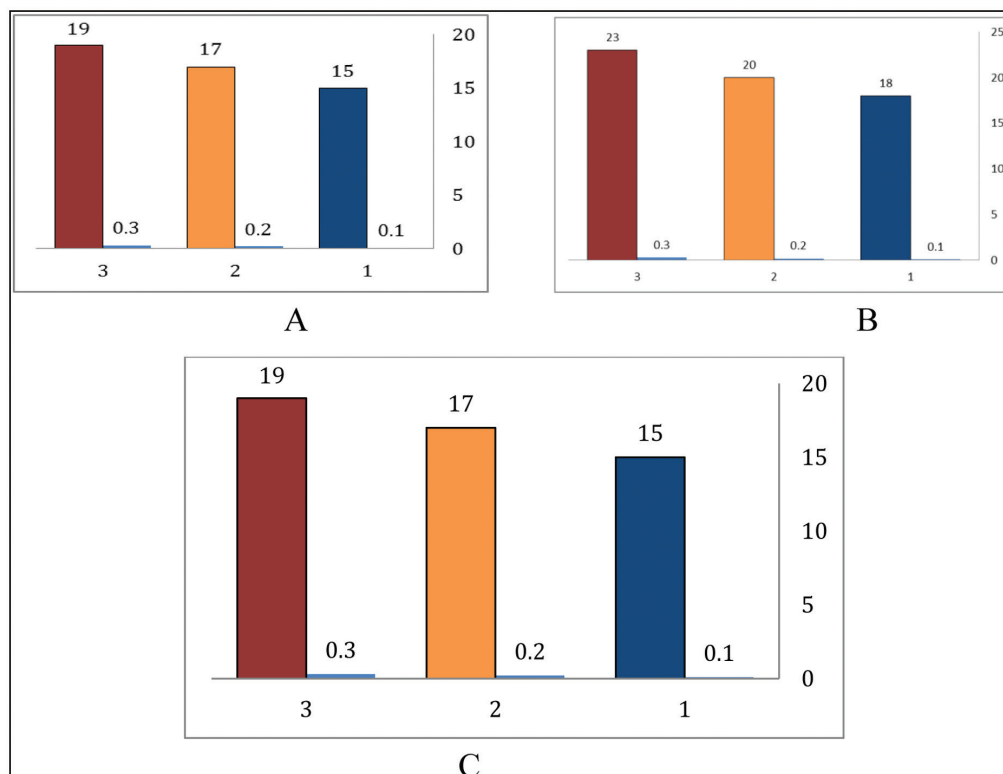


Figure 3: (A–C) Synergistic effect between silver nanoparticles and *Saccharomyces cerevisiae* extract concentrations

Table 1: The effects of AgNps on the growth of *P. acnes*

AgNp Conc. Extract. Conc.	0.1	0.2	0.3
	Inhibition zone		
50%	18	20	23
40%	16	19	20
30%	15	17	19

depending on the concentrations added to the medium, with the 50% concentration showing the highest inhibition diameter at a rate of 18mm in the bacteria under study, whereas the inhibition diameter was at an average rate of 18mm. For each of the bacteria, the highest rate of inhibition was at a concentration of 40% and a diameter of 15mm, while the lowest rate of inhibition was at a concentration of 30% and a diameter of 12mm. 15–30mm, which is attributed to the high proportion of polysaccharides, which enhance osmosis and the absence of a high humidity for bacterial growth, as well as the high acidity, which is also partially responsible for the strong yeast extract's ability to prevent microbial development

Effect of silver nanoparticle concentration on *Propionibacterium acnes*

The results showed that silver nanoparticles, AgNp, were effective in inhibiting acne-causing isolates (*P. acnes*) at concentrations of 1%, 2%, 3%, and 4%; the maximum inhibition rate was achieved at the 4% concentration. When concentrated, the average retarding diameter is 20 mm, and when concentrated by 3%, it is 17 mm [Table 1].

The results showed that combining silver nanoparticles with yeast extract had a clear symbiotic effect in inhibiting acne-causing bacteria that outperformed the percentage of inhibition achieved by the extract or nanoparticles alone, indicating that the nanomaterial was effective in enhancing the extract's inhibitory ability in inhibiting the growth of microorganisms, allowing the extract to be used alone. The AgNps are used as an alternative to antibiotics as they prevent the development of bacteria in a healthier and more efficient manner.

DISCUSSION

As a result, silver-based nanoparticles have established a place in medical (catheters, implants, and prosthesis),^[8] as well as in commercial items (textiles and deodorants).^[9] There have been multiple reports of microbes forming magnetic nanoparticles from their corresponding salts.^[10] Although silver nanoparticles (SNPs) may be generated both intracellularly and extracellularly, the extracellular technique is more favorable owing to environmental control, large-scale synthesis, and simple processing processes.^[11]

We used *S. cerevisiae*, an industrial yeast, to biosynthesize monodispersed and, more importantly, stable AgNps in our investigation. Although this bacterium has previously

been known to produce SNPs, the anti-fungal effects of the produced nanoparticles have yet to be established.^[12] *Saccharomyces cerevisiae* is most known for its involvement in bread, beer, and wine fermentation, both traditional and industrial. It can also be used as a nutritional supplement and a treatment for diarrhea caused by antibiotics. Cases of diagnosed *S. cerevisiae* infections have increased in recent years, possibly due to an increase in the number of immunocompromised individuals and advancements in diagnostic procedures. *Saccharomyces cerevisiae*, on the other hand, has long been thought to be a nonpathogenic and harmless organism.^[13] *Propionibacterium acnes* has been found in human skin wounds in previous studies.^[14] In adult patients with shoulder sepsis and prosthetic hip infections, *P. acnes* and *S. aureus* were shown to be co-isolated.^[15] We suggest that *P. acnes* reaches the dermis when a deep incision is caused by pathogen infection, despite the fact that it predominates on the skin surface and is exposed to ambient oxygen. *Propionibacterium acnes* may be triggered to ferment carbon sources (such as glycerol and glucose) generated naturally in the skin by the anaerobic milieu seen in deep-seated abscesses. *Propionibacterium acnes* got its name from its capacity to ferment carbohydrates into propionic acid, an antibacterial short-chain fatty acids.^[16]

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Conflicts of interest

There are no conflicts of interest.

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