Cite this as: Xiao-yu GAO, Xing-cheng LIAO, Ruo-lai WU, Ting LIU, Hai-xing WANG, Ling-li LU, 2017. Purified isolation of vacuoles from *Sedum alfredii* leaf-derived protoplasts. *Journal of Zhejiang University-Science B (Biomedicine & Biotechnology)*, 18(1):85-88.
http://dx.doi.org/10.1631/jzus.B1600138

Purified isolation of vacuoles from *Sedum alfredii* leaf-derived protoplasts

Key words: Hyperaccumulation, *Sedum alfredii*, Protoplast, Vacuole, Isolation, Purification

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Research Summary

This study aims to develop a method for isolating and purifying protoplasts/vacuoles from fresh leaves of a Cd hyperaccumulator plant species, *Sedum alfredii*.

Main processes

◆ Preheating cellulase and macerozyme at 50℃ for 5 min significantly accelerated the cell wall degradation(a).

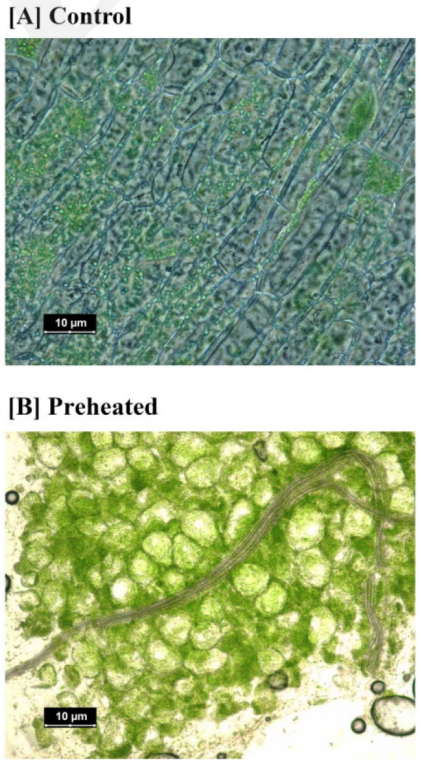


Figure 1 Microscope images of hydrolyzed leaf tissues of *Sedum alfredii* after vibration.

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Main processes

◆ For the most optimal conditions for mesophyll protoplast isolation, this was followed by 2-hour-long vibration.

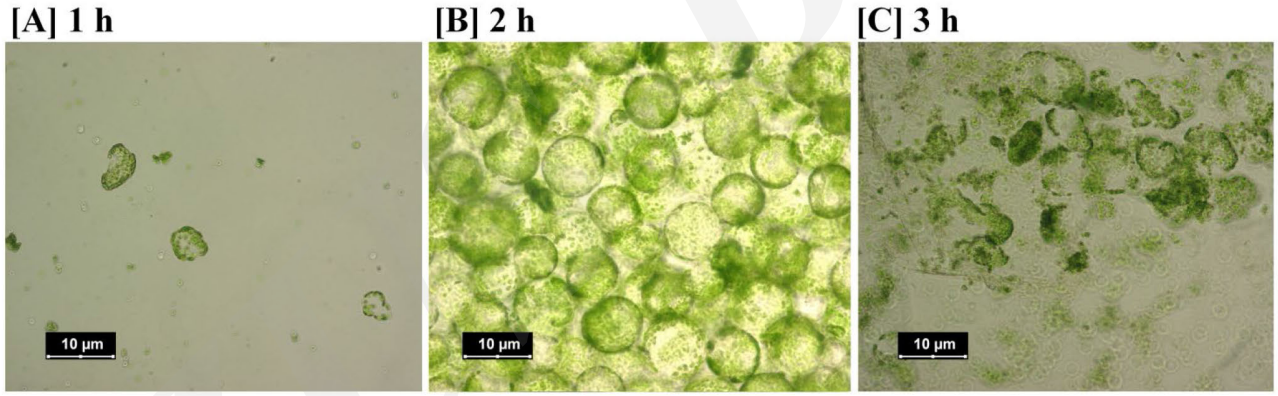


Figure2 Microscope images of resulted protoplasts (A, B, and C) of *Sedum alfredii* after vibration.

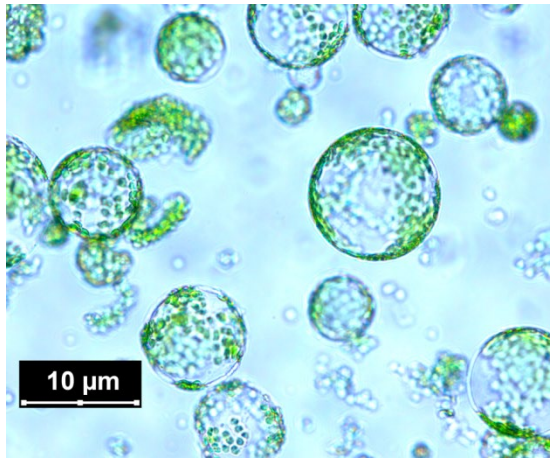


Figure 3 Microscopic image of resulted protoplasts from young leaves of *Sedum alfredii* after centrifugation.

Main processes

◆ The protoplast lysate for vacuole isolation was diluted, where by 0.675 mM was identified as the most appropriate CHAPS level.

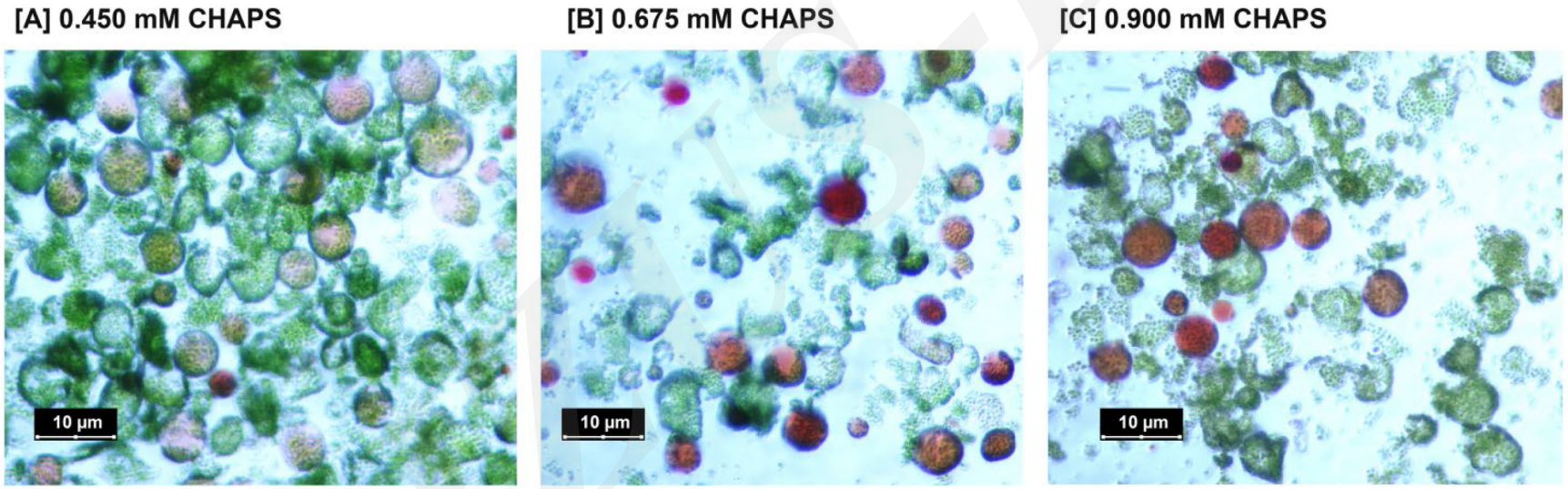


Figure 4 Microscope images of vacuoles and protoplasts after treated with protoplast lysate for 1 hour. The vacuoles were marked using neutral red.

Main processes

◆ For the best vacuole purification results, we established that 0.8 M was the most optimal mannitol level in vacuole buffer in terms of vacuole protection during centrifugation, whereas Ficoll concentration of 10% was adopted in the density gradient centrifugation.

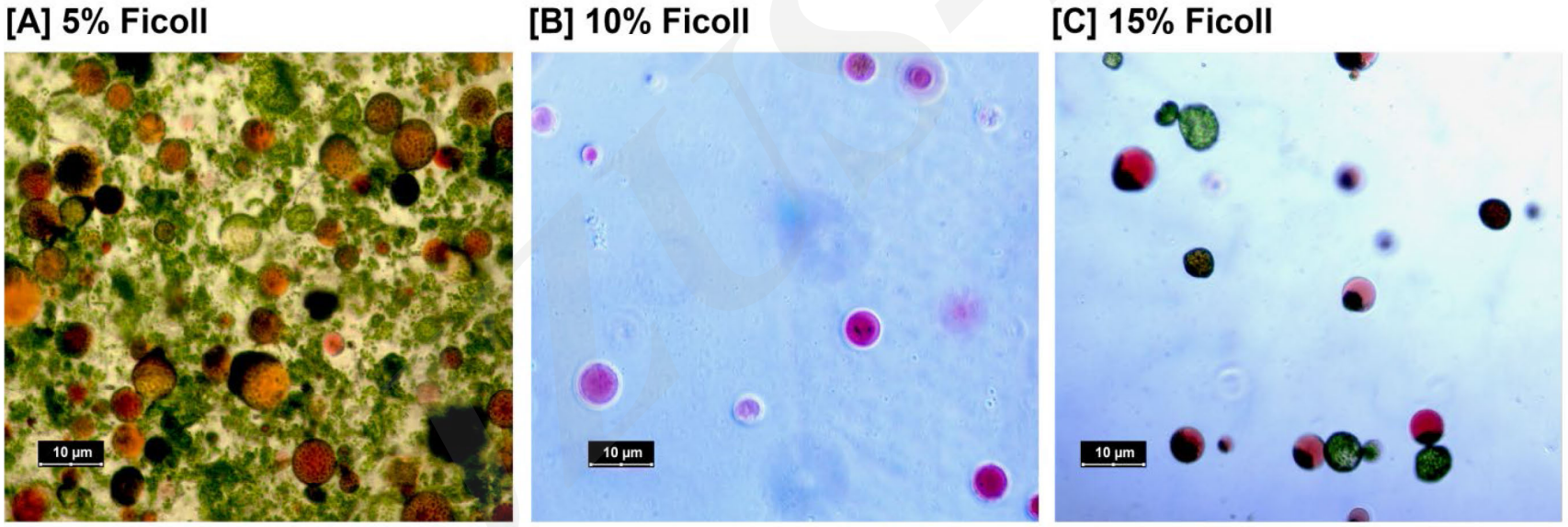


Figure 5 Microscope images of vacuoles after centrifuged at 1500 g and 10°C for 5 min. The vacuoles were marked using neutral red