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Research Article
Characteristics of High Ammonium-tolerant *Arthrobacter* sp. LM1KK Isolated from High Ammonia Odorous Region of Laying Hens Farm in the Tropical Area

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Abstract

Background: Animal manure may contributes global environmental problems especially unpleasant odor from the emission of ammonia. It challenges the development of proper management processes for safe and efficient disposal. **Objective:** The aim of this experiment was to identify isolates screened from soil in the odorous region of chicken hens farms in a tropical area and to evaluate their capability for growing in ammonium and animal's urine high content medium. **Materials and Methods:** Indigenous isolates originally obtained from soil sample collected at ammonia high-emitted area of a tropical country Indonesia using a 1/100 nutrient agar with high concentration of $(NH_4)_2SO_4$. Selected strain was identified based on morphology and biochemical identifications, as well as molecular identification (16S rRNA sequence homology). Reduction of ammonium was measured from cultured medium using Nessler reagent and spectrophotometer. **Results:** Ammonium sulfate-responsive, strain LM1KK was successfully isolated and identified based on morphology, physiology and biochemical identifications, as well as molecular identification based on 16S rRNA sequence. The cells of strain LM1KK are rods, aerobic and motile. Colonies (1-2 mm in diameter) on a plate culture are yellowish, circular and smooth. Based on the morphology, physiological test and its 16S rRNA gene sequence, strain LM1KK was identified as species *Arthrobacter nitroguajacolicus*. The 16S rRNA gene sequence analysis demonstrated this isolate is showing 98% similarity to species of *Arthrobacter nitroguajacolicus* MNPB4T. **Conclusion:** This bacterium has a higher optimal growth temperature of 30°C. Strain LM1KK can tolerate ammonium at high concentration and has the ability for growing in the urine contained medium that should make it applicable for use in deodorization plants for enhancing the efficiency of deodorization. Ammonium was oxidized 26.86 mg L^{-1} by strain LM1KK during 48 h cultivation period.

Key words: Nitrifying bacteria, ammonia-tolerant bacteria, *Arthrobacter* sp., deodorization

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Intensive and sustainable animal production is crucial for an agricultural-based economic country such as Indonesia. Agrobiological waste, such as animal manure, contributes global environmental problems, challenge the development of proper management processes for safe and efficient disposal. Among animal waste produced from livestock production, poultry excreta and dairy feces contributes significantly to the emission of gaseous ammonia (NH_3) as a source of greenhouse gas emission[1,2].

These ammonia emissions cause some environmental problems because of their odor, toxicity and contribution to eutrophication and acid rains[3]. Some potential consequences associated with exceeding threshold concentrations of both oxidized and reduced forms of N in the environment include: (1) Some respiratory diseases caused by exposure to high levels of fine particulate aerosols, (2) Contamination of drinking water by nitrate, (3) Decreasing of water quality and eutrophication of surface water, (4) Changes in vegetation or ecosystem because of high N concentrations, (5) Climatic changes due to increases in nitrous oxide (N_2O) concentration, (6) N saturation of forest soils and (7) Soil acidification through nitrification[4].

Various methods have been studied and investigated to reduce odor nuisance from animal waste, including dietary and management strategies to minimize the generation and volatilization of ammonia[5] application of plants to trap ammonia emissions[6] and developing of deodorizing system[7]. Among them, biological additives employ microorganisms has attracted attention for their ease of use, fast in action and relatively lower expenses[8,9]. This ammonia removing treatment is based on the transformation of volatile N to non-volatile N comprising of nitrification and denitrification processes by chemoautotrophic nitrifying and denitrifying bacteria.

Nitrification-denitrification is one of the important processes involved in the N cycle. In many ecosystems, ammonia-oxidizing bacteria and archaea (AOB and AOA, respectively) oxidize NH_3 to nitrite NO_2^-, whereas NO_2^- is further oxidized to nitrate NO_3^- by Nitrite Oxidizing Bacteria (NOB)[7,3,10,11]. Mainly autotrophic nitrifiers are believed to be responsible for this process. The major grouping of AOB belongs to the subclass Beta-proteobacteria and comprises genera such as *Nitrosomonas*, *Nitrosococcus*, *Nitrosospira*, *Nitrosovibrio* and *Nitrosolobus*[12]. A few species of marine *Nitrosococcus* are belong to the Gamma-proteobacteria. All currently know AOA belong to the new phylum Thaumarchaeota[13]. However, nitrification is also employed during heterotrophic growth of some bacteria, such as *Bacillus methylotrophicus*[14], *Alcaligenes faecalis*[15], *Pseudomonas stutzeri*[10] and *Thiosphaera pantotropha*[16]. Heterotrophic nitrification is thought to be performed in a similar ways to the autotrophic process: NH_4^+ is initially converted to NH_2OH by the enzyme ammonia monooxygenase and then followed by the oxidation of NH_2OH to NO_2^- by the enzyme hydroxylamine oxidoreductase (HAO), then NO_2^- is further oxidized[17] to NO_3^-. Genus *Arthrobacter* was firstly investigated to be involved in nitrification system[18] at 1972.

Composting is a general method to decompose animal feces, crop residues, organic industrial waste and other organic matter. During aerobic composting, decomposer is important to improve the quality of compost product. Studies on nitrification during composting has been performed[3,7]. Fungi are another kind of decomposer, besides bacteria and archaea, for which the role and development are not clear in the composting process. It is observed that the composting self-heating pile may reach a temperature too extreme for their survival[19]. They would thus be eliminated during the thermophilic stage and recovered when the temperature decreases[20]. However, because of their ability to decompose some slowly biodegradable materials like cellulose and lignin, fungi may play a significant role during the maturation stage[19]. Moreover, some fungi exhibit heterotrophic nitrification activity linked to lignin degradation. Their role involved in N transformations during composting needs to be considered[21].

In order to deal with the N losses and NH_3 emissions from aerobic treatment to understand the N dynamic and to study the microorganisms involved in N transformations are indispensable. The first objective of the present study was to isolate and identified strain microbe from the soil that have a deal with nitrogen tolerant and to understand the role of an isolated strain in nitrification.

MATERIALS AND METHODS

Media and culture: A 1/100 nutrient agar with 500 mg L^{-1} $(NH_4)_2SO_4$ was used for screening ammonium-responsive microorganisms. Cultures were performed for 7 days at 30°C. Liquid culture was carried out using 100 mL of 1/100 nutrient broth with 500 mg L^{-1} $(NH_4)_2SO_4$ in 250 mL Erlenmeyer flasks and cells were grown aerobically at about 30°C with a reciprocal shaker (120 rpm). Bacterial growth was monitored at Optical Density (OD) 600.

Screening of ammonium-responsive microorganisms: Indigenous isolates originally obtained from ammonia high emitted area of a tropical country Indonesia. Soil samples

(1.0 g) collected from various spots at a high odorous poultry farm in tropical areas were suspended in 9.0 mL sterile pure water and diluted properly. A portion of the cell suspension was spread on a 1/100 nutrient agar plate with 500 mg L^{-1} $(NH_4)_2SO_4$. Bacteria colonies appearing on the plate were picked and purified. Each purified colony was inoculated on an agar plate with and without 500 mg L^{-1} $(NH_4)_2SO_4$. Microorganisms displaying a peculiar growth on the agar with $(NH_4)_2SO_4$ were selected as ammonium-responsive microorganisms. Isolates were purified by plating on 1/100 nutrient broth (0.01% meat extract, 0.01% polypeptone and 0.005% NaCl, pH 7.2) supplemented with 500 mg L^{-1} $(NH_4)_2SO_4$ and inoculated at 30°C for 48 h in aerobic condition.

Identification of an ammonium-responsive bacterium: Morphological, biochemical and physiological characteristic of the ammonium-responsive bacterium were investigated according to Bergey's Manual of Systematic Bacteriology[22]. Genomic DNA of the bacterium was extracted by conventional methods[23]. The 16S rRNA was amplified by PCR using universal primers. The purified PCR product was sequenced using BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster city CA, USA) with an ABI prism 310 genetic analyzer (Applied Biosystems) and analyzed using the BLAST version 2.2.18 (blast) to compare with the public database of DDBJ (http://blast.ddbj.nig.ac.jp).

Scanning electron micrograph: To prepare the sample for Scanning Electron Microscope (SEM), the 1/100 diluted nutrient broth containing 3.5% agar and 0.1% gelatin was used. Glass fiber filter (GA100, Advantec) was cut into 5 mm^2 and sterilized. The glass fiber was put on the 1/100 diluted nutrient agar medium and a small portion of the sterilized agar medium was dropped gently on the glass filter to make a thin layer and solidified. Cells precultured in the nutrient broth were poured on the glass fiber. Then the glass fiber was incubated at 30°C for 24-48 h. Next, the cells on the glass filter were fixed with fixation solution that consisted of 630 µL of 0.2M HCl, 1.008 g of NaCl, 0.214 g of sodium cacodylate, 1 g of glutaraldehyde and filled up to 100 mL with distilled water. The glass filter was put into Eppendorf tube (1.5 mL) and filled gently with 1 mL of fixation solution and stored in a refrigerator (4°C) for 30 min. By using micropipette, fixation solution was removed and 60% acetone was then poured to remove water in the sample. The tube was then kept at room temperature for 15 min. Then the solution was removed and replaced by 80% acetone and further changed by 100% acetone. The treatment by 100% acetone was repeated twice. After the removal of 100% acetone, 1 mL N-amyl acetate (Pentyl acetate) was added gently and stored at room temperature for 20 min. After drying, the sample was coated with platinum. The sample was placed on the tape that placed in gold plate. The sample was pushed with pinset. This coating process was performed by the JEOL JEC-3000FC auto fine coater. The sample was observed by SEM (JEOL JSM-6510LA).

Genomic DNA isolation: For genomic DNA isolation, an overnight 1 mL cell culture was harvested and suspended in 500 µL DNA extraction buffer (2.5 M NaCl, 500 mM EDTA, 1 M tris-HCl, 10% SDS, pH 7.5). The solution was then mixed by tiping over the tube followed by incubation at 65°C for 5 min and repeated twice. After incubation, 250 µL phenol:chloroform:isoamyl alcohol (P:C:I) was added to solution followed by vortex for 5 min and centrifugation at 13000 rpm for 10 min to separate DNA from other cellular components. The top aqueous phase was transferred to other new Eppendorf tube and then added by 30 µL 3M $NaCH_3COO$ and 750 µL 100% ethanol and incubated at -20°C for 30 min. Precipitated DNA was harvested by centrifugation at 13000 rpm for 10 min and washed with 250 µL 70% ethanol and resuspended in tris-EDTA (TE) buffer. The isolated DNA was resolved by electrophoresis on a 0.7% agarose gel in TAE buffer at 100 V for 30 min, then visualized by ethidium bromide staining at UV-illuminator. The size was calculated by using λ-Eco T_{14}I DNA Marker (Takara).

Amplification of 16S rRNA gene by Polymerase Chain Reaction (PCR): The PCR was carried out to amplify ~1500 bp fragment of the 16S rRNA gene using oligonucleotide primers designed based on the region of 16S rRNA gene for bacteria and 18S rRNA for yeast. A combination of forward primers 16S forward (5-AGAGTTTGATCCTGGCTCAG-3) and 16S reverse (5-GGYTACCTTGTTACGACTT-3) were applied to amplify 16S rRNA sequence of bacteria. A pair of 18S forward (5-ACCCGCTGAACTTAAGC-3) and 18S reverse (5-TACTACCACC AAGATCT-3) were used to amplify target gene of yeast. The PCR was performed using PCR thermal cycler (Applied Biosystem, USA) with total volume of 50 µL consists of: 35 µL dH_2O (water free nuclease), 1 µL DNA template, 1.5 µL forward primer, 1.5 µL reverse primer and 5 µL 10×polymerase buffer, 1 µL Taq polymerase (Takara, Japan) and 5 µL dNTP. The condition of PCR was set as follows: Pre denaturation at 94°C for 2 min, denaturation at 94°C for 15 sec, continued by annealing at 50°C for 30 sec, elongation 68°C for 2 min (35 cycles) and postelongation 68°C for 5 min. Amplified bands were resolved by electrophoresis in a 0.75% (w/v) agarose gel in TAE buffer and visualized with ethidium bromide staining in UV-illuminator. Size was calculated by using λ-Eco T_{14}I DNA Marker (Takara, Japan).

DNA sequencing and phylogenetic analysis: The purified DNA of selected isolates was sequenced using DNA analyzer (Applied Biosystems). The observed sequences were used to search closed similarity sequences in the NCBI database by using BLAST and the identities of isolates were calculated on the basis of the highest score (>98%). Sequences were aligned and assembled using the sequencher 4.7 program (GENE CODES) to construct a phylogenetic tree by the neighbor-joining algorithm was performed using ClustalW and viewed using Figtree v1.4.0.

Assessment of growth and ammonium reduction: Shaking culture experiments were conducted to observe the effect of $(NH_4)_2SO_4$ or cow's urine addition at different concentration of 1, 3, 5, 7, 10 and 15% (g/v) on growth. Cow's urine collected from Friesian Holstein Cows at Faculty of Animal Science Universitas Gadjah Mada were mixed with autoclaved sterile 1/100 meat extract medium to 100 mL in total volume. One percent of overnight strain preculture were then inoculated to medium in 250 mL Erlenmeyer flasks and incubated at 30°C. The growth of an isolate was observed spectrophotometry at a wavelength of 600 nm (Shimadzu UV-1601PC spectrophotometer). Furthermore, the ability of the strain in reduce ammonia was performed by centrifuged culture samples at 8000 rpm for 15 min (Eppendorf centrifuge 5804R). Ammonia concentration was determined by Nessler assay at a wavelength 425 nm.

RESULTS

Isolation and selection of microorganisms: About 100 strains were isolated from soils from two laying hens industry that categorize as high ammonia emission region and strain LM1KK belongs to that group. Because environmental conditions are usually nutritionally poor, a 1/100 nutrient agar was used for screening ammonium-responsive strains. We have isolated a soil bacterium, which located at high ammonia odorous region of laying hens farm in tropical area, responsive to the addition of 500 mg L^{-1} $(NH_4)_2SO_4$ as addition of nitrogen source in nutrient agar from soil at Yogyakarta city.

This strain that showed good growth in liquid culture was submitted to this present study. The growth of strain LM1KK at 1/100 nutrient broth was promoted by the addition of 500 mg L^{-1} in culture medium at 120 rpm shaker at 30°C after incubation at 4 days. Colony diameter of the strain was also confirmed increased in size when growing in 1/100 nutrient agar.

Morphological and physiological characteristics: The morphology of strain LM1KK colonies on nutrient agar plate were light yellow, circular, convex, smooth and wet (Fig. 1). Spores are not formed. Morphological observation using scanning electron microscope (JEOL JSM-6510LA) of pure strains was in Fig. 2. It is found that cells of LM1KK were short rod, 0.3-0.7×1.2-1.8 μm in size. The morphological, biochemical and physiological characteristics of strain LM1KK were shown in Table 1. The cells of the strain are Gram-negative bacteria, confirmed as oxidase negative, non-nitrate reduction and non-indole production. Furthermore, catalase reaction, starch hydrolysis, H_2S production and citrate utilization were positive. This following substrate were utilized by the strain for respiration: D-glucose, D-mannose and dl-malic acid, but D-mannitol, n-acetyl-D-glucosamine, maltose and D-gluconate were not used as substrate by the strain. According to the data, strain LM1KK was confirmed belong to genus *Arthrobacter*.

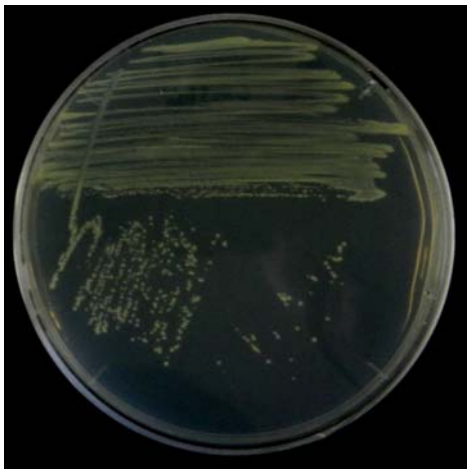

Fig. 1: Colony of strain LM1KK growing on 1/100 meat extract medium contain 0.01% yeast extract, 0.01% polypepton and 0.005% NaCl with 1.5% agar. Medium was suplemented with 500 mg L^{-1} $(NH_4)_2SO_4$

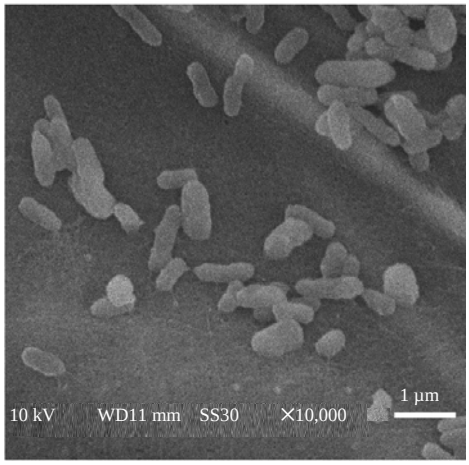

Fig. 2: Scanning electron micrograph of strain LM1KK grown in 1/100 meat extract medium

Table 1: Morphological, biochemical and physiological characteristics of *Arthrobacter* sp. LM1KK

Shape	Rod shape	Urease	–
Size of cells	0.3-0.7×1.2-1.8 μm		
Size of colonies	1-2 mm	Gelatine hydrolysis	–
Gram staining	–	Galactosidase	+
Spore forming	–	Substrate utilization	
Motility	+	D-glucose	+
GC content	57.4 mol%	D-mannose	+
Colony morphology	Circular	D-mannitol	–
	Smooth	n-acetyl-D-glucosamine	–
	Low convex	Maltose	–
	Luster	D-gluconate	–
	Semitranslucent		
Catalase	+	dl-malic acid	+
Oxidase	+	Citrate	+
O/F test	Oxidative		
Nitrate reduction	–	Optimum temperature	30-35°C
Indol production	–	Optimum pH for growth	7.0-7.5
D-glucose acidification	–	Maximum tolerance of $(NH_4)_2SO_4$	579,74 mM
Arginine dihydrolase	+	Ability for growing in liquid urine medium addition of 1-15%	No significant different

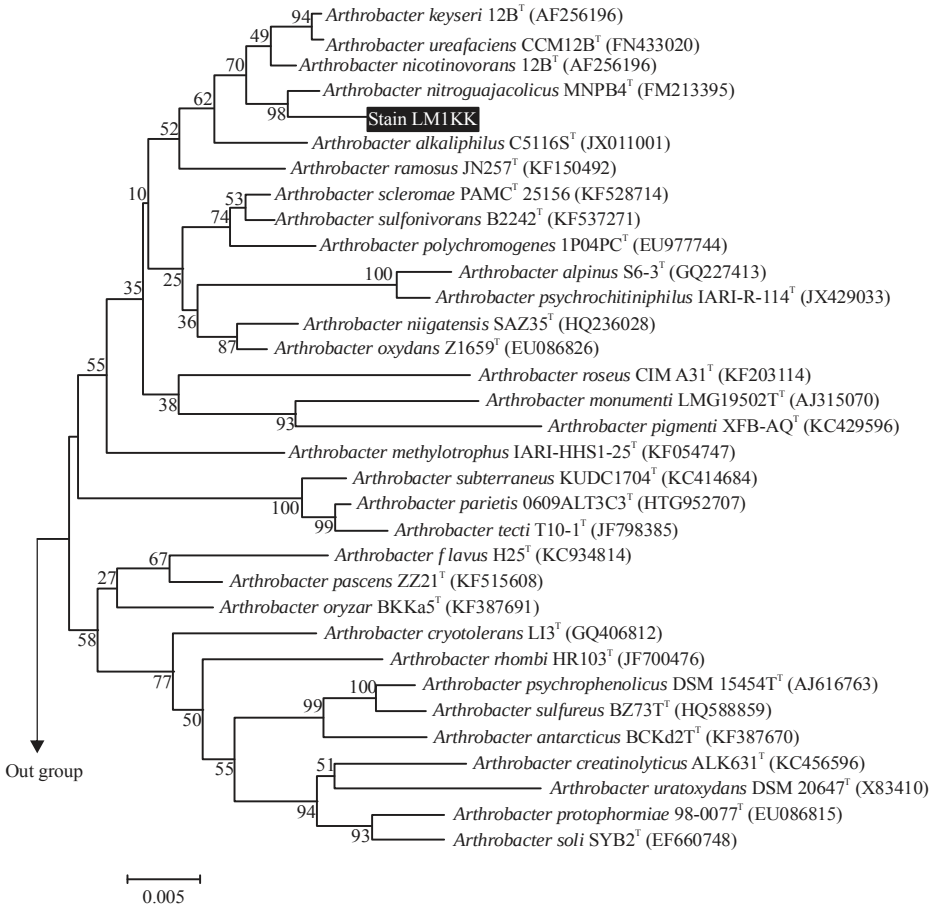

Fig. 3: Neighbour-joining phylogenetic analysis based on 16S rDNA gene sequences showing the positions of strains LM1KKT among the type strains of selected *Arthrobacter* species available from GenBank NCBI/EMBL/DDBJ (accession No. are given in parentheses). Bar, 0.01 substitutions per nucleotide position

Phylogenetic analysis of 16S rRNA: The partial 16S rRNA sequence of strain LM1KK was determined and a phylogenetic tree was constructed based on the 16S rRNA sequence (Fig. 3). The sequence of 16S rRNA was deposited in the GenBank

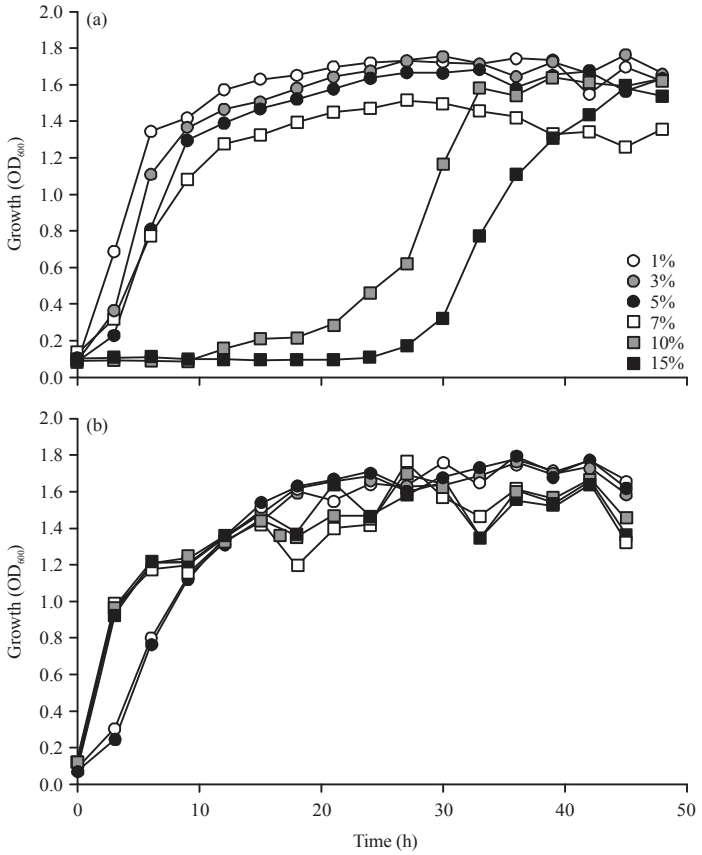

Fig. 4(a-b): Growth of strain LM1KK on (a) Ammonium and (b) Cows urine supplemented medium. Strain LM1KK was cultivated in 1/100 dilution meat extract medium

database with accession No. LC008302. From comparative ribosomal DNA gene sequence as well as phylogenetic analysis, LM1KK was deduced to be located in the genus *Arthrobacter* close to *Arthrobacter nitroguajacolicus* MNPB4^T (FM213395) (Fig. 3). Some species close to strain LM1KK are *A. ureafaciens* CC12B^T (FN433020), *A. nicotinovorans* S32115^T (AB648947) and *A. keyseri* 12B^T (AF256196) were also suggested take a role in organic wastes processing, that suppose they act some part in the composting reaction. By examining physiological and biochemical characteristics and comparing the 16S rRNA gene sequence, the strain was identified as a strain of *Arthrobacter* and designed as *Arthrobacter* sp. LM1KK.

Growth and ammonia removal by strain LM1KK: For being ammonia mitigation agent, *Arthrobacter* sp. LM1KK should have ability to grow in manure that contains a high concentration of animal urine in the environment. Thus, research regarding the high concentration effect of $(NH_4)_2SO_4$ and cow's urine on the growth of strain LM1KK was observed in 1/100 meat extract medium at 120 rpm aerobic shaking condition. In Fig. 4, the addition of $(NH_4)_2SO_4$ at different concentration gave different growth profiles of strain LM1KK. The growth of the strain in the liquid medium with 1, 3, 5 and 7% ammonium addition was slightly lower comparing with control (without $(NH_4)_2SO_4$). Furthermore, the addition of 10 and 15% of ammonium in medium gave impact on the delayed growth of strain LM1KK as well as prolongation of the adaptation phase until 10 h. Different phenomena were observed when strain LM1KK is growing with different concentration of cow's urine. Fresh urine cows which collected from Friesian Holstein Cow Farm at Faculty of Animal Science Universitas Gadjah Mada didn't give significant effect to the growth profiles of strain LM1KK comparing with control observed spectrophotometry until 48 h of cultivation period. High concentration of animal's urine didn't show the prohibition effect on the growth of strain LM1KK.

Ammonium removal ability of strain LM1KK in 1/100 meat extract culture medium was investigated in shaking condition at 30°C and the patterns of ammonium removal was shown in Fig. 5. Strain LM1KK could oxidize 26.86 mg L^{-1} ammonium after 48 h cultivation time.

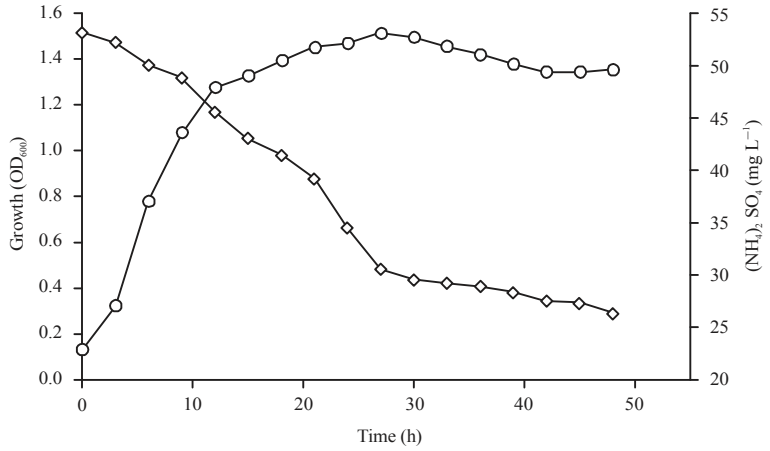

Fig. 5: Growth curve of strain LM1KK in the presence of 7% $(NH_4)_2SO_4$ (black circle) and reduction of $(NH_4)_2SO_4$ during cultivation period

DISCUSSION

At atmospheric pressure, ammonia (NH_3) is a colorless gas, which is lighter than air and possesses a strong, penetrating odor. Gaseous NH_3 is emitted from livestock production due to the excretion of nitrogen (N) from undigested protein from animal feed. Livestock manures contain N in organic forms. Excess N in the feed and inefficient utilization of crude protein or amino acids in animal diets is the source of N in excreted urine and feces. Most of the N (up to 97%) is excreted as urea in the urine of sheep, cows and pigs, while the rest is excreted as undigested organic N in the feces[8,24]. Within hours to a few days, urea is then hydrolyzed to NH_4^+ in a process catalyzed by the microbial enzyme urease originating mainly from feces[4]. In contrast, the microbial breakdown of organic N in feces into NH_4^+ in a process named as ammonification or mineralization requires months or even years to effect. The NH_4^+ resulting from either urea hydrolysis or organic N decomposition or both is the one susceptible to volatilization from manure depending on pH and temperature conditions. In laboratory scale study conducted by Ohta and Ikeda[25] the optimum conditions for emission of odor from animal manure were observed at pH 8.6-10, temperature 35-40°C and moisture content 42-63%.

Treatments using microorganisms have been extensively used in municipal livestock waste for degradation of organic matter[26]. Furthermore, microbial treatments are emerging to treat livestock waste since degradation of organic matter in livestock waste relies on microorganisms[27]. In the other hand, using microorganisms as additive to encounter odor as well as ammonia emission from livestock production is become promising methods due to cheap and fast in action. Biological treatment processes for the ammonia mitigation from animal production are either based on the assimilation and immobilization of volatile N or the transformation of volatile N into non-volatile inorganic N as fertilizer[4]. The conversion of volatile N to non-volatile is a major biological treatment process comprising of coupled nitrification and denitrification processes that occur in the various environment. Nitrification is one of the key processes involved in the N cycle. In various ecosystems, ammonia-oxidizing bacteria oxidize NH_3 to nitrite (NO_2^-), whereas NO_2^- is further oxidized to nitrate (NO_3^-) by nitrite-oxidizing bacteria. The major grouping of ammonia-oxidizing bacteria belongs to the subclass Beta-proteobacteria[28].

As a tropical country, Indonesia has continuously warm conditions which suppose to be a potential habitat for nitrifying bacteria. We had isolated soil bacterium from the odorous region of layer hens farm at Yogyakarta city and based on biochemical and physiological observation as well as gene analysis of 16S rRNA the strain was identified as *Arthrobacter* sp. LM1KK. Strain LM1KK has the ability to grow in high concentration ammonium contained medium. A phylogenetic tree (a neighbor-joining tree) analysis, based on the 16S rRNA gene sequences, showing the relationship of strain LM1KK with other ammonia-oxidizing bacteria was constructed by the same method as described in a previous report[29].

This strain can grow well in both yeast extract and cow's urine medium. The reaction rate of nitrification of strain LM1KK is low compared to that of another strain such as *Pseudomonas stutzeri*[10] YZN-001 or *Alcaligenes faecalis*[15]. Strain LM1KK could oxidize ammonium 26.86 mg L^{-1} during 48 h of the cultivation period. Therefore, during the

nitrification, we suggest this strain transformed ammonium nitrogen to oxidize into N (nitrite and nitrate) and then these compounds biologically reduced to N_2 by denitrifying bacteria that concern us for the next research. The selected bacterium LM1KK was highly tolerant of $(NH_4)_2SO_4$, suggesting that strain LM1KK is adaptable to animal wastes being composted or in animal's manure.

The key in using bacterial cultures for deodorization of manure is to have the added bacteria become the predominant strain in the manure. For the added bacteria to the best growth, the real environment should appropriate from the optimum growth range for the bacteria. The indigenous flora (not only for those reducing odors) of the wastes always grew better than the inoculated microorganisms. In addition, the selected microorganisms may even use other organic compounds in preference to the malodorous substances when inoculated in wastes. Since predominance of the added bacteria is critical to the treatment, the quantity of bacterial material is questionable. Usually, the indigenous microorganisms are present in high concentration and are able to grow rapidly. Therefore, massive inoculation has to be exercised to accelerate the development of the added bacteria. Such massive inoculation can be achieved only on a laboratory scale, not at the farm level where the number of manure to be treated are considerable.

Nitrification is the more important step and usually receives more attention in the biological treatment of wastewaters for removal of NH_3. The selected bacterium LM1KK was highly tolerant of $(NH_4)_2SO_4$, suggesting that strain LM1KK is adaptable to animal wastes being composted or in animal's manure.

According to Liao and Bundy[30], microbial digestive additives contain bacteria or enzymes that eliminate odors and suppress gaseous pollutants by their biochemical digestive processes. Bourque *et al.*[31] conducted study on microbiological degradation of odorous substances of swine manure on a laboratory scale under aerobic conditions. The bacterial culture under study was inoculated into sterilized animal manure and incubated for a maximum of 6 days at 29°C. They found that three bacterial species (*Acinetobacter calcoaceticus*, *Alcaligenes faecalis* and *Arthrobacter flavescens*) could completely degrade all types of VFAs in swine manure while *Corynebacterium glutamicum* and *Micrococcus* sp., could only degrade acetic and propionic acids. Another laboratory experiment was done by Jolicoeur and Morin[32] which reported that *Acinetobacter calcoaceticus* could degrade VFAs in both sterilized and non-sterilized animal slurry incubated at 22°C within pH 6.2-8.6 for 21 days.

Since the source of the odorous compounds is mainly microbial in origin, a sustained, rational research initiative is required using well-developed classical anaerobic microbiology technology, combined with modern molecular techniques and the latest analytical/sensory methodology, to determine the fundamentals controlling the production of malodor[33].

This strain was expected to be involved most treatments with several variations of physical, chemical or components of both physical and chemical unit processes to provide suitable conditions for the processes to occur efficiently and cost-effectively in mitigation of ammonia. We suggest that during nitrification, this strain transformed total ammoniacal nitrogen to oxidize into N (nitrite and nitrate). These compounds are then biologically reduced to environmentally benign N gas (N_2) by denitrifying bacteria as our next concern in performing research.

CONCLUSION

The indigenous ammonia-oxidizing bacterium has successfully isolated from biological deodorization plants at poultry farm Yogyakarta Indonesia as tropical area and it was identified as *Arthrobacter* sp. LM1KK. The strain could growth both in ammonium high contain medium and animals urine medium with efficiently remove ammonium during the nitrification process. It indicates this strain could serve as microbial manure deodorization additive.

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