Review Form 3

Journal Name:	**Journal of Advances in Biology & Biotechnology**
Manuscript Number:	**Ms_JABB_122814**
Title of the Manuscript:	**Biosynthesis and Characterization of Zinc Nanoparticles Using Pseudomonas and Actinobacteria**
Type of the Article	**Research Article**

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PART 1: Review Comments

Compulsory REVISION comments	**Reviewer's comment**	**Author's Feedback** *(Please correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)*
Please write a few sentences regarding the importance of this manuscript for the scientific community. Why do you like (or dislike) this manuscript? A minimum of 3-4 sentences may be required for this part.	**I salute the authors for their efforts in conducting this research. In this paper the Authors studied the Biosynthesis and characterization of zinc nanoparticles using strains of Pseudomonas and Actinobacteria. The article is acceptable one but will be better if the comments raised below can be addressed. There are a lot of mistakes, most of which are linked to the writing of certain scientific terms and the use of abbreviations that have been misused. In addition, I have several queries regarding this work. Comments raised are done using the page and the title or subtitle line number.**	
Is the title of the article suitable? (If not please suggest an alternative title)	**Title to rephrase: Add the word Strains Of to the title. The title becomes: Biosynthesis and Characterization of Zinc Nanoparticles Using Strains Of Pseudomonas and Actinobacteria.**	
Is the abstract of the article comprehensive? Do you suggest the addition (or deletion) of some points in this section? Please write your suggestions here.	Corrections to be made -Page 1, line 3 of the abstract: Add the abbreviation (ZnNPs) for zinc nanoparticles -Page 1, line 3 of the abstract: … Pseudomonas and actinobacteria... Should be … Pseudomonas and Actinobacteria... --Page 1, line 3 of the abstract: Replace .. Collection and screening of Pseudomonas and actinobacterial isolates.. with … Collection and screening of Pseudomonas and Actinobacterial isolates … -Page 1, line 6 of the abstract: Replace .. (EDX), ..with ..energy-dispersive X-ray spectroscopy (EDS) -Page 1, line 7-8 of the abstract: Replace .. The zinc nanoparticles (ZnNPs) synthesized through AUDP209 (Pseudomonas) and AUDT636 (actinobacteria)… with … The ZnNPs synthesized through Pseudomonas (AUDP209) and Actinobacteria (AUDT636) isolates… -Page 1, line 9-10 of the abstract: Replace … SEM images revealed the spherical shape of the biosynthesized zinc nanoparticles (ZnNPs),…with… SEM images revealed the spherical shape of the biosynthesized ZnNPs,… -Page 1, line 11 of the abstract: of the zinc nanoparticles should be of the ZnNPs -Page 1, line 11-12-13 of the abstract: In the last three lines of the abstract we do not give the absorbances in the IR but we give the conclusions drawn from the FTIR analysis ZnNPs biosynthesized through AUDP209 showed the absorption peaks at 2360 cm-1, similarly, biosynthesized ZnNPs using AUDT636 showed the absorption peaks at 1581 cm-1, 1419 cm-1, 1014 cm-1, 887 cm-1, 771 cm-1, 675 cm-1, 648 cm-1. Should be FTIR spectroscopy confirmed the organic compounds present in the microbial extracts responsible for the capping and stabilizing of the biosynthesized ZnNPs. Keywords: -Page 1, line 14: Replace Biosynthesis, Nanoparticles, Nanotechnology, Zinc with Biosynthesis, Zn-Nanoparticles, Pseudomonas and Actinobacteria, Characterization	
Are subsections and structure of the manuscript appropriate?	**The manuscript is well structured but there is an error in the numbering of the titles in the Materials and Methods section. Please see the Optional/General comments section.**	

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Please write a few sentences regarding the scientific correctness of this manuscript. Why do you think that this manuscript is scientifically robust and technically sound? A minimum of 3-4 sentences may be required for this part.	**Introduction** **•The introduction of the manuscript was well written and effectively highlighted the use of nanoparticle biosynthesis, but the choice of zinc was not justified.** **Materials and methods** **• Most of the methods need the references to support** **• Provide details of the microbiological strains used, their origin and how they were isolated** **•Provide details of the equipment used (e.g. incubator-shaker?)** **•Provide details of the products and reagents used (e.g. zinc acetate dihydrate?)** **•Specify the concentrations of the solutions used** **Results and discussion** **•More references must be used to discuss the obtained results** **•Why the choice of these two microbial strains? we do not find an answer in the results and discussion section!** **•The characterization was more or less well done, but the choice of methods was not well justified.** **•Can the authors comment on the bioactive molecules present in microbial extracts of actinobacteria and Pseudomonas responsible for the reduction of Zn ions and acting both as covering and stabilizing agents for the synthesis of Zn nanoparticles and suggest future experiments to demonstrate these molecules?** **Conclusion** **The conclusion is well written but needs some corrections** **Figure Legends** **The same title is repeated in all the figures (eight in number) except for the name of the technique, which changes. The authors should make an effort to find more appropriate titles.**	
Are the references sufficient and recent? If you have suggestions of additional references, please mention them in the review form. **-**	**References** **The references are recent but must be (in the text or in the references section) according to the review form.** **The used references are insufficient. The authors summarize their main findings, but only relate them to a limited number of previously published studies.**	

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Minor REVISION comments **Is the language/English quality of the article suitable for scholarly communications?**	The style and quality of the English language of this article is adapted to scientific communication	
Optional/General comments	**Comments raised are done using the page and the title or subtitle line number.** **Introduction** -Page 1, line 1: Write … Nanotechnologies can contribute to a new technological revolution in agriculture … instead of …. Nanotechnology may help bring about a new technological revolution in agriculture. -Page 1, line 6: Replace… release of nanoparticles…with… release of nanoparticles (NPs)… -Page 1, line 6: Replace… by nanoparticles entering …with… by NPs entering … -Page 1, line 8: Write via in italic -Page 2, line 6: Replace… nanoparticles …with… NPs … -Page 2, line 9-11: From the sentence below, remove the word viruses and add at least one reference . Microorganisms (fungi, viruses, bacteria, yeast, and actinomycetes) can be thought of as biological nanofactories since they can take in metal ions from the environment and change them into elemental compounds. -Page 2, line 14-15: From the sentence below, remove the double quote marks and write biosynthesis without capital letter and replace EDX with EDS “Biosynthesis of zinc nanoparticles using two different microbial strains and characterized through UV-Vis Spectroscopy, PSA, SEM, EDX, XRD and FTIR”. **Materials and methods** • Most of the methods need the references to support • Provide details of the microbiological strains used, their origin and how they were isolated •Provide details of the equipment used (e.g. incubator-shaker?) •Provide details of the products and reagents used (e.g. zinc acetate dihydrate?) •Specify the concentrations of the solutions used -Page 2, subtitle **2.1**: Write .. Biosynthesis and characterization of ZnNPs.. instead of .. Biosynthesis and characterization of zinc nanoparticles 2.1.1 Biosynthesis of zinc nanoparticles using Pseudomonas strain AUDP209 -Page 2, subtitle **2.1.1:** Write .. Biosynthesis of ZnNPs using *Pseudomonas* strain AUDP209..instead of.. Biosynthesis of zinc nanoparticles using *Pseudomonas* strain AUDP209 -Page 2, subtitle 2.1.1, line 2-3: Replace .. set at 30 °C and 150 rpm, for a period of two days to facilitate growth.. with .. set at 30°C and 150 rpm for two days to allow bacterial growth. -Page 2, subtitle 2.1.1, line 3-4: Write… At the end of each incubation period, the liquid medium was centrifuged at 10,000 rpm for 15 minutes to remove cell debris…instead of… The end of each incubation period, the liquid medium was centrifugation at 10,000 rpm for 15 minutes to remove the cell debris. Page 2, subtitle 2.1.1, line 4: Specify the concentration of a stock solution of zinc acetate dihydrate -Page 2, subtitle 2.1.1, line 6: Write … 30°C.. instead of 30 °C -Page 2, subtitle 2.1.1, line 6-7: Replace .. by adding 1 per cent NaOH... with .. by adding NaOH 1%... and specify the type of percentage -Page 2, subtitle **2.1.2:** Write .. Biosynthesis of ZnNPs using Actinobacterial strain AUDT636..instead of.. Biosynthesis of of zinc nanoparticles using actinobacterial strain AUDT636 -Page 3, subtitle **2.1.2**, line 2-3: Replace .. set at 30 °C and 150 rpm, for a period of seven days to facilitate growth.. with .. set at 30°C and 150 rpm for seven days to allow bacterial growth.	

	-Page 3, subtitle **2.1.2**, line 3-4: Write… At the end of each incubation period, the liquid medium was centrifuged at 10,000 rpm for 15 minutes to remove cell debris…instead of… The end of each incubation period, the liquid medium was centrifugation at 10,000 rpm for 15 minutes to remove the cell debris. -Page 3, subtitle **2.1.2**, line 4: Specify the concentration of a stock solution of zinc acetate dihydrate -Page 3, subtitle **2.1.2**, line 6: Write … 30°C.. instead of 30 °C -Page 3, subtitle **2.1.2**, line 6-7: Replace .. by adding 1 per cent NaOH... with .. by adding NaOH 1%... and specify the type of percentage -Page 3, subtitle **2.2.3**, line 6-7: Replace ..energy-dispersive X-ray spectroscopy (EDX)...with.. energy-dispersive X-ray spectroscopy (EDS) -Page 4, subtitle **2.2.3**, line 2: Write … range of 5-10 kV. …instead of… range of 5-10 kV.The -Page 4, subtitle **2.2.5**: Replace subtitle **2.2.5 X-ray diffraction** ..with.. **2.2.4 X-ray diffraction** -Page 4, subtitle **2.2.5**: Write … After reduction, the ZnNPs powder was subjected to X-ray diffraction (XRD) analysis to examine its crystal structure. …instead of… The ZnNPs powder, after reduction, exposed to X-ray diffraction (XRD) analysis to examine its crystalline structure. -Page 4, subtitle **2.2.6**: Replace subtitle **2.2.6 Fourier transform infrared spectroscopy**..with.. **2.2.5 Fourier transform infrared spectroscopy** -Page 4, subtitle **2.2.6,** line 1-2: Replace .. The biosynthesized zinc nanoparticles powder was utilized for FTIR analysis using an FTIR spectrometer.... with .. The biosynthesized ZnNPs powder was utilized for FTIR analysis using a Bruker TENSOR 27 FTIR spectrometer -Page 4, subtitle **2.2.6,** line 3-4: The Bruker TENSOR 27 spectrometer was used for this analysis, with a measuring wavelength ranging from 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1}. ..should be.. The spectra are recorded between 4000 and 400 cm^{-1} with a resolution of 4 cm^{-1}. **Results and discussion** The authors summarize their main findings, but only relate them to a limited number of previously published studies. •More references must be used to discuss the obtained results •Why the choice of these two microbial strains? we do not find an answer in the results and discussion section! •The characterization was more or less well done, but the choice of methods was not well justified. •Can the authors comment on the bioactive molecules present in microbial extracts of actinobacteria and Pseudomonas responsible for the reduction of Zn ions and acting both as covering and stabilizing agents for the synthesis of Zn nanoparticles and suggest future experiments to demonstrate these molecules? -Page 4, subtitle **3.1**: **3.1 Biosynthesis of zinc nanoparticles using microbial extracts**..should be.. **3.1 Biosynthesis of ZnNPs using microbial extracts** -Page 5, subtitle **3.1**, line 1: The ZnNPs instead of The zinc nanoparticles -Page 5, subtitle **3.1.2**: Size 10, not 11 -Page 5, subtitle **3.1.2**: In this subtitle Actinobacterial instead of actinobacterial -Page 5, subtitle **3.1.2**, line 2-4: Replace.. The reduction was confirmed visually by monitoring the change in colour from colourless to milky white colour using actinobacteria as reported by Sanjivkumar et al., 2022) (Fig 2). ..with.. The reduction was confirmed visually by monitoring the change in colour from colourless to milky white colour (Fig 2) using Actinobacteria as reported by Sanjivkumar et al. (2022). -Page 5, subtitle **3.2**: In this subtitle ZnNPs instead of zinc nanoparticles -Page 5, subtitle **3.2.1**, line 2: of NPs. instead of nanoparticles (NPs). -Page 5, subtitle **3.2.1**, line 3-4:	

	where NPs were biosynthesized instead of where zinc nanoparticles were biosynthesized -Page 5, subtitle **3.2.1**, line 5: NPs instead of zinc nanoparticles -Page 5, subtitle **3.2.2**, line 2: The DLS method instead of The dynamic light scattering method -Page 5, subtitle **3.2.2**, line 4: caused by Brownian motion instead of caused by brownian motion -Page 5, subtitle **3.2.2**, line 6-7: Replace ZnO-NPs were synthesized using the *Pseudomonas* strain, found that the average diameter of biosynthesized ZnO-NPs was 48 nm (Eltarahony et al., 2018)...with..Eltarahony et al. (2018) state that ZnO-NPs synthesised using the *Pseudomonas* strain have an average diameter of 48 nm. -Page 5, subtitle **3.2.2**, line 8: by DLS instead of by dynamic light scattering -Page 6, subtitle **3.2.3**, line 1: Write ..The surface morphology of NPs is determined using a SEM,..istead of.. The surface morphology of nanoparticles is determined using a scanning electron microscope, -Page 6, subtitle **3.2.3**, line 2: EDS isntead of energy dispersive X-Ray Spectroscopy -Page 6, subtitle **3.2.3**, line 2-4: In the present experiment, ZnNPs biosynthesised using AUDP209 and AUDT636 were characterised by SEM and EDS. ..instead of.. In the present experiment, characterization of biosynthesized ZnNPs using AUDP209 and AUDT636 by SEM and EDX. -Page 6, subtitle **3.2.3**, line 4: and EDS..instead of.. and EDX -Page 6, subtitle **3.2.3**, line 4: According to Rajivgandhi *et al.* (2018), ZnNPs biosynthesised from actinomycetes have an irregular shape and a percentage elemental weight of 81.24% for zinc and 18.76% for oxygen. ..instead of.. Rajivgandhi *et al.* (2018), the zinc nanoparticles biosynthesized from actinomycetes have irregular shape and an elemental weight percentage was 81.24% for zinc and 18.76% for oxygen. -Page 6, subtitle **3.2.3**, line 9: Write Actinomycetes. ..instead of.. actinomycetes, -Page 6, subtitle **3.2.3**, line 11-12: Write ..Jayabalan *et al.* (2019) used *Pseudomonas putida* for the biosynthesis of spherical zinc nanoparticles. ..instead of.. Jayabalan *et al*. (2019), *Pseudomonas putida* was used in the biosynthesis of spherical shape zinc nanoparticles. -Page 6, subtitle **3.2.4**, line 4: (103) instead of 103) -Page 6, subtitle **3.2.4**, line 5: respectively. instead of respectively, -Page 6, subtitle **3.2.4**, line 8: (103) instead of 103) -Page 6, subtitle **3.2.4**, line 10: XRD peaks instead of X-Ray Diffraction peaks -Page 6, subtitle **3.2.4**, line 11: plane of (100), (002), (101), (102), (110), [103) and (112) instead of plane of [100], [002], [101], [102], [110], [103] and [112] -Page 6, subtitle **3.2.4**, line 13: have an important role instead of have a important role -Page 7, subtitle **3.2.5**, line 2: fourier-transform infrared spectroscopy should be FTIR analysis -Page 7, subtitle **3.2.5**, line 3: . However, ..instead of.. , similarly, -Page 7, subtitle **3.2.5**, line 4-5: , 675 cm^{-1}, 648 cm^{-1} (Fig 8). ..Should be.. , 675 cm^{-1} and 648 cm^{-1} (Fig 8). -Page 7, subtitle **3.2.5**, line 10: The peaks in the range from 900 to 400 cm^{-1} ..instead of..The peaks in the range from 400 to 900 cm^{-1} -Page 7, subtitle **3.2.5**, line 13: The peak observed at 2360.20 cm^{-1}, 2341.12 cm^{-1} and 2067 cm^{-1} ..instead of.. The peak observed at 2360.20,	

	2341.12 and 2067 cm^{-1} -Page 7, subtitle **3.2.5**, line 14: The FTIR ..instead of.. The FT-IR -Page 7, subtitle **3.2.5**, line 16: this result ..instead of.. this results -Page 7, subtitle **3.2.5**, line 17: Actinobacteria ..instead of.. actinobacteria -Page 7, subtitle **3.2.5**, line 19: Nps ..instead of.. nanoparticles -Page 7, subtitle **3.2.5**, line 22: The FTIR spectra ..instead of.. The FT-IR spectra -Page 7, title **4.**, line 1: Nps ..instead of.. nanoparticles -Page 7, title **4.**, line 2: Actinobacteria ..instead of.. actinobacteria -Page 8, title **4.**, line 1: From the sentence below, remove the words ..and iron.. and abbreviate zinc nanoparticles as ZnNPs for synthesis of zinc and iron nanoparticles. -Page 8, title **3.**, line 2: Replace.. in the colour of the reactant mixture, later the synthesized samples..with.. ..in the colour of the reactant mixture. Later, the synthesized samples.. -Page 8, title **3.**, line 4: Actinobacteria ..instead of.. actinobacteria -Page 8, title **3.**, line 5: Respectively. Particle size analyser ..instead of.. respectively, Particle size analyser -Page 8, title **3.**, line 5: the ZnNPs..instead of.. the zinc nanoparticles -Page 8, title **3.**, line 6: Actinobacteria strain AUDT636, to be 48.7 nm and 54.9 nm, respectively. ..instead of.. actinobacteria strain AUDT636, to be 48.7 nm and 54.9 nm, respectively, **Conclusion** -Page 8, title **4.**, line 7-9: Replace.. SEM analysis showed spherical morphology and EDX showed elemental weight percentage of zinc was 70.80 per cent and 58.20 per cent, respectively and oxygen was 21.86 per cent and 25.19 per cent, respectively for ZnNPs were biosynthesized from AUDP209 and AUDT636, With.. SEM analysis revealed a spherical morphology of ZnNPs biosynthesised from AUDP209 and AUDT636. In addition, EDS showed that the elemental weight percentage of zinc was 70.80% and 58.20%, respectively, and that of oxygen was 21.86% and 25.19%, respectively. -Page 8, title **4.**, line 9-13: Replace.. The sharp peaks obtained in the XRD pattern of zinc nanoparticles biosynthesized through Pseudomonas strain AUDP209 and actinobacteria strain AUDT636 revealed the crystalline nature of the synthesized samples and FTIR spectroscopy confirmed the organic compounds present in the microbial extracts responsible for the capping and stabilizing of the biosynthesized zinc nanoparticles. With.. The sharp peaks obtained in the XRD diagram of the ZnNPs revealed the crystalline nature of these NPs. Finally, FTIR spectroscopy confirmed the organic compounds present in the microbial extracts responsible for the encapsulation and stabilisation of these biosynthesised ZnNPs. **Figure Legends** zinc nanoparticles which must be written in abbreviated ZnNPs form and actinobacteria with capital A to preserve the homogeneity of the manuscript	

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PART 2:

	Reviewer's comment	**Author's comment** *(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)*
Are there ethical issues in this manuscript?	*(If yes, Kindly please write down the ethical issues here in details)*	

Reviewer Details:

Name:	**Benkhaled Abderrahim**
Department, University & Country	**University of M'sila, Algeria**