RESEARCH ARTICLE

Isolation and identification of *Sphingomonas* sp. from chicken cecum and its ammonia-degrading activity

Mingcheng Wang, Ye Wang, Gailing Wang, Linglong Xu, Enzhong Li*

College of Biological and Food Engineering, Huanghuai University, Zhumadian, Henan 463000, China

Received: March 23, 2021; **accepted:** April 23, 2021.

Emission of ammonia is one of the main causes for the bad odor of the chicken manure. The ammonia does not only impose threats on animal health but also is associated with stimulations to chicken mucous membrane, such as conjunctiva and cornea. The mucous membrane damage increases chicken susceptibility to pathogenic microorganisms, for example the Newcastle disease virus and mycoplasma, etc., and eventually results in reduced chicken production. In this study, one bacterial strain degrading ammonia was isolated from the chicken cecum with the use of anaerobic nitrification medium and aerobic denitrification medium. This strain was yellow-colored, Gram-stain-negative, and strictly aerobic. Phylogenetic analysis based on 16S rRNA gene sequence indicated that the bacterial strain is a member of the genus of *Sphingomonas*, designated *Sphingomonas* sp. Z392. Afterwards, *Sphingomonas* sp. Z392 was fed to the chicken in water with a concentration of 0.4×10^6 CFU/mL. After feeding, the organic nitrogen, ammonium nitrogen, and nitrate nitrogen contents in chicken manure were determined by the Kjeldahl method, the indophenol blue method, and the phenol-disulfonic acid method, respectively. The results showed that the content of organic nitrogen in chicken manure decreased significantly ($P<0.05$) after addition of Z392 into the feeding water for 10 days, while the content of ammonium nitrogen in fresh chicken manure increased at first but decreased significantly after 24 h ($P<0.05$) and the content of nitrate nitrogen increased significantly ($P<0.05$). The research indicated that the addition of *Sphingomonas* sp. into chicken feeding water had obvious effects on degrading the ammonia, deodorizing the manure, and purifying the environment.

Keywords: *Sphingomonas* sp.; chicken cecum; identification; ammonia-degrading; deodorization.

***Corresponding author:** Enzhong Li, College of Biological and Food Engineering, Huanghuai University, Zhumadian, Henan 463000, China. Phone: +86 396 285 3517. Email: enzhongli@163.com.

Introduction

With the rapid development of the poultry husbandry, the bad odor produced from livestock manure is ranked the second one (only after noise pollution) among the six major public hazards associated with husbandry [1]. The toxic gases, such as ammonia (NH_3) and hydrogen sulfide (H_2S), emitted by the chicken manure constitute a serious pollution to the air and threaten to the chicken productivity. Among these gases, ammonia is a direct stimulus of the mucous membrane in the respiratory tract, conjunctiva, and cornea causing mucosal damage, which directly compromises chicken resistance to the pathogenic bacteria, especially the *Escherichia coli* infection. Consequently, the reduced resistance to avian diseases will further induce secondary Newcastle diseases or Mycoplasma gallisepricum and finally result in declined chicken production [2].

Currently, many studies on deamination and deodorization procedures have been reported.

However, they are mainly focused on physiochemical methods, which represent high cost of deamination and high risk of secondary pollution. Microbial deodorization technique now becomes the spotlight of the study on this field because of its high efficiency, high environmentally friendly profile, and low energy consumption [3]. In a previous study, the researchers found that the combined action of ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) was able to convert ammonium nitrogen into nitrate nitrogen, and thus reduced the emission of ammonia in chicken manure [4]. Since the heterotrophic nitrifying bacteria are successfully isolated, the deamination technique developed for them has been widely studied, which is considered as a novel direction for biological method of ammonia removal [2].

Sphingononas sp. is highly tolerant to poor nutrition and available against various simple molecules. It is noted for its various physiological functions, such as high metabolic capacity on degraded organic complex [5], the degradation activity on dye wastes and organochlorine pesticides [6], rubber desulphurization [7], the improved production of welan gum [8, 9]. There also have been intensive studies on it covering many fields including food processing, oil exploitation, building materials [5]. Therefore, it attracts the increasing interest from the investigators. However, there are few reports on its applications on deamination, deodorization, and environmental protection.

In this study, a bacterial strain with ammonia-degrading was isolated from the chicken cecum with the use of anaerobic nitrification medium and aerobic denitrification medium. Subsequently, this bacterial strain was identified by 16S rRNA sequencing. Its ammonia-degrading function was also studied in order to present a novel effective way of biological deodorization for husbandry manure.

Material and methods

Preparation of chicken cecum content

The chickens were killed painlessly, and the abdominal cavity was opened aseptically. The amount of 10 g cecal contents were collected and placed in the conical bottle containing 100 mL of sterilized saline. The mixture was then shaken thoroughly so that the bacteria in the chicken cecum content were fully dissolved in phosphate buffered saline. After static incubation for 20 minutes, the obtained supernatant was collected for the subsequent experiments.

Screening, isolation, and purification of bacterial strain

The supernatant of chicken cecum content was diluted in a sequential manner at a concentration ratio of 10^0 to 10^{-6}, respectively. Equal volume of diluted samples was spread on anaerobic nitrifying medium [0.5 g of $(NH_4)_2SO_4$, 5.62 g of sodium succinate, 50 mL of balanced salt solution (5.0 g of K_2HPO_4, 2.5 g of $MgSO_4{\cdot}7H_2O$, 2.5 g of NaCl, 0.05 g of $FeSO_4{\cdot}7H_2O$, and 0.05 g of $MnSO_4{\cdot}4H_2O$ were dissolved in distilled water up to 1 L), and 20 g of agar were dissolved in 1 L of distilled water at pH 7.0] with a glass spreading rod under aseptic condition. The procedure was repeated in triplicate for each concentration and the petri dishes were remained still for 30 minutes and then placed reversely in an anaerobic incubator (YQX-II) (Shanghai Xinmiao medical equipment manufacturing Co., Ltd, Shanghai, China) at 37°C for 48 h. After single bacterial colonies were formed on the surface of culture media, the morphologies of the colonies were recorded and identified by gram stain.

The isolated strains Z392 was inoculated on aerobic denitrification medium (1 g of KNO_3, 0.5 g of KH_2PO_4, 0.5 g of $FeCl_2{\cdot}6H_2O$, 0.2 g of $CaCl_2{\cdot}7H_2O$, 1.0 g of $MgSO_4{\cdot}7H_2O$, 8.5 g of sodium succinate, and 20 g of agar were dissolved in 1 L of distilled water at pH 7.0) and the cultivation procedure was repeated in triplicate for each concentration. The petri dishes were remained still for 30 minutes, and cultured in an inverted manner in an aerobic incubator (SPX-150L)

(Suzhou Guofei Laboratory Instrument Co., Ltd, Suzhou, Jiangsu, China) at 37°C for 48 h. The morphology of the colonies was recorded as above.

The isolated colonies obtained on aerobic denitrifying medium were purified by streak plate method. A sterile agar plate was marked off into four sectors (A, B, and C). The mixed microorganisms were fully dispersed on the surface of sector A. After flaming inoculating loop and rotating plate 90 degree, the loop was streaked across the agar surface A to B and the bacteria were inoculated to sector B. The above steps were repeated once until sector C was inoculated. After incubation, more and more bacteria are rubbed off until individual separated organisms are deposited on the agar, and then the purpose of pure species separation was achieved. For long-term maintenance, the culture was preserved at -80°C in nutritional broth supplemented with 20% (v/v) glycerol.

16S rRNA gene sequencing and phylogenetic analysis

Genomic DNA of the isolated strain was extracted according to the kit's instruction (TaKaRa Biotechnology, Dalian, Liaoning, China). The 16S rRNA gene was amplified by using Polymerase chain reaction (PCR) with forward primer 27F (5'-AGAGTTTGATCMTGGCTCAGC-3') and reverse primer 1492R (5'-GGTTACCTTGTTACGACTT-3') [10]. The PCR program was pre-denatured at 94°C for 5 min, 35 cycles of 95°C for 30 s, 55°C for 40 s, 72°C for 1 min 30 s followed by extended at 72°C for 10 min. The PCR product was purified using E.Z.N.A. gel extraction kit (Omega Bio-tek, Norcross, GA, USA). Then purified PCR product was ligated to the pMD18-T vector (TaKaRa Bio Inc., Kusatsu, Shiga, Japan) in solution I at 16°C by following manufacturer's instructions, and finally transformed into *Escherichia coli* strain DH5α competent cells (Sangon Biotech, Shanghai, China) by heat shock. Three positive clones inserted with target fragment were confirmed by PCR, and sequenced by Nanjing Genscript Biotechnology Co., Ltd. (Nanjing, Jiangsu, China). The result of 16S rRNA sequencing was then submitted to GenBank nucleotide sequence database for BLAST. The phylogenetic and molecular evolutionary analyses were constructed using the maximum-likelihood algorithm method in the MEGA 6.0 program with 1,000 bootstrap replicates [11].

Bacteria morphological observation

The pure culture of bacteria was directly adhered with conductive adhesive on slide for morphological observation. The samples were sprayed with platinum (Pt) (15 mA, 100 s) and then placed in the electron microscope (TESCAN VEGA3, Brno, Kohoutovice, Czech Republic), and observed with a lower accelerating voltage of 10 kV.

Growth curve of the strain and determination of the total bacterial count at stable stage

The bacterial suspension was inoculated in the nutritional broth at a ratio of 1:100 at 37°C and then placed on a shaker at 200 r/min for cultivation with three replicates. Sampling was conducted at a time interval of 2 h. The optical density (OD) of each sample was determined by using spectrophotometer (Model-722) (Qingdao Juchuang Environmental Protection Group Co., Ltd, Qingdao, Shandong, China) under a wavelength of 600 nm. The OD_{600} was then plotted against inoculation time to obtain the OD-time relationship curve [12]. The total bacterial count at the stable stage was counted using the plate counting method. A brief procedure was as follows: the bacteria were properly diluted and fully dispersed into lots of individual bacteria. A certain amount of diluted sample solution was coated on the plate. After cultivation, each single cell was grown and propagated to form a colony visible to the naked eye, that is, a single colony should represent a single bacterium. The total number of bacteria in sample was equal to the number of colonies times the dilution factor.

Feeding test

The feeding test was conducted among 7 groups of experimental chickens. Group I was the control group, and Group II to VII were the test groups.

For each test group and the control group, 3 parallel tests were conducted simultaneously. In each group, twenty 75-day-old hemp-feather chickens were randomly selected. The different groups of experimental chickens were kept in individual cage in separated poultry house sized 3.56 m × 2.38 m × 2.96 m (length × width × height). The temperature of the poultry house was kept at 28±2°C with natural light exposure. The experimental chickens were implemented with free food and water intake. *Sphingomonas* sp. was added into the feeding water for the different test groups (as shown in Table 1). After 10 days of continuous feeding, the concentrations of in-room ammonia gas were measured with an ammonia gas detector.

Table 1. Dose of *Sphingomonas* sp. added into the feeding water.

Groups	Dose of *Sphingomonas* sp. ($\times 10^6$ CFU/mL)
Group I	0
Group II	0.2
Group III	0.4
Group IV	0.6
Group V	0.8
Group VI	1.0
Group VII	1.2

Quantitative determination of organic nitrogen, ammonium nitrogen, and nitrate nitrogen content in chicken manure

In Group III, 0.4×10^6 CFU/mL of *Sphingomonas* sp. was added into the feeding water for chickens. After 10 days of feeding, 20 g chicken manure was collected from group I and III, respectively. At 0 h, 24 h, 48 h, 72 h, and 96 h, the chicken manure was collected to measure the content of organic nitrogen, ammonium nitrogen, and nitrate nitrogen. Before using the Kjeldahl method to determine organic nitrogen, the moderate chicken manure was digested in digestive furnace and distilled in distillation unit according to the requirement methods [13]. While determining the content of ammonium nitrogen and nitrate nitrogen, the chicken manure was added into ultra-pure water at a solid-liquid ratio of 1:5 and shaken thoroughly, and then, subjected to 1 h vibration extraction at 40°C with vibration speed of 150 r/min on the shaker. Afterwards, the stock liquid was pipetted into a centrifuge tube, which was then centrifuged with a speed of 12,000 r/min for 20 minutes at 4°C. 2 mL of resultant supernatant was collected for later tests. Subsequently, the contents of ammonium nitrogen and nitrate nitrogen in chicken manure were evaluated through the indophenol blue method [13] and phenol-disulfonic acid method [14, 15], respectively.

Data processing and statistical analysis

The data was expressed as mean ± SD, and SPSS (version 16.0) (IBM, Ammon, New York, USA) was used for statistical analysis of the experimental data. These data have been used to determine the denitrifying performances of *Sphingomonas* sp. Any difference with $P<0.05$ was considered as statistically significant.

Results

Morphological characterization and phylogenetic analysis of the bacterial strain

The cream yellow-colored bacterial colonies grew well on anaerobic nitrifying and aerobic denitrifying medium. As shown in Figure 1A, colonies were round, opaque with a smooth, flat surface and neat edges. Scanned electron photomicrograph of bacteria revealed a spherical structure with a smooth surface (Figure 1B). The size of the bacteria was 0.5 to 1.0 μm × 2.0 to 4.0 μm.

The nearly complete length of 16S rRNA gene sequence for the strain Z392 was 1,337 bp (GenBank accession number: MN108136). The phylogenetic tree based on 16S rRNA gene sequences from GenBank showed that the strain Z392 formed a lineage within the members of the family *Sphingomonadaceae* and clustered with *Sphingomonas oligophenolica* (GenBank accession number: AB018439) (Figure 2). This

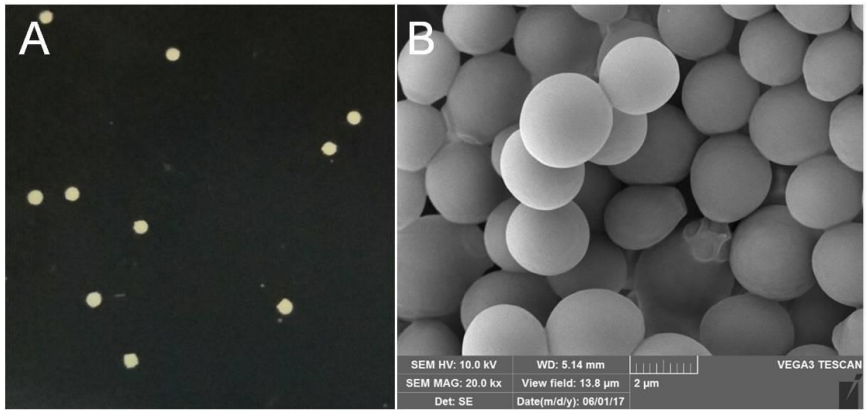

Figure 1. Z392 colony and bacterial morphology. A: colony morphology. B: the image under the electron microscopy with 20,000 times magnification.

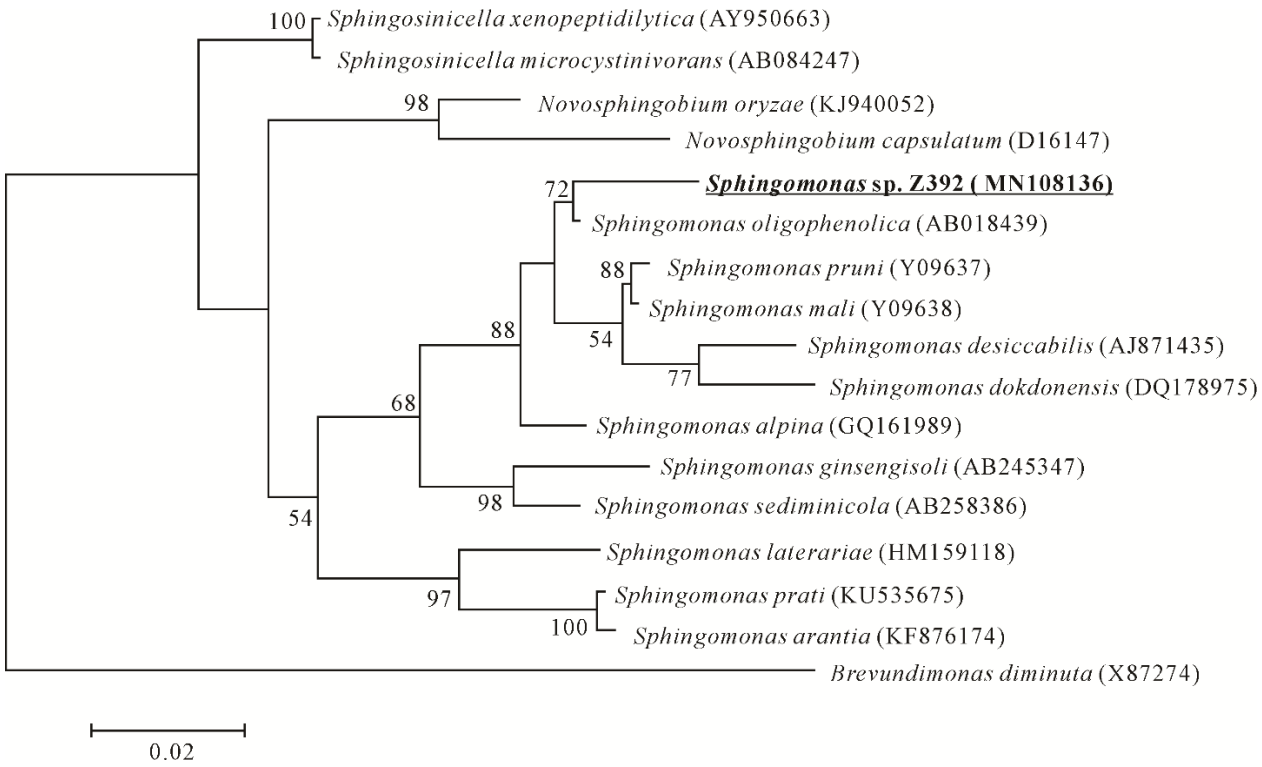

Figure 2. Maximum likelihood tree based on nearly complete 16S rRNA gene sequences. The numbers at the nodes indicate the percentage of 1,000 bootstrap replicates, only values >50% are shown. GenBank accession numbers are given in parentheses. *Brevundimonas diminuta* was used as an out-group. The scale bar represents 0.02.

strain was named as *Sphingomonas* sp. Z392. It was preserved in Biological engineering training center of Huanghuai University in Zhumadian, Henan, China.

Growth curve of the strain and determination of the total bacterial count at stable stage

The lag phase of *Sphingononas* sp. was 2 h, while its logarithmic phase was 3-39 h. It reached stationary phase after being inoculated for 40 h

and the total bacterial count at stable stage was 1.76×10^6 CFU/mL (Figure 3).

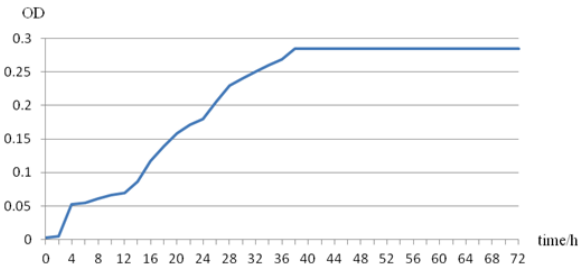

Figure 3. The growth curve of *Sphingononas* sp..

Feeding test

In order to study the deamination and deodorization effects of *Sphingomonas* sp., the ammonia gas contents in poultry house were measured after 10 days of addition of different doses of *Sphingomonas* sp. in feeding water, and the health status of the chickens was observed. The results of ammonia gas content measurement showed that the ammonia gas content in Group II was slightly lower than that of the control group, but there was no statistical significance ($P>0.05$), while the ammonia gas content in Group III and IV decreased significantly ($P<0.05$). The results of chickens' health observation indicated that chickens in Group II, III and IV were healthy without any evidence of clinical pathological changes. Unshaped stool was observed in chickens from Group V and VI, while obvious diarrhea was observed in Group VII (Table 2). Due to the obviously abnormal health status observed in experimental chickens, feeding of *Sphingomonas* sp. was terminated for Group V-VII.

Quantitative determination of organic nitrogen, ammonium nitrogen, and nitrate nitrogen contents in chicken manure

In order to investigate the deamination and deodorization mechanisms of *Sphingomonas* sp., chicken manure was collected from group I and III at 0 h, 24 h, 48 h, 72 h, and 96 h, respectively, after 10 days of addition of *Sphingomonas* sp. into the feeding water. The organic nitrogen, ammonium nitrogen, and nitrate nitrogen contents in chicken manure samples were determined using the Kjeldahl method, the indophenol blue method, and phenol-disulfonic acid method, respectively. The results showed that after feeding 0.4×10^6 CFU/mL of *Sphingomonas* sp., the organic nitrogen content in the chicken manure was significantly lower than that in the control group ($P<0.05$) (Figure 4A), while the ammonium nitrogen content in fresh chicken manure increased at first but decreased significantly after 24 h ($P<0.05$) (Figure 4B). The content of nitrate nitrogen was significantly higher than that in the control group ($P<0.05$) (Figure 4C).

Discussion

The nitrogen contents in the poultry manure are mainly originated from three sources, namely, surface nitrogen, fecal nitrogen, and endogenous urinary nitrogen. The formation of surface nitrogen is mainly due to the shedding of feathers, hooves, skins, and saliva during chicken growth. The amount of nitrogen content from this aspect is extremely small and negligible. Fecal nitrogen is mainly involved in three conversion pathways, i.e., the organic nitrogen, ammonium nitrogen, and nitrate-nitrogen [16]. For endogenous urinary nitrogen, it is mainly the urea and the uric acid.

Due to the structural characteristics of the chicken digestive tract, the feed could only retain in chicken body for a very short time. Some nutrients in the feed, especially the protein and amino acids, can hardly be completely digested before being excreted from the body. They thus become the main source of organic nitrogen content in chicken manure, which provides rich nitrogen source for the microbial reproduction. While microorganisms are growing and reproducing upon the nitrogen source provided by organic nitrogen, they also convert the organic nitrogen into the ammonium nitrogen [2]. The anaerobic ammonia oxidizing bacteria use ammonium nitrogen as the nitrogen source and

Table 2. Changes in ammonia gas contents in poultry house after addition of *Sphingomonas* sp. in feeding water.

Group	Ammonia gas content in poultry house (mg/L)	Health status of cooks
Group I	91.3±1.53	No abnormality
Group II	87.3±2.05	No abnormality
Group III	78.6±1.39*	No abnormality
Group IV	77.9±2.17*	No abnormality
Group V	-	Unshaped stool
Group VI	-	Unshaped stool
Group VII	-	Diarrhea

Note: Group I was the control, while Group II-VII were test groups which received different doses of *Sphingononas* sp. in feeding water. *indicates significant difference vs control group (P<0.05). Due to the obviously abnormal health status observed in experimental chickens, feeding of *Sphingomonas* sp. was terminated for Group V-VII.

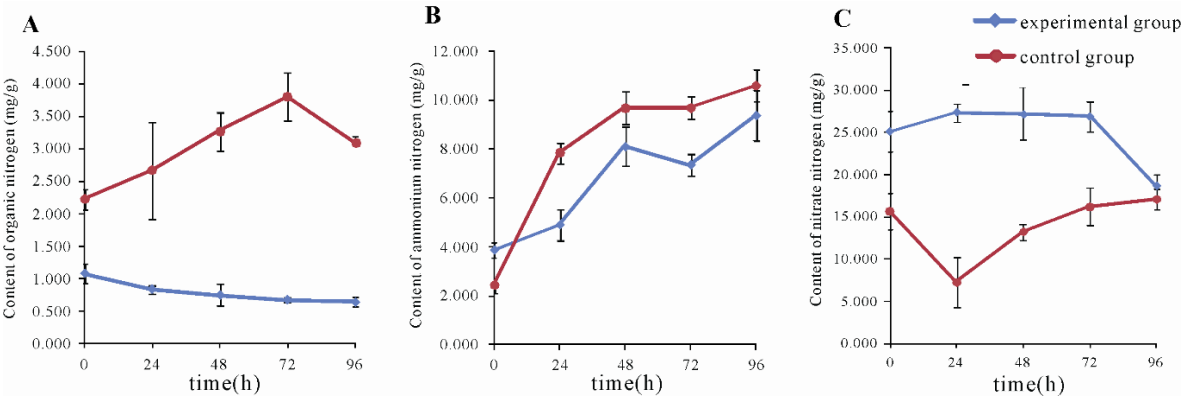

Figure 4. Change in organic nitrogen, ammonium nitrogen, and nitrate nitrogen contents in chicken manure. After feeding of *Sphingomonas* sp., organic nitrogen content in chicken manure was significantly reduced (**A**) (P<0.05), while the ammonium nitrogen content in the fresh manure increased at first but decreased significantly after 24 h (**B**) (P<0.05), and the nitrate nitrogen content increased significantly (**C**) (P<0.05).

convert it into NO_2^--N, while nitrite oxidizing bacteria use NO_2^--N as nitrogen source and convert it into NO_3^--N.

In the process of chicken manure storage, extensive smelling substances will be emitted, which produce a serious threat to the health of people and animals. Among these odorous gases with complex components, the ammonia gas does not only induce respiratory diseases of poultry, but also may cause chicken poisoning or death in severe cases. Controlling of fecal nitrogen accumulated in the form of NH_4^+-N is the key for odor management and improves the retention rate of nitrogen [17]. In order to reduce the emission of ammonia gas in chicken manure, herein a bacterial strain (*Sphingomonas* sp. Z392) was isolated using the anaerobic nitrification medium and aerobic denitrification medium. Subsequently, a feeding test was conducted, and the results showed that when the amount of *Sphingomonas* sp. Z392 added into the feeding water was elevated to 0.8×10^6 CFU/mL for 2-3 days, unshaped stool or even diarrhea was observed in the experimental chickens. Therefore, the feeding test was terminated, and no ammonia gas measurement data was obtained from these groups. This indicated that the *Sphingomonas* sp. Z392 content in drinking water higher than 0.8×10^6 CFU/mL was likely to harm the health of chicken and caused clinical diseases in severe cases.

Under anaerobic conditions, *Sphingomonas* sp. Z392 was not only able to utilize organic nitrogen as the nitrogen source, but also to use NH_4^+ as the

only nitrogen source [18-20]. In order to investigate the deamination effect of *Sphingomonas* sp., the ammonia concentrations in each experimental poultry house were also measured. The results showed that the amount of ammonia in the poultry house decreased after addition of 0.4-0.6×10^6 CFU/mL of *Sphingomonas* sp. Z392 into the feeding water, in particular, statistically significant declination of ammonia was observed in Group III and IV. The results showed that the addition of *Sphingomonas* sp. Z392 into the feeding water could effectively reduce the emission of ammonia gas in chicken manure.

In order to further explore the mechanism of ammonia removal by *Sphingomonas* sp. Z392, the organic nitrogen, ammonium nitrogen, and nitrate nitrogen in chicken manure samples were quantitatively determined. The results showed that *Sphingomonas* sp. Z392 could reduce the content of ammonia in chicken manure by reducing the content of organic nitrogen, ammonium nitrogen, and nitrate nitrogen, which had a significant effect. This could be attributed to two reasons. One is that the yield of ammonium nitrogen decreased with the decrease of organic nitrogen content. *Sphingomonas* sp. Z392 could transform or promote other microorganisms to transform ammonia nitrogen into nitrate nitrogen because they consume a lot of ammonia nitrogen as the nitrogen source for growth and reproduction. The second is that, under anaerobic conditions in the chicken digestive tract, *Sphingomonas* sp. Z392 could use organic nitrogen and ammonia nitrogen as nitrogen source [20], while under aerobic conditions *in vitro*, nitrite (NO_2^--N) could be converted into nitrate (NO_3^--N) under the action of *Sphingomonas* sp. Z392, so as to effectively reduce the production of ammonia in chicken manure and play the role of ammonia and deodorization.

Acknowledgement

This work was supported by the Science and Technology Development Plan Project of Henan province of China (172102110201 and 212102110005), and the major science and technology project of Zhumadian City, Henan, China (17702).

References

1. Matsuzaka E, Nomura N, Nakajima-Kambe T, Okada N, Nakahara T. 2003. A simple screening procedure for heterotrophic nitrifying bacteria with oxygen-tolerant denitrification activity. J Biosci Bioeng. 95(4):409-411.
2. Salim HM, Patterson PH, Ricke SC, Kim WK. 2014. Enhancement of microbial nitrification to reduce ammonia emission from poultry manure: a review. Worlds Poult Sci J. 70(04):839-856.
3. Matusiak K, Borowski S, Opaliński S, Bakuła T, Kołacz R, Gutarowska B. 2015. Impact of a microbial-mineral biopreparation on microbial community and deodorization of manures. Acta Biochim Pol. 62(4):791-798.
4. Sorokin DY, Lücker S, Vejmelkova D, Kostrikina NA. 2012. Nitrification expanded: discovery, physiology, and genomics of a nitriteoxidizing bacterium from the phylum *Chloroflexi*. ISME J. 6:2245-2256.
5. Gou M, Qu YY, Yang H, Zhou JT, Li A, Guan XY, *et al*. 2008. *Sphingomonas* sp.: A novel microbial resource for biodegradation of aromatic compounds. Chin J Appl Environ Biol. 14(2):276-282.
6. OuYang ZC, Wang YH, Li XN, Gao YF, Jiang GB, Fang YS. 2008. Test of pesticide degradability by *Sphingomonas yanoikuyae* XJ Strain. J South China Agric Univ. 29(2):47-49.
7. Cui XX, Zhao SH, Wang BW. 2016. Microbial desulfurization for ground tire rubber by mixed consortium-*Sphingomonas* sp. and *Gordonia* sp. Polym Degrad Stab. 128:165-171.
8. Liu YT, Dong XQ, Wang W, Zhang YG, Ji WK. 2016. Optimization of fermentation medium for welan gum production by *Sphingomonas* sp. China Brewing. 35(1):73-77.
9. Zhu H, Sun SW, Li H, Chang AP, Liu YC, Qian J, *et al*. 2019. Significantly improved production of Welan gum by *Sphingomonas* sp. WG through a novel quorum-sensing-interfering dipeptide cyclo(L-Pro-L-Phe). Int J Biol Macromol. 126:118-122.
10. Frank JA, Reich CI, Sharma S, Weisbaum JS, Wilson BA, Olsen GJ. 2008. Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA gene. Appl Environ Microbial. 74:2461-2470.
11. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol Biol Evol. 30:2725-2729.
12. Wang GL, Hu L, Wang MC, Li EZ. 2017. Identification of pathogens in the groundwater of laying hen flocks and selection of sensitive drugs. J Biotech Res. 8:27-32.

13. Zhang CM, Sun Y, Yang XS, Hao ZZ, Bao K. 2008. Measuring method of organic nitrogen content in paper and paperboard. China Pulp Paper Industry. 16:70-71.
14. de Vera GAD, Gernjak W, Weinberg H, Farré MJ, Keller J, vonGunten U. 2017. Kinetics and mechanisms of nitrate and ammonium formation during ozonation of dissolved organic nitrogen. Water Research. 108(1):451-461.
15. Wang MC, Wang GL, Li CF, Li FL, Jin XJ, Xu ZJ. 2018. Isolation and identification of ammonia reducing *Bacillus megaterium* from chicken cecum. J Biotech Res. 9:1-7.
16. Hayes ET, Curran TP, Dodd VA. 2006. Odour and ammonia emissions from intensive poultry units in Ireland. Bioresour Technol. 97(7):933-939.
17. Ferket PR, van Heugten E, van Kempen TATG, Angel R. 2002. Nutrional strategies to reduce environmental emissions from nonruminants. J Anim Sci. 80:168-182.
18. Eriksson M, Sodersten E, Yu ZT, Dalhammar G, Mohn WW. 2003. Degradation of polycyclic aromatic hydrocarbons at low temperature under aerobic and nitrate reducing conditions in enrichment cultures from northern soils. Appl Environ Microbiol. 69(1):275-284.
19. Kim BC, Sohn CK, Lim SK, Lee JW, Park W. 2003. Degradation of polyvinyl alcohol by *Sphingomonas* sp. SA3 and its symbiote. J Ind Microbiol Biotechnol. 30(1):70-74.
20. Tang YQ, Li M, Zou YN, Lv MY, Sun JM. 2018. Mechanism of aerobic denitrifiers and calcium nitrate on urban river sediment remediation. Int Biodeterior Biodegradation. 126(1):119-130.