Electronic Supplementary Material (ESI) for RSC Advances.

NosZ gene cloning, reduction performance and structure of *Pseudomonas citronellolis* WXP-4 nitrous oxide reductase

Liyong Hu,[a] Xiaoping Wang,[a] Cong Chen,[a] Jianmeng Chen,[a] Zeyu Wang,[b] Jun Chen,[b,*] Dzmitry Hrynshpan,[c] Tatsiana Savitskaya,[c]

[a] College of Environment, Zhejiang University of Technology, Hangzhou, 310014, China

[b] Interdisciplinary Research Academy, Zhejiang Shuren University, Hangzhou, 310015, China

[c] Research Institute of Physical and Chemical Problems, Belarusian State University, Minsk, 220030, Belarus

*Corresponding author: Jun Chen

E-mail: bec@zjut.edu.cn

Phone/Fax: +86-571-88285209

S1.1 His tag protein purification

The Ni-Agarose Resin was added to the chromatographic column. After the gel was separated from the solution, ethanol slowly flowed out from the bottom outlet liquid (1 mL packing for purification of 20-30 mg protein). After adding 5 column volumes of deionized water to rinse the column, the column equilibrated by 10 column volumes of Binding Buffer.

Then, Binding Buffer was used to dilute the sonicated crude enzyme solution equally and load it on the column at an appropriate flow rate to collect the flow-through solution. Other proteins were washed away by adding 15 column volumes of Soluble Elution Buffer, and then appropriate amount of Soluble Elution Buffer were used to elute and collect N_2OR.

S1.2 SDS-PAGE

40 μL of protein solution and 10 μL of 5×SDS Loading buffer were mixed, and boiled in water bath for 10 mins. 4%-20% of the precast gel was putted into the protein electrophoresis tank correctly, and 1 L Tris-MOPS-SDS electrophoresis buffer was used for electrophoresis. After carefully pull out the comb in the gel, 7 μL protein sample were added to the gel hole. The SDS-PAGE electrophoresis system (Basic Bio-rad Laboratories) ran at 140 V for 70 minutes until the sample reaches the bottom of the gel to complete the electrophoresis. Finally, the gel was placed in a protein staining and decolorizing instrument for 10 mins.

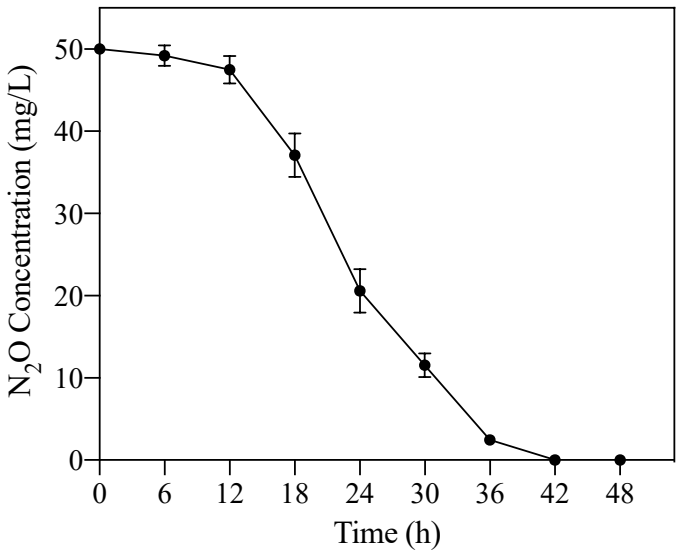

Fig. S1 N_2O reduction performance of *P. citronellolis* WXP-4.

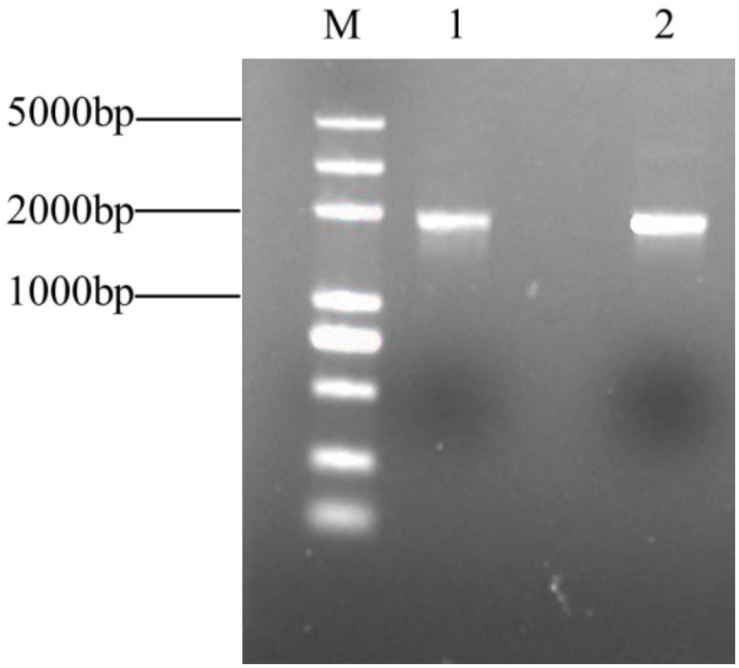

Fig. S2 Agarose gel electrophoresis of the *nos*Z gene PCR products. M: marker; 1and 2: PCR products of *nos*Z gene.

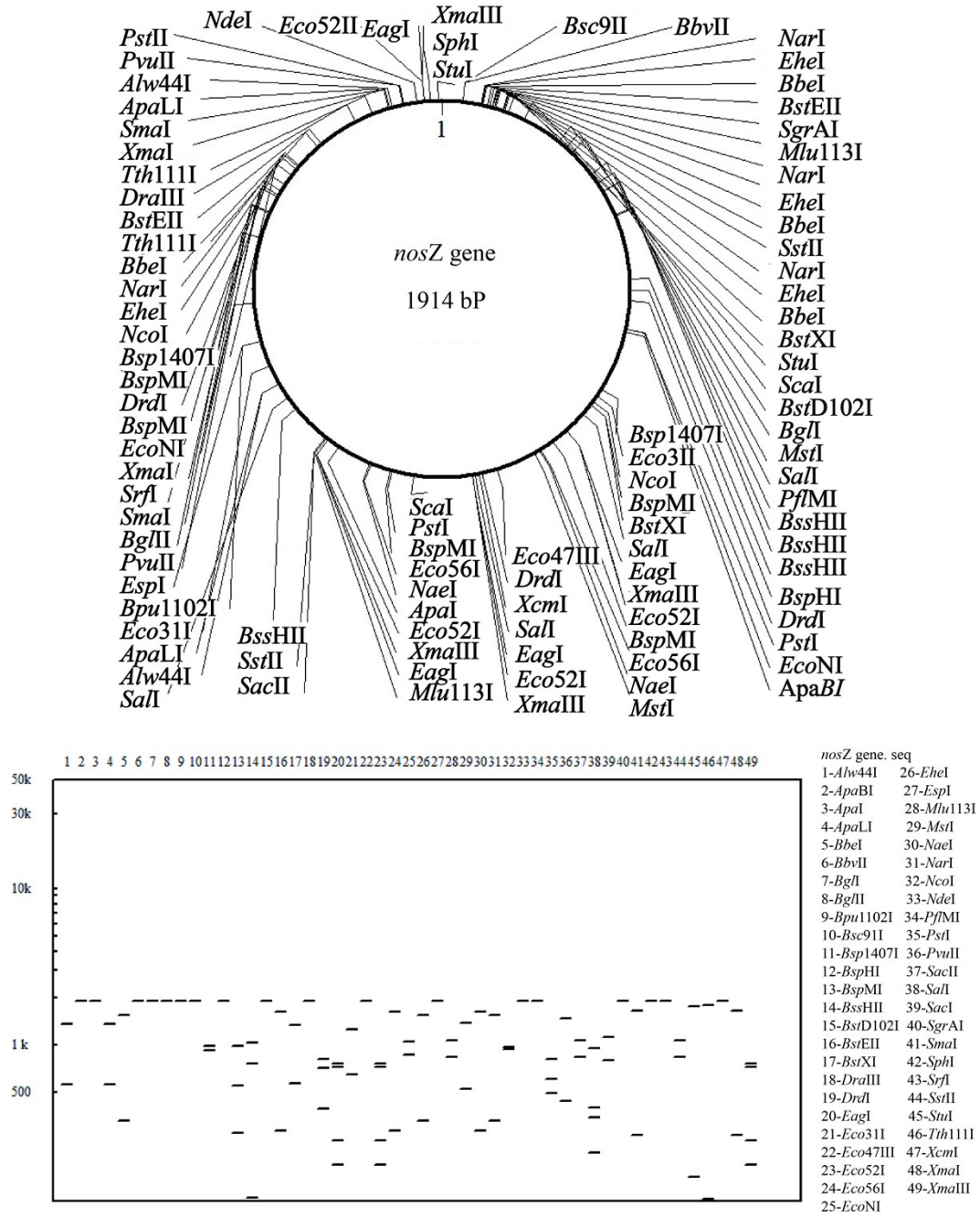

Fig. S3 Restriction map of *nos*Z gene. Electrophoretic simulation of single enzyme digestion of *nos*Z gene.

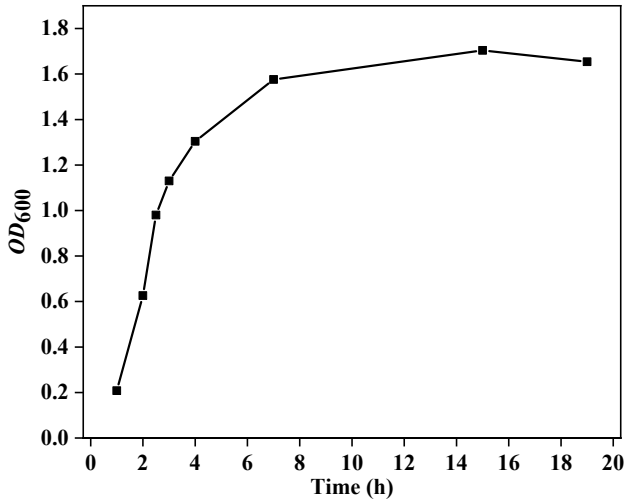

Fig. S4 Growth curve of *E. coli* BL21(DE3)-pET28a-*nos*Z.