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The Role of Nanotechnology in the Control of Bacterial Contamination in Plant Tissue Culture: A Review

Esraa A. H. Al Samir[1], Intisar Albandar[2], Muntaha J. Kadhim[3] and Shaimaa A. Alsamir[4*]

[1, 2, 4]Department of Biology, College of Science, University of Basrah, Basrah, Iraq
[3] Date Palm Research Center- University of Basrah, Basrah, Iraq
*Corresponding Author: Shaimaa A. Alsamir
ORCID ID: 0000-0002-6528-3676

Abstract

Successful “micro-propagation” requires avoiding microbial contamination of plant tissue cultures at every stage of growth. Bacterial contaminants (both Endophytic and epiphytic organisms) can lead to the loss of propagated plants at every stage of development. Plants infected with bacterial contamination will suffer from impaired rooting and multiplication, and may even die. Plant death results in the loss of plant tissue, which is particularly detrimental for the tissue culture of economically important plants such as potatoes and date palms. Early detection of bacterial contamination is difficult, as it is present within plant tissues, leading to the loss of plant tissue before the contamination is detected and treated. Bacterial contamination can result from various causes, including the use of improper sterilization techniques or the use of improperly sterilized equipment. To produce sterile plant cultures, various criteria must be known, such as the source of contamination and the type of pathogen. These criteria can be minimized, and sterilized plant cultures can be produced using antibiotics or other advanced techniques. It has been found that methods of preventing bacterial contamination using chemical compounds cause harm to both the transferred and growing plant tissues and to workers in plant tissue culture laboratories or fields. Therefore, nanotechnology using various types of nanoparticles has emerged as a promising new solution to this intractable and widespread problem in plant tissue cultures. Nanoparticles may serve as an alternative to antibiotics, particularly when bacteria develop resistance to existing antibiotics.

Keywords: *Plant tissue culture*, *Contamination*, *Bacteria*, *Nanoparticles*, *Nanotechnology*

Introduction

Sources of Bacterial contamination

It is difficult to recognize the sources of contaminated cultures [1]. However, it was evident that bacteria causing contamination in plant tissue cultures may originate from laboratory environments, explants, ineffective sterilization techniques, or operators [2]. Moreover, it was detected that the species of plant and the type of explant can determine bacterial contamination [3]. One of the most important features of bacterial contamination is" decreased shoot proliferation, unstable growth or tissue necrosis" [4,5]. Figure 1 shows an image of a plant contaminated with bacteria that grew as creamy colonies on a nutrient agar plate. Figure 2 illustrates the colonies of three types of bacteria found in contaminated palm tree tissue.

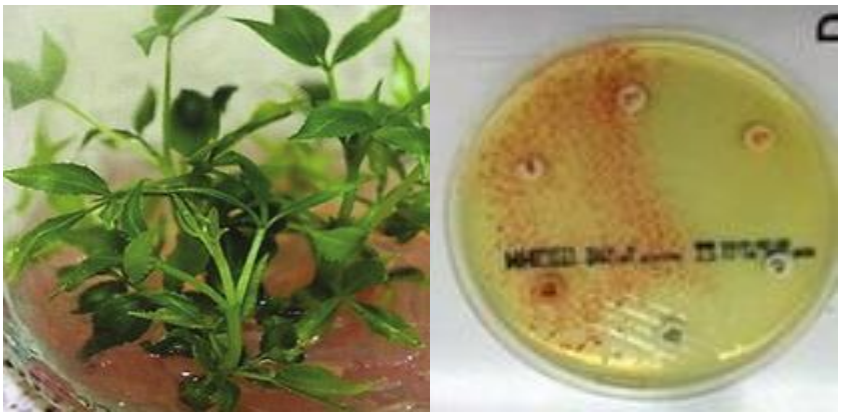

Figure 1. The potato culture is contaminated with endogenous bacterial growth at the base of the explants and the red colonies of contaminated bacteria [6]

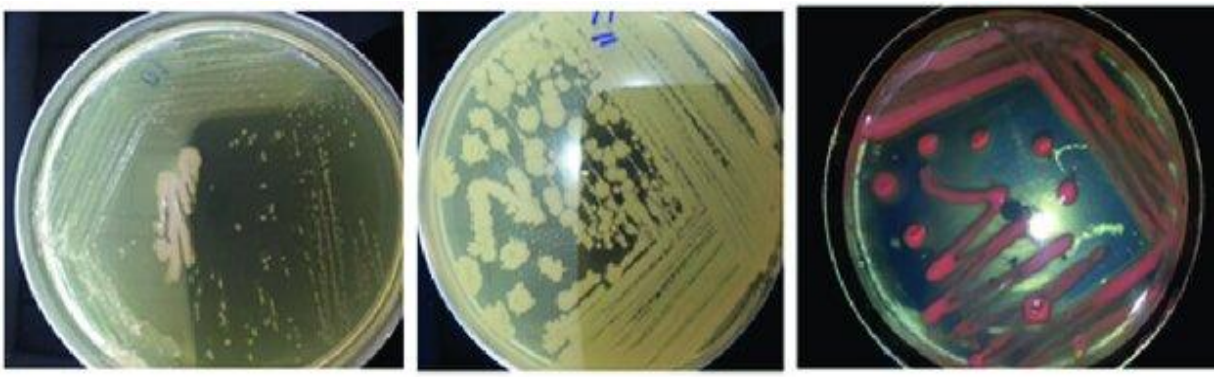

Figure 2. Figure 2. Bacterial colonies isolated from contaminated plant tissue culture, showing different bacterial species [7]

In plants, there are two kinds of bacteria, which are “Epiphytes or Endophytes’’ [8]. Various types of disinfectants can effectively remove epiphytic bacteria, as they are commonly found on the surface of plants [9, 10]. [11] found that “Endophytic bacteria ” didn’t cause any symptoms of disease during a part of the life cycle or the entire life cycle. In contrast, bacteria living inside healthy plants can be hazardous, leading to decreased growth and rooting rates, and can also show resistance to surface disinfectants [3].

Prevention of bacterial contamination

To prevent contamination, it must take into account the importance of each step in plant tissue culture (choosing explant, preparation of media, sub-culturing, plant incubation) [12]. *In vitro* plant cultures, most bacterial contaminations are present in the” culture vessels, laboratory media, and inefficient sterilization of explants, which happens during transmission by thrips and mites or during handling the plant material [13].

The plant contamination can be reduced by maintaining laboratory cleanliness during the subculturing of plant cultures and ensuring the entire plant tissue culture process is conducted aseptically. [12] pointed out that “ bacterial contamination” can be decreased by adjusting the air source and laboratory room cleanliness during subculturing of plant cultures. Furthermore, the populations of epiphytic organisms (stock plants) applied for plant tissue cultures should be grown under non-endangered environments (greenhouses and growth chambers) [12, 14]. [2] emphasized using the watering stock plants with filtered water to reduce bacterial contamination. Moreover, [15] suggested that bacterial characterization is necessary to identify the sources of contamination, determine contamination level from each source, and develop strategies to eradicate or stop contaminants.

Antibiotics as a traditional solution for plant tissue bacterial contamination

In plant tissue culture, bacterial contamination, which is caused by” Endophytic bacteria,” can not be removed by any superficial sterilization methods; therefore, it is a substantial issue that demands antibiotic therapy [6]. The antibiotics used in micropropagation should be non-resistance-inducing, stable, unaffected by pH, soluble, inexpensive, dissolved in the media, bactericidal, highly active, suitable for combination use, and nontoxic to human health [16].

The studies revealed that antibiotics are used to eliminate bacteria when a contaminant is indicated, or they can be used in combination as prophylactics in tissue culture medium [16]. Additionally, the studies revealed that bacterial resistance develops when a single antibiotic is used repeatedly [2, 7]. Antibiotics are classified into four main categories: inhibitors of bacterial cell wall synthesis, those acting by mode of action, DNA replication blockers, and inhibitors of bacterial protein synthesis [16]. Antibiotics can also be grouped by chemical structure into: aminoglycosides, β-lactams, glycopeptides, quinolones, macrolides, lacosamides, polymyxins [17].

Knowing the kinds of bacteria present—whether they are Gram-positive or Gram-negative—is necessary in order to choose an appropriate antibiotic. Therefore, some simple biochemical tests and initial characterization with Gram staining are necessary [18], and no single antibiotic is effective against all bacteria. [17] showed that many kinds of antibiotics are used to treat plant tissue cultures, such as carbenicillin, gentamicin, cephalothin, polymyxin, streptomycin, rifampicin, and timentin have been used, and sometimes combinations of antibiotics may be used, which have positive effects against bacterial contamination.

Figure 3 shows different types of bacterial strains that are isolated from the contaminated plant tissues and tested for sensitivity using five types of antibiotics. The figure shows that different bacterial isolates showed different patterns of sensitivity against these antibiotics, which reflect their ability to affect the plant tissue.

On the proper media, different bacterial strains were isolated from contaminated plant tissues. After purifying the isolates, one colony was cultivated on the nutrient broth medium. Following an overnight incubation period at 37°C, the culture was spread out on Muller-Hinton agar plates, and using sterile forceps, the five different kinds of antibiotic discs were placed on the agar plate's surface. To determine how sensitive the bacterial isolates were to the antibiotics, the plates were then incubated at 37 °C. The diameter of the inhibition zones surrounding the antibiotic discs was then determined.

Figure 3. Antibiotic sensitivity test for bacteria isolated from contaminated plant tissue cultures [17].

Identification and characterization

To avoid bacterial plant tissue contamination, it's necessary to identify bacterial colonies, which provide important information about the sources of contamination [17]. There are numerous methods for isolating and cultivating bacteria. We can purify non-tamable bacteria using standard bacteriological procedures and characterize them through biochemical tests, such as motility, gelatinase, Gram stain, and oxidase [19]. A reliable systematic bacteriology source, which is crucial for characterizing genera and species to identify bacteria, is used in this procedure [20]. These traditional detection techniques need time, and in some cases, bacterial growth can delay micropropagation while

Molecular recognition based on direct deoxyribonucleic acid investigation is subtle enough for most bacterial sets, rendering these time-consuming methods redundant. At the genus level, the most common way for bacterial identification is the 16S ribosomal RNA (rRNA) [21]. The detection of bacteria in plant tissue cultures has been done using this technique for the *Prunus avium* [22], *Cynara scolymus* L (Artichoke) [23], *Vitis vinifera* [24], *Echinacea purpurea* (Echinacea) [25], and in many propagated ornamental plants, Dendranthema, Rosa, Spathiphyllum, Pelargonium, Ficus, and Dahlia [21]. [26, 27] recommend using multiple tests for accurate identification of bacteria in micropropagation.

Nanotechnology as an advanced strategy for plant tissue bacterial contamination

Nanotechnology is essential for improving several facets of plant tissue culture [28]. Recently, nanoparticle applications have auspiciously directed the removal of bacterial contaminants from plant tissue cultures and revealed the useful role in callus proliferation, carnal embryogenesis,

organic tissue development, genetic alteration, and production of secondary metabolites [29-31]. Additionally, nanoparticles function as enhancers to promote the large-scale production of bioactive chemicals, rapid shoot development, and root protraction [32] (Figure 4).

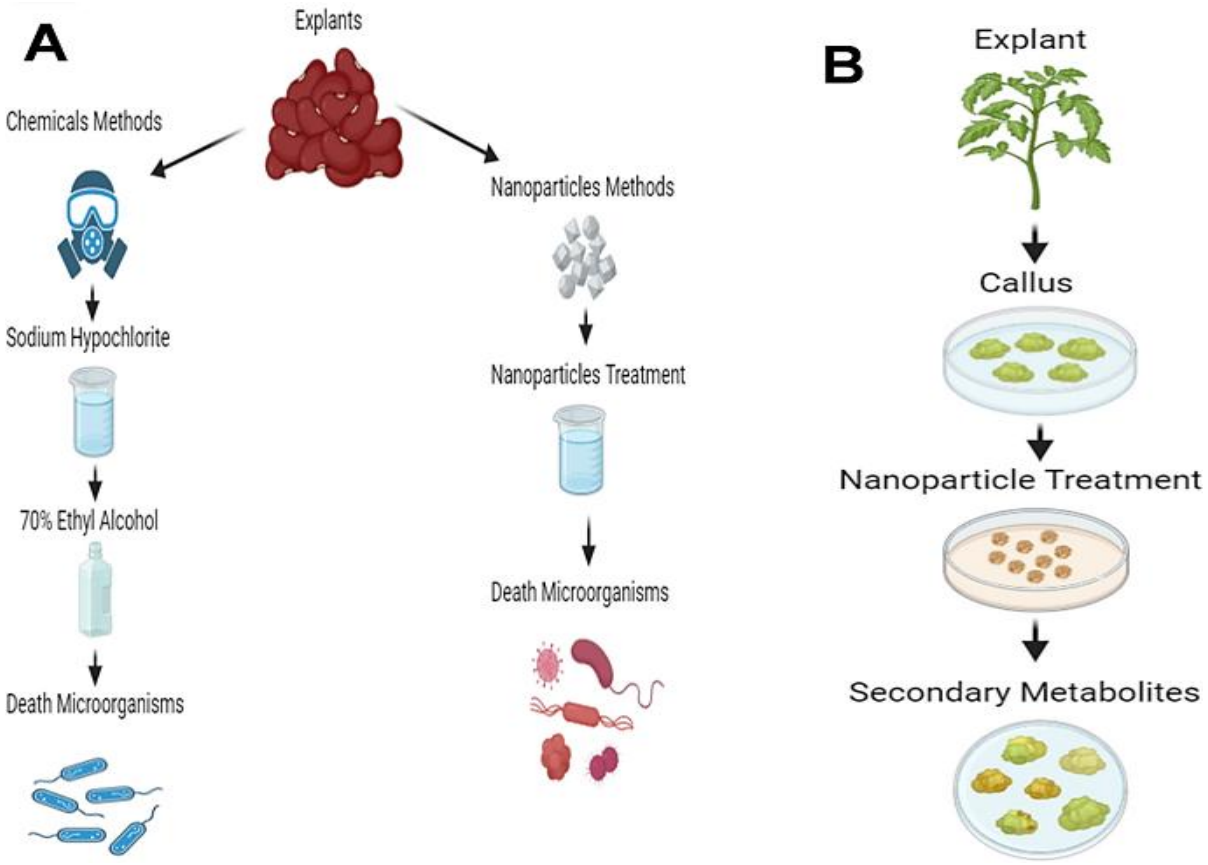

Figure 4. Treatment of explant with different types of nanoparticles. A comparison of two methods of treating bacteria isolated from contaminated plant tissue: chemical treatment and nanoparticle therapy. Figure A illustrates that chemical treatment requires wearing a face mask and employing hazardous chemicals. While nanoparticles, particularly green-manufactured ones, are safe to use and do not require chemicals, B: Applying nanoparticles to the medium with the callus. The use of nanoparticles improved callus development and efficiently generated secondary metabolites [32]

Furthermore, nanoparticles are involved in reducing the oxidative stress in plant cells, thus encouraging the development of more calluses and higher levels of secondary metabolites [33]. The nanoparticle's potential to promote more effective, long-lasting, and

improved technological settlement in in vitro culture is highlighted by its combined presence in plant tissue culture [34]. The connotations of nanoparticles include reducing dependence on natural resources and minimizing environmental impacts, with nanotechnology considered a transformative strategy for sustainable plant tissue biotechnology [35].

Nanoparticles with an average size of 1–100 nm و have had a significant impact on the field of plant tissue culture due to their distinct physical and chemical characteristics. Different types of nanoparticles, such as silver (AgNPs), Gold (AuNPs), Magnesium (MgNPs), Copper (CuNPs), Zinc/ Zinc oxide (ZnNPs/ ZnONPs), and Titanium dioxide (TiO2NPs), have appeared as innovative critical tools in the development of traditional plant tissue culture [36-38]. These nanoparticles are available in various forms, such as small particles, powders, clusters, and tubes, and come in different sizes. The most recognized property of nanoparticles (NPs) is their ability to kill various types of bacteria and microorganisms [39]. They act as antibacterial agents by contributing numerous functionalities, depending on their distinctive physicochemical characteristics [40], and also inhibit browning [41]. Figure 5 illustrates the applications of nanoparticles in plant tissue culture [42]. The figure illustrates how nanoparticles can be used in plant tissue for a variety of purposes, including preventing contamination by either adding nanoparticles to the prepared media of plant tissue before explant transfer or to the cultured explant after it has grown. Additionally, by preserving nutrients, improving nutrient absorption, maintaining moisture in media, and enhancing secondary metabolites, nanoparticles can promote the growth of plant tissue.

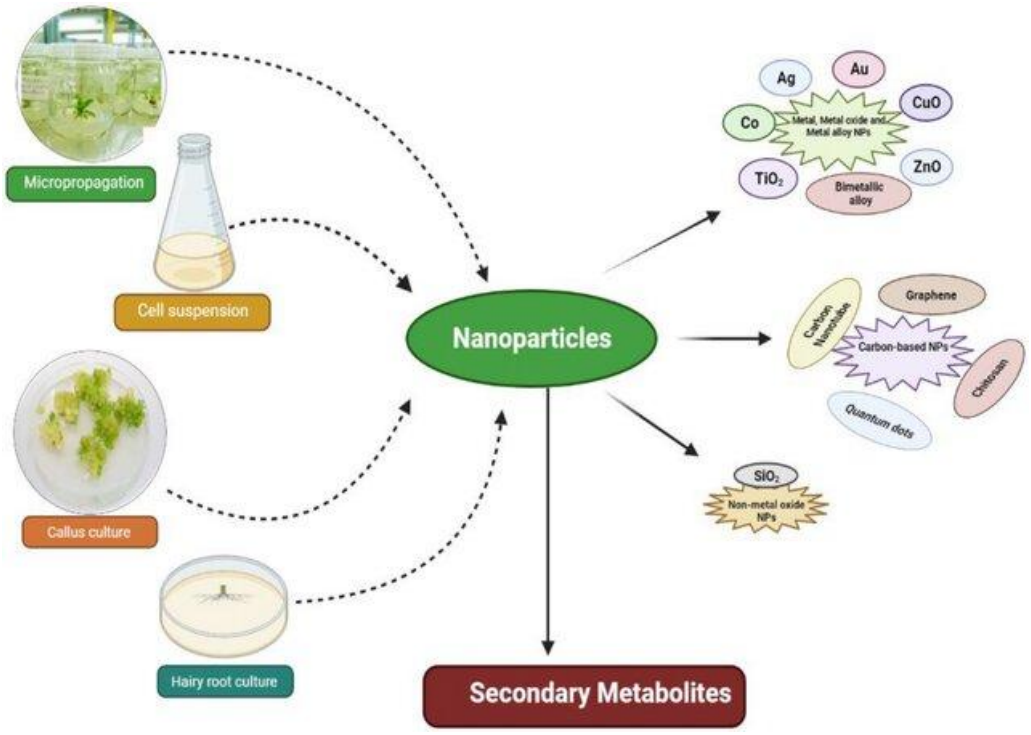

Fig. 5Different applications of Nanoparticles' in plant tissue culture [42]

Silver nanoparticles are commonly used in various fields, including medical and environmental applications [43], and primarily in plant tissue agriculture [44]. AgNPs can be used to disinfect the surface of explants and have been shown to reduce microbial contamination in various plants. Silver NPs' antibacterial properties appear to have been thoroughly investigated [45]. Although AgNPs have a strong capacity to eradicate microorganisms without impairing plants, numerous studies have examined their impact on plant disinfection, revealing both beneficial and detrimental effects on plants [46]. Since plants are a fundamental component of the world and a source of diet, it was suggested that more research must be done on the effects of NPs on explants removed from various plant species to establish an appropriate technique for applying AgNPs in plant disinfection [47]. Therefore, it is crucial to understand how AgNPs affect plants, particularly since their widespread use raises the possibility that they will be discharged into the ecosystem [48-50]. Numerous studies have documented the use of gold nanorods (GNRs) and gold nanostars (GNSs) in tests to eradicate bacteria [51]. Furthermore, ZnO and Cu nanoparticles showed high effectiveness against microorganisms [52, 53]. It was discovered that the nanoparticles kill bacteria

by generating reactive oxygen species (ROS) [54, 55].

The introduced review focused on demonstrating the effect of microorganisms on plant tissue culture and the most commonly used materials to mitigate this effect. Moreover, the review indicated the role of nanotechnology, using different types of nanoparticles, to reduce or eliminate microbes due to their unique properties resulting from a high surface-to-volume ratio. Furthermore, nanoparticles could be used to enhance the survival rate of explants and remove contamination.

Conclusions

Bacterial contamination, in micropropagation, “plant tissue culture” ", is still considered a continuing threat; however, there are many methods for reducing this contamination. First, Laboratories must ensure that all processes are performed in a sterile environment to determine or avoid bacterial contamination. Second, during the culture cycle and at the initiation stage, indexing culture is important because it reduces the number of contaminants that escape detection; furthermore, it uses antibiotics to determine bacterial contamination. However, nanotechnology has advanced to improve this sector of biotechnology by enhancing plant growth, increasing its resistance to insects, and eliminating bacterial contamination that continues to threaten plant tissue culture.

Conflicts of Interest: The authors declare that they have no conflicts of interest.

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دور تقنية النانو في السيطرة على التلوث البكتيري في زراعة الأنسجة النباتية: مراجعة

اسراء عبد الرزاق حميد السامر[1] ،انتصار البندر[2] ، منتهى جواد كاظم[3] ، شيماء عبد الرزاق السامر[4]

[4,2,1] قسم علوم الحياة, كلية العلوم, جامعة البصرة, البصرة , العراق

[3] مركز ابحاث النخيل ، جامعة البصرة، البصرة، العراق

الباحث المراسل: شيماء عبد الرزاق السامر

الباحث المراسل ORCID ID **:** 0000-0002-6528-3676

الخلاصة

تتطلب الزراعة النسيجية الناجحة منع التلوث الميكروبي في مزارع أنسجة النبات في كل مرحلة من مراحل النمو. يمكن أن تؤدي الملوثات البكتيرية (الداخلية والخارجية) إلى فقدان النباتات المتكاثرة, حيث ان النباتات المصابة بالتلوث البكتيري ستعاني من ضعف بعملية التجذير وعملية التضاعف ويمكن ان تموت. ان موت النباتات يتسبب في خسارة النسيج النباتي والذي يعود بالضرر الكبير خاصة في حالة زراعة الانسجة النباتية لنباتات اقتصادية مثل البطاطا وتمر التخيل. وقد لوحظ انه من الصعب التعرف على "التلوث البكتيري" مبكرًا لوجوده داخل أنسجة النبات وبالتالي فقدان الانسجة النباتية قبل اكتشاف التلوث ومعالجته. ان التلوث البكتيري قد ينتج عن اسباب كثيرة منها تقنيات التعقيم الرديئة أو استخدام المعدات غير المعقمة بشكل جيد. لإنتاج مزرعة نباتية معقمة، يجب معرفة معايير مختلفة، مثل مصدر التلوث ونوع مسببات الأمراض، لتقليل تلوث وإنتاج مزرعة نباتية معقمة باستخدام المضادات الحيوية أو غيرها من التقنيات المتقدمة. وقد وجد ان طرق منع التلوث البكتيري باستخدام المركبات الكيمياوية يتسبب بأضرار على كل من الانسجة النباتية المنقولة والنامية والعاملين في مختبرات او حقول زراعة الانسجة النباتية. لذلك ظهرت تقنية النانو باستخدام انواع مختلفة من الجسيمات النانوية كحل واعد جديد لهذه المشكلة المستعصية والمنتشرة في مزارع الانسجة النباتية. وقد تستخدم الجسيمات النانوية كبديل للمضادات الحيوية خاصة عندما تتطور البكتيريا المقاومة للمضادات الحيوية المتوفرة.