Detection of plant-synthesized nanoparticles and their antibacterial capacity

Salem W. and Schild S.
University of Graz, Institute of Molecular Biosciences, BioTechMed-Graz, Austria

- **Nanoparticles are potential cost-effective antibiotics**
- **Detect nanoparticles and their antibiotic capacity using one instrument**
- **Acquire absorbance spectra that identify nanoparticles due to plasmon surface resonance**

Introduction

Metallic nanoparticles became subject of intensive research because of their potential antibiotic properties. Nanoparticles such as silver, gold or zinc oxide particles are easily and cost-effectively synthesized by blending metal salts with plant extracts that reduce the metal. However, the resulting nanoparticles differ in size and antimicrobial capacity depending on the plant extract used. Hence, different extracts, varying in the plant or the part of the plant used for the extract, are currently investigated in regard to their capacity to form nanoparticles and their antimicrobial efficacy. The formation of nanoparticles can be verified by UV-Vis spectroscopy due to surface plasmon resonance of the particles that lead to a characteristic spectrum defined by the underlying metal and particle size. Subsequent analysis of nanoparticles on microbial growth is typically tested by methods based on absorbance changes. Here, we present how the spectrometer-based BMG instruments are used to quickly confirm Ag and ZnO nanoparticle formation and their inhibitory effect on the diarrhea-causing bacteria *Vibrio cholerae* and enterotoxic *Escherichia coli* (ETEC).

Materials & Methods

Nanoparticle formation

- Plant extract (from fruits and leaves of *Calotropis procera*)
- $Zn(NO_3)_2$ and $AgNO_3$ (Sigma-Aldrich)
- LB (lysogeny broth) growth medium
- Disposable UV-Vis cuvettes (Sarstedt Article 742)

Ag and ZnO nanoparticles were synthesized by adding $AgNO_3$ or $Zn(NO_3)_2$ to aqueous extracts of plants or leaves of the bush C. procera and subsequent heating and stirring of the mixtures. Generation of particles was verified upon washing by UV-Vis spectrum analysis.

Biofilm assay

- Microplates (96 wells, clear, U-bottom, Sterilin™)
- Microbial strains (*V. cholerae* and ETEC)
- 0.1 % crystal violet (Carl Roth T123.1)

A potential inhibitory effect of the nanoparticles on biofilm generation was determined by growing the bacteria in microplates, addition of nanoparticles, thorough washing and crystal violet-staining of the biofilm. Upon solubilization with 96 % EtOH, absorbance was measured at 595 nm.

Instrument settings

Nanoparticle detection	Biofilm assay
SPECTROstar® *Nano*	
22 flashes	
Absorbance spectrum 220-800 nm	Absorbance at 595 nm
Scan resolution 1 nm	

Results & Discussion

The formation of Ag and ZnO nanoparticles that were synthesized with the help of fruit or leave plant extracts was verified by UV-Vis spectrometry. Silver nanoparticles showed a characteristic absorbance spectrum with a peak around 340 nm, which was expected based on material and size (diameter of 100-150 nm) of the particle. Generation of zinc oxide nanoparticles was verified by a characteristic absorption spectrum with a peak around 370 nm. The phyto-synthesis approach yielded different nanoparticle concentrations with fruit and leave extracts. Broadly speaking, the leave extract resulted in a higher nanoparticle concentration than the fruit extract and the difference was displayed in a higher absorption for leave-extract-synthesized nanoparticles.

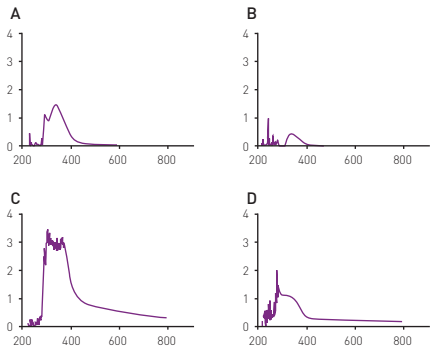

Fig. 1: UV-vis spectra of plant-synthesized silver (A, B) and zinc oxide (C, D) nanoparticles. Nanoparticles were synthesized using leave (A, C) or fruit extract (B, D) from *C. procera*.

Having proven the formation of nanoparticles, they were tested regarding their ability to impair biofilm formation of the diarrhea-causing bacteria *Vibrae cholerae* and enterotoxic *Escherichia coli*.

Staining the biofilm with crystal violet and solubilizing it with ethanol results in a homogenous absorbance of

the chromophore which is proportional to the biofilm. Reading the assay on a SPECTROstar ^{Nano} revealed that silver nanoparticles synthesized with leave extract inhibit *V. cholera* and ETEC-driven biofilm formation (Fig 2.). Furthermore, zinc oxide nanoparticles inhibit ETEC driven biofilm formation irrespective of the extract (fruit or leave) used for synthesis (Fig 2).

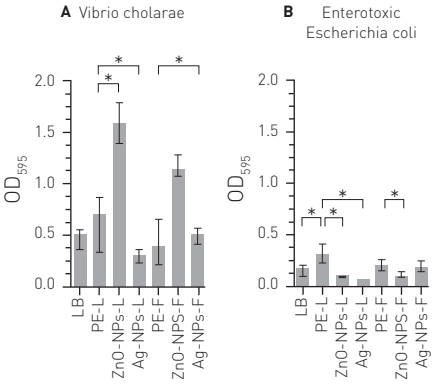

Fig. 2: Impact of nanoparticles on biofilms of V. cholerae and ETEC. Addition of LB broth (LB), plant extracts from leaves (PE-L) or fruits (PE-F) served as a control. Biofilm formation was quantified by crystal violet staining and subsequent determination of the OD595. Shown are the medians from at least eight independent measurements. The error bars indicate the interquartile range. Significant differences between the data sets are marked by asterisks ($P < 0.05$; Kruskal–Wallis test and post hoc Dunn's multiplecomparisons).

Conclusion

Antibacterial nanoparticles can cost-effectively be synthesized by reducing metal salts with the help of plant extracts making it attractive for treating drinking water. Verification of nanoparticle formation as well as its effect on microbial growth, in particular on biofilm formation, is reliably measured by the SPECTROstar ^{Nano} absorbance reader. The spectrometer-based instrument acquires absorbance spectra in less than a second, making it the ideal instrument for quickly detecting nanoparticles by UV-Vis spectra.

References

1. Salem, W., Leitner, D.R., Zingl, F.G., Schratter, G., Prassl, R., Goessler, W., Reidl, J., Schild, S. (2015) Antibacterial activity of silver and zinc nanoparticles against Vibrio cholerae and enterotoxic Escherichia coli. *Int. J. Med. Microbiol.* **305**:85–95