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Isolation and Identification of a Lactic Acid Bacterial Strain KJ-108 and Its Capability for Deodorizing Malodorous Gases Under Anaerobic Culture Conditions

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Abstract A number of different sources, such as composts, leachates, and pig feces samples, were collected from different pig farms in Korea, and several microorganisms were screened for their ability to deodorize the malodorous gases. Consequently, a novel malodorous gases-deodorizing bacterial strain, KJ-108, was isolated, because it was highly abundant in nitrate-supplemented minimal medium (MM-NO_3^-) under anaerobic culture conditions. Airtight crimp-sealed serum bottles containing MM-NO_3^- medium were inoculated with KJ-108. Nitrate concentration was decreased rapidly after 20 h of incubation, and incubation was carried out until nitrite production reached almost zero. Taxonomic identification, including 16S rDNA base sequencing and phylogenetic analysis, indicated that the isolate had 100% homology in its 16S rDNA base sequence with *Lactobacillus pentosus*. Among the volatile fatty acids, acetic acid contained in large amounts in fresh piggery slurry was decreased by about 40% after 50 h incubation with strain KJ-108. *n*-Butyric acid, *n*-valeric acid, and isovaleric acid were gradually decreased, and isobutyric acid and capronic acid were dramatically eliminated at the initial period with the treatment. Moreover, NH_3 removal efficiency reached a maximum of 98.5% after 50 h of incubation, but the concentration of H_2S was not changed.

Key words: Deodorization, malodorous gases, piggery slurry, lactic acid bacterium

With development of the livestock industry, large amounts of domestic animal excrement have accumulated. The livestock wastes in Korea are amassed at the rate of four million tons (dry matter) annually [12]. As a result, the excrement has created environmental pollution, such as the contamination of water resources and the production of obnoxious odors. Odor pollution causes significant health problems for workers and even nearby residents [22]. In addition, malodorous gases can lead to industrial disasters, and therefore, lowers production efficiency, causing economical losses. In particular, ammonia (NH_3), hydrogen sulfide (H_2S), and menthanethiol (CH_3SH) of piggery wastewaters are irritating, smelly substances with low odor thresholds [11, 14]. These three unwanted gases are usually liberated in livestock farming and wastewater treatment [4, 7, 22, 27]. Many technologies have been used to treat these malodorous compounds from air contaminated by piggery slurry [5, 10, 11, 17]. As regulatory measures are required to control for malodorous compounds, the demand for cost-efficient air pollution control technology will increase. Currently, treatment by microorganisms has drawn great attention, because they cost less than conventional methods and have a comparable removal efficiency of offensive gases [17]. Therefore, in the present study, isolation and identification of microorganisms able to deodorize malodorous compounds were undertaken, and removal characteristics of obnoxious gases from piggery slurry or wastewater were investigated.

Materials and Methods

Media for Screening Microorganisms

The piggery slurry agar medium, minimal medium (MM), and nitrate-supplemented minimal medium (MM-NO_3^-) were used for isolation of microorganisms. The piggery slurry medium was composted with 300 g of air-dried pig feces and 15 g agar per 1 l. pH was adjusted to 7.0 before

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addition of agar and then autoclaved for 20 min at 15 psi pressure and 121°C. The MM contained NH_4Cl, 0.3 g; KH_2PO_4, 1.5 g; and $Na_2HPO_4 \cdot 7H_2O$, 7.9 g; per 1 l. MM-NO_3^- included the same as MM but with an added 2.0 g of KNO_3. Both media were adjusted to pH 7.0 with 5 N NaOH. Na-succinate, an intermediate in the TCA cycle, was used as a sole carbon source and as the terminal electron receptor to support maximum growth of the bacteria.

Isolation of Microorganisms to Deodorize Malodorous Gases

Various samples were collected from different sources, such as composts, leachates, and pig feces, for screening of microorganisms available for deodorizing of piggery slurry from different pig farms in Korea. Thirty specimens were collected, and 0.5 g of each sample was mixed with 4.5 ml of distilled water and left for 2 h to settle. The mixed suspensions were diluted to 10^{-1}–10^{-9} and 100 µl of each diluted sample was sprayed onto pig slurry agar medium [30% (w/w) air-dried pig feces and 60% (wt/wt) moisture contents] and incubated in BBL anaerobic jars (Anaerobic System, Difco) at 30°C for 24 h. From these plates containing 30–300 colonies, each colony formed on the plates was streaked on a tryptic soy agar (pancreatic digest of casein 15 g, papaic digest soybean meal 5 g, NaCl 5 g, and agar 15 g per 1 l) plate to further purify it by single colony isolation. The purified isolates that exhibited good growth on the plates were selected and then transferred to MM or MM-NO_3^- agar plates in an AnaeroPack system (Mitsubisui Gas Co., Tokyo, Japan) at 30°C to investigate the reduction of nitrate. The nitrate removal test or nitrite formation was carried out in MM-NO_3^- broth medium in crimp-sealed serum bottles under anaerobic conditions. The isolates exhibiting urease activity, a potent inhibitor of aceticlastic methanogens, on a urea test agar medium [10], were excluded. Determination of optimal pH and temperature for growth of bacterial strains was carried out on piggery slurry medium. The morphological characteristics of the isolates were observed with a Zeiss microscope (Zeiss, Jena, Germany).

Determination of Biochemical and Physiological Characteristics of the Five Isolates

The biochemical and physiological characteristics were determined by the method of Gerhardt *et al.* [9], and carbon utilization, optimum pH, and temperature for growth, a hemolysis test, homo-hetero fermentation test, and Gram straining were also investigated.

Test for Antibiotic Sensitivity

Antibiotics are commonly added to animal feed to treat pig infections, therefore, it is important to choose antibiotic resistant bacteria for use in wastewater treatment systems. Bacterial suspensions of the isolates precultured for 24 h were spread onto the surface of Antibiotic Medium II (Difco) agar plates with sterile swabs to create a bacteria lawn. Thirteen different kinds of antibiotic discs (Difco) were then placed on the bacterial lawn using sterile forceps. The plates with a bacterial lawn and antibiotic discs were incubated at 30°C for 24–48 h to verify their antibiotic resistance. The antibiotics used for this study were bacitracin, cabenicillin, cephalothin, chloramphenicol, clindamycin, erythromycin, gentamycin, kanamycin, lincomycin, penicillin G, rifampin, streptomycin, and tetracycline.

Growth and Nitrate Reduction of the Five Isolates on Nitrate Agar Plates Under Anaerobic Conditions

Five isolates, KJ-005, KJ-007, KJ-108, KJ-1033, and KJ-1260, were inoculated onto nitrate plates (beef extract, 3 g; peptone, 5 g; potassium nitrate, 1 g; agar, 15 g; per 1 l) and incubated in anaerobic jars to monitor nitrate reduction under anaerobic culture conditions. Nitrite production was determined by the method of Murray *et al.* [18]. A 0.1 ml solution of sulfanilamide, composed of 5 g of sulfanilamide in 500 ml 10% HCl solution, was added to the colonies grown in 0.1 ml NED solution, including 0.5 g *N*-(1-naphthyl) ethylenediamine dihydrochloride in 500 ml demineralized water, and immediately mixed. The color was measured after 10 min.

Anaerobic Growth and Nitrate Reduction of the Five Isolates in MM or MM-NO_3^- Liquid Medium

Thirty ml of MM or MM-NO_3^-, containing 100 mM Na-succinate as a sole carbon and energy source, was added to 60-ml crimp-sealed serum bottles. A 1.5 ml cell suspension of the five isolates was further added to bottles and the suspension was incubated at 30°C. Optical density of cells, and nitrite and nitrate measurements were periodically carried out.

Growth and Nitrate Reduction of the Isolate KJ-108 and KJ-1033 in MM or MM-NO_3^- Liquid Medium Under Anaerobic Culture Conditions

Fifty ml of MM or MM-NO_3^-, containing 100 mM Na-succinate as a sole carbon and energy source, was added into 125-ml crimp-sealed serum bottles. A bacterial suspension of KJ-108 and KJ-1033 was inoculated into triplicate serum bottles and incubated at 30°C. Gas samples were taken out from the headspace of the crimp-sealed serum bottles to analyze nitrogen. Optical density of cells, and nitrite and nitrate measurements were periodically carried out.

Analysis of 16S rDNA Sequence to Identify the Isolate KJ-108

The chromosomal DNA was isolated by the method described elsewhere [28]. The amplification of the 16S rDNA was conducted using two primers according to Stackebrandt and Liesack [24], 5'-GAGTTTGATCCTGG-

CTCCAG-3' and 5'-AGAAAGGAGGTGATCCAGCC-3'. A PCR was run for 35 cycles in a DNA thermal cycler [Genetic analyzer 377 (Perkin-Elmer, Boston, U.S.A.], employing the thermal profile according to Yoon *et al.* [28]. The 16S rDNA sequence of the isolate KJ-108 was aligned using CLUSTAL W software [19] and the evolutionary distance matrices were calculated with the DNADIST program within the PHYLIP package [8]. The sequence of representative species of the genus *Lactobacillus* and related taxa were cited using the GenBank Database. The values of 16S rDNA similarity were calculated from the alignment, while the evolutionary distances were calculated using a Kimura two-parameter correction. A phylogenetic tree was constructed using the neighbor-joining method [23], based on the calculated distance matrix.

Comparison of the Volatile Fatty Acids Removal Efficiency by the Isolate KJ-108 and KJ-1033

The volatile fatty acids, which are the main compounds of piggery slurry odor, were analyzed quantitatively by a gas chromatography using fresh pig feces prepared as follows. A 500 g wet matter of fresh pig feces was added to 1,000 ml of tap water, left to stand for 10 min at room temperature, and filtered through a sheet of gauze. A 30 ml aliquot of fresh pig feces medium was incubated with 12 h-old cultures of KJ-108 and KJ-1033, having a cell density of 5×10^7 cells ml^{-1}, in 60-ml serum bottles. The bottles were then sealed by self-sealing butyl-rubber stoppers and aluminum seals and incubated at 37°C for up to 70 h, along with uninoculated controls in anaerobic jars. The gas in the headspace from inoculated and uninoculated bottles was analyzed for volatile fatty acids by gas chromatography (GC; Model 263-50, Hitachi Ltd., Tokyo, Japan) with a flame-ionized detector. Helium was used as a carrier gas at a rate of 30 ml min^{-1}. The column oven temperature was kept at 190°C and the injection port was maintained at 210°C.

Changes of Ammonia and Hydrogen Sulfide Concentration by the Isolate KJ-108

A 30 ml aliquot of fresh pig feces medium was incubated with 12 h-old culture of *C. perfringens* AB&J, having 5×10^7 cells ml^{-1} density, in 60-ml serum bottles. The bottles were then sealed by self-sealing butyl-rubber stoppers and aluminum seals, and incubated at 37°C for up to 70 h along with uninoculated controls in anaerobic jars. The gas in the headspace from inoculated and uninoculated bottles was analyzed for the presence of ammonia and hydrogen sulfide at 10 h intervals by gas chromatography (GC; Model 263-50, Hitachi Ltd., Tokyo, Japan).

Analytic Methods for Oxygen and Nitrogen Determination

Ten grams of a sample was suspended in 100 ml of distilled water, left to stand for 10 min at room temperature, and pH was measured. Nitrite was determined by absorbance at 543 nm [18], and nitrate was measured by the spectrophotometric screening method [1] by calculating the difference between O.D. at 220 nm and 2 × O.D. at 275 nm. For O_2 and N_2 determination, 20 µl of samples were taken from the headspace of the serum bottles by a pressure-lock syringe (Supelco, Inc., Bellefonte, PA, U.S.A.) for gas chromatographic analysis (GC; Hitachi Ltd., Tokyo, Model 263-50) with a flame-ionized detector.

RESULTS AND DISCUSSION

Isolation and Characteristics of the Five Isolates

No less than a thousand bacterial isolates were collected from the colonies from different sources, such as composts, leachates, and pig feces. The isolates were examined for whether or not they could grow well on MM or MM-NO_3^- agar plates. Consequently, five isolates exhibiting good growth on MM-NO_3^- agar plates were isolated from composts and pig feces under anaerobic conditions: Two bacterial strains, KJ-005 and KJ-007, were isolated from composts, whereas the rest of three bacteria, KJ-108, KJ-1033, and KJ-1260, were isolated from pig feces. Morphological, physiological, and biochemical characteristics of the five isolates are listed in Table 1, and the antibiotic sensitivity in Table 2. The isolate KJ-108 showed intermediate reaction only to lincomycin. The results of growth on MM or MM-NO_3^- and nitrate agar plates are shown in Table 3. All the isolates, except KJ-005, were able to reduce nitrate on solid agar plates. However, none of the isolates could grow on MM agar plates, because of lack of nitrate to act as the terminal electron acceptor for anaerobic respiration. As seen in Fig. 1, the isolate KJ-108 was able to predominately grow on MM-NO_3^- liquid medium without any additives under anaerobic culture conditions: After 30 h of incubation, the isolate KJ-108 reached its maximum cell density, while the isolate KJ-1033 reached it after 60 h. The final absorbance at 660 nm of isolates KJ-007 and KJ-1260 never exceeded 0.4. Because of the lower cell density of the isolate KJ-005 under the same incubation conditions, removal efficiency of its unwanted gases was the worst among the five isolates. Therefore, the isolate KJ-108 was selected for further investigation of malodorous compounds removal under anaerobic conditions. Based on its physiological, biochemical, and morphological characteristics, the isolate KJ-108 was found to be very similar to lactic acid bacteria, such as *Lactobacillus* sp. The isolate KJ-108 did not produce any clear zones surrounding the colonies on blood agar plates: Namely, a hemolysis test of the KJ-108 was negative (Table 1). There are a few reports on the biodegradation of obnoxious compounds from wastewaters by microorganisms. For example, *Arthrobacter oxydans* [21], *Tolura* sp. [2],

Table 1. Morphological and biochemical characteristics of the five isolates.

Characteristics	Isolate				
	KJ-005	KJ-007	KJ-108	KJ-1033	KJ-1260
Shape	Rod	Rod	Short rod	Rod	Rod
Gram stain	-	-	+	-	-
Optimum temperature for growth	30	35	37	30	30
Optimum pH for growth	6.0	5.5	6.0	6.0	6.5
Growth at 45°C	-	+	+	-	+
Growth at 15°C	+	+	+	+	+
Motility	+	+	+	+	+
Catalase	-	+	-	+	+
Hemolysis	-	-	-	-	-
Homo-hetero fermentation	Hetero	Hetero	Homo	Hetero	Hetero
Carbohydrate utilization					
Amygdalin	-	-	+	-	-
Arabinose	-	+	-	+	+
Cellulose	-	-	+	-	-
Esculin	-	-	+	-	-
Fructose	+	-	+	+	+
Galactose	+	+	+	+	+
Glucose	-	+	+	+	+
Gluconate	+	-	+	-	+
Lactose	+	+	+	+	-
Maltose	+	+	+	+	-
Mannitol	+	-	+	+	+
Mannose	+	+	+	-	+
Melibiose	+	-	+	-	-
Raffinose	+	-	+	+	-
Rhamnose	+	+	+	+	-
Ribose	+	+	+	-	+
Sorbital	+	-	+	-	-
Sucrose	-	-	+	+	-
Xylose	+	+	+	+	-
On demand	FAA	FAA	FAA	FAA	FAA

FAA, facultative anaerobic.

Table 2. Antibiotic sensitivity of the five isolates.

Antibiotics	Bacterial strain				
	KJ-005	KJ-007	KJ-108	KJ-1033	KJ-1260
Bacitracin	S	R	R	R	R
Cabenicillin	S	R	R	S	R
Cephalothin	S	R	R	R	I
Chloramphenicol	S	S	R	R	R
Clindamycin	S	S	R	R	R
Erythromycin	S	I	R	S	R
Gentamycin	S	R	R	S	I
Kanamycin	S	R	R	S	R
Lincomycin	S	R	I	R	R
Penicillin G	S	R	R	R	R
Rifampin	S	S	R	R	S
Streptomycin	R	S	R	S	R
Tetracycline	S	S	R	S	R

S: Sensitivity; R: Resistance; I: Intermediate reaction.

Thiobacillus thioparus [4], *Arthrobacter oxydans* [5], *Nitrosomonas europaea* [3], *Vibrio alginolyticus* [11], *Pseudomonas putida* [6], *Bacillus stearothermophilus* [15], and blue-green algae [13] were used for removal of unwanted gases. Thus, the current study seems to be the first to report that lactic acid bacteria are also able to degrade piggery slurry or wastewater odors.

Growth and Nitrate Reduction of the Five Isolates in MM or MM-NO_3^- Liquid Medium Under Anaerobic Culture Conditions

None of the isolates could grow in MM medium under anaerobic culture conditions. Nevertheless, all the isolates grew well in MM-NO_3^- medium, except the isolate KJ-005 (Fig. 1). Thus, nitrate reduction by KJ-005 was almost zero. At 20 h of incubation, the nitrate removal efficiencies of isolates KJ-007, KJ-108, KJ-1033, and KJ-1260 were 22.2%, 93.8%, 26.3%, and 38.2%, respectively (Fig. 2).

Table 3. Cell growth or nitrate reduction of the five isolates on MM, MM-NO_3^-, and nitrate agar plates under anaerobic culture conditions.

Strain	MM agar plate		MM-NO_3^- agar plate		Nitrate agar plate	
	Cell growth	Nitrate reduction	Cell growth	Nitrate reduction	Cell growth	Nitrate reduction
KJ-005	-	-	-	-	-	-
KJ-007	-	-	+	+	+	-
KJ-108	-	-	++	++	++	++
KJ-1033	-	-	+	+	+	+
KJ-1260	-	-	+	+(-)	+	+(-)

++, strong reaction; +, slight reaction; +(-) blow detection because very weak reaction; -, no reaction.

Meanwhile, KJ-1033 revealed the highest nitrate removal efficiency at 60 h of incubation, and the KJ-007 and KJ-1260 exhibited 33.3% and 42.8% at 40 h, respectively. Figure 3 shows that nitrite production by isolate KJ-007 and KJ-1260 was maximal at 60 h, indicating that the isolate KJ-007 and KJ-1260 could reduce nitrate to nitrite. Nitrite concentration produced by isolates KJ-108 and KJ-1033 was almost zero at the end of the experiment. These results show that nitrate was reduced to nitrite and even further to nitrogen gases by the isolates KJ-108 and KJ-1033. Indeed, the nitrogen gas production was evidenced by bubbles appearing inside the crimp-sealed serum bottles.

Growth and Nitrate Reduction of the Isolate KJ-108 and KJ-1033 in MM or MM-NO_3^- Liquid Medium Under Anaerobic Culture Conditions

Based on the nitrate and nitrite removal efficiencies of the five isolates (Figs. 2 and 3), isolates KJ-108 and KJ-1033 were selected for investigation of oxygen consumption. Both isolates showed good growth in the MM-NO_3^- liquid medium, but KJ-1033 appeared to grow better than KJ-108 with high oxygen consumption (Table 4). The initial oxygen concentrations in bottles inoculated with isolates KJ-108 and KJ-1033 were determined by GC measurement. For 70 h of incubation, the oxygen concentration in bottles of MM-NO_3^- inoculated with KJ-108 decreased from 3.19 to 2.75 $mmol^{-1}$, while nitrate was reduced simultaneously to nitrogen gas. A similar course was also observed with the isolate KJ-1033. These suggest that isolates KJ-108 and KJ-1033 could reduce nitrate to nitrogen gas without accumulation of nitrite under anaerobic culture conditions. The results presented in Table 4 indicate that the concentration of oxygen in the headspace of sealed serum bottles was not a limiting factor during the entire investigational period. Accordingly, isolate KJ-108 had a better anaerobic denitrification capability than KJ-1033 (Fig. 4). The denitrification was considered as an anaerobic process by previous investigators [16, 25, 29]. Stouthemer [25] and Zumft *et al.* [29] reported that three groups of

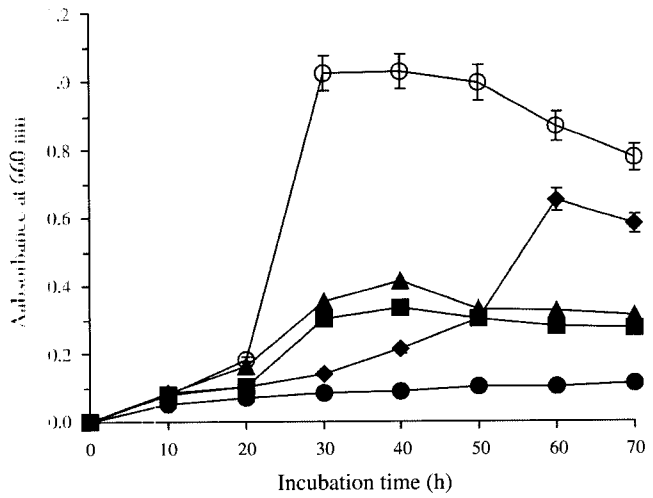

Fig. 1. Time course of the five bacterial isolates grown in MM-NO_3^- liquid medium under anaerobic conditions at 30°C and pH 7.4.

The initial cell concentration of 1,000 µl was adjusted to approximately 1.3 at 660 nm. Symbols are (●), KJ-005; (■), KJ-007; (○), KJ-108; (◆), KJ-1033; (▲), KJ-1260.

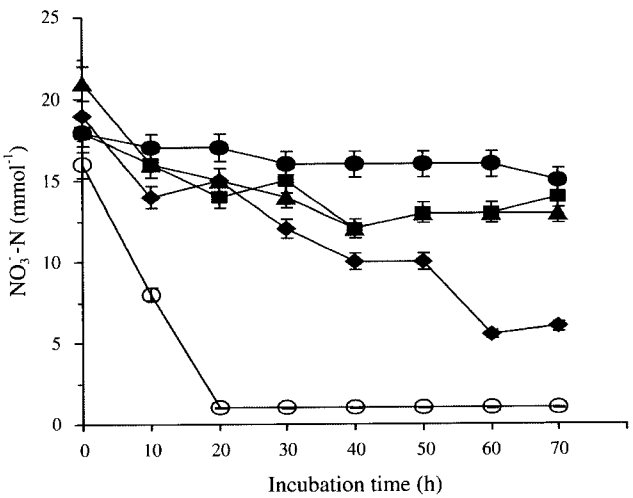

Fig. 2. Removal of NO_3^--N by the five bacterial isolates, KJ-005 (●), KJ-007 (■), KJ-108 (○), KJ-1033 (◆), and KJ-1260 (▲), incubated in MM-NO_3^- liquid medium under anaerobic culture conditions.

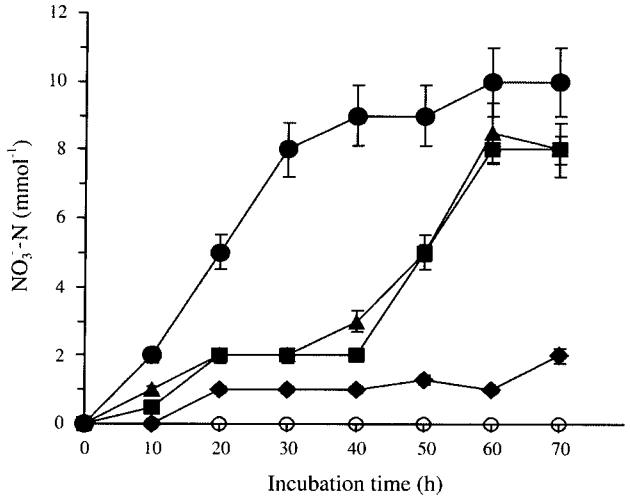

Fig. 3. Removal of NO_3^--N by the five different bacterial isolates, KJ-005 (●), KJ-007 (■), KJ-108 (○), KJ-1033 (◆), and KJ-1260 (▲), incubated in MM-NO_3^- liquid medium under anaerobic culture conditions.

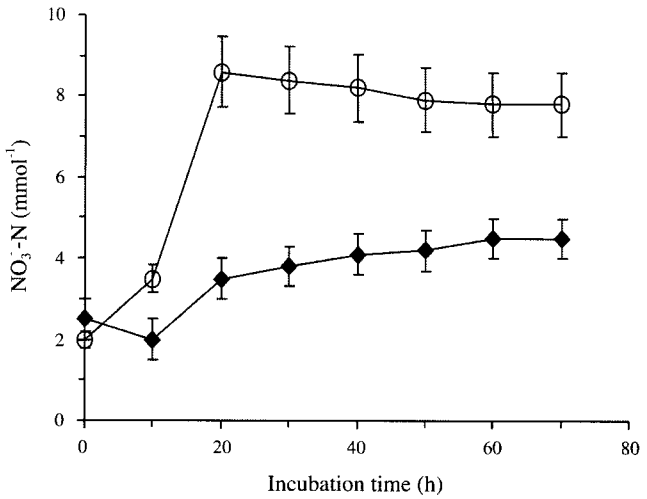

Fig. 4. Nitrogen gas production by isolates KJ-108 (○) and KJ-1033 (◆) incubated in MM-NO_3^- liquid medium under anaerobic culture conditions.

denitrification by different *Pseudomonas stutzeri* strains under anaerobic conditions were based on the denitrification mechanism. The first group consisted of 50% of isolates and reduced nitrate to form nitrogen gas without nitrite accumulation. The second group was composed of 25% of isolated strains, which performed two steps ($NO_3^-\rightarrow NO_2^-$ and then $NO_2^-\rightarrow N_2$) with a distinct lag phase between these two denitrification steps. The third group consisted of the remaining isolates that accumulated low concentration of nitrite [20]. Accordingly, the isolate KJ-108 belongs to the first group. Su *et al.* [26] suggested that promotion of the nitrite and nitrate removal efficiency by *Pseudomonas stuzeri* SU2 in piggery wastewater treatment under aerobic conditions is also very important. However, in the present study, nitrite and nitrogen were found to be removed only during the anaerobic treatment step. Thus, the isolate KJ-108 should be studied further and applied to *in situ* piggery slurry to biologically remove more nitrogen.

Table 4. Oxygen consumption by isolates KJ-108 and KJ-1033 in MM or MM+NO_3^- broth incubated under anaerobic culture conditions.

Incubation time (h)	Concentration of oxygen in atmosphere (mmol^{-1})			
	KJ-108		KJ-1033	
	MM	MM-NO_3^-	MM	MM-NO_3^-
0	2.94±0.23	3.19±0.18	3.09±0.19	2.98±0.33
10	2.92±0.22	3.17±0.21	2.66±0.11	2.85±0.12
20	2.89±0.24	3.15±0.15	2.410.13	2.60±0.38
70	2.60±0.20	2.75±0.23	2.17±0.18	1.67±0.36
Consumption (%)	11.56	13.79	29.77	43.96

Elimination of the Volatile Fatty Acids by the Isolates KJ-108 and KJ-1033

Gas chromatographic analyses of the volatile fatty acids in fresh pig feces medium are shown in Fig. 5. The volatile fatty acids odors, which are the main compounds of piggery wastewater, were diminished by isolates KJ-108 and KJ-1033. Among the volatile fatty acids, a large amount of acetic acid present in fresh piggery slurry was decreased by about 40% after 40 h of incubation with the isolate KJ-108. *n*-Butyric acid, *n*-valeric acid, and isovaleric acid were gradually decreased, and isobutyric acid and capronic acid were dramatically eliminated in the initial phase. The minor change of acetic acid by the treatment with isolate KJ-108 under anaerobic conditions could be due to the degradation of the volatile fatty acids to low molecular acetic acid. Then, the removal efficiency of acetic acid was found to be low. With the treatment of KJ-1033, all of the volatile fatty acids investigated were only slightly decreased or not changed, except, capronic acid which was dramatically decreased after 40 h of incubation. In contrast, a large amount of acetic acid contained in fresh poultry feces was decreased by 90% after 3 days treatment with fungus *Tolura* sp. [2]. However, its growth rate, that is one disadvantage, was extremely low. Furthermore, the period of acclimation to reach a steady state sometimes took about one month, indicating that it is not suitable for *in situ* piggery wastewater treatment. In conclusion, therefore, the isolate KJ-108 appears to be a better and more useful candidate for piggery slurry treatment to remove offensive gases.

Changes in Ammonia and Hydrogen Sulfide Concentration by the Isolate KJ-108

The changes in ammonia and hydrogen sulfide concentrations and the removal ratios are shown in Fig. 6. Ammonia

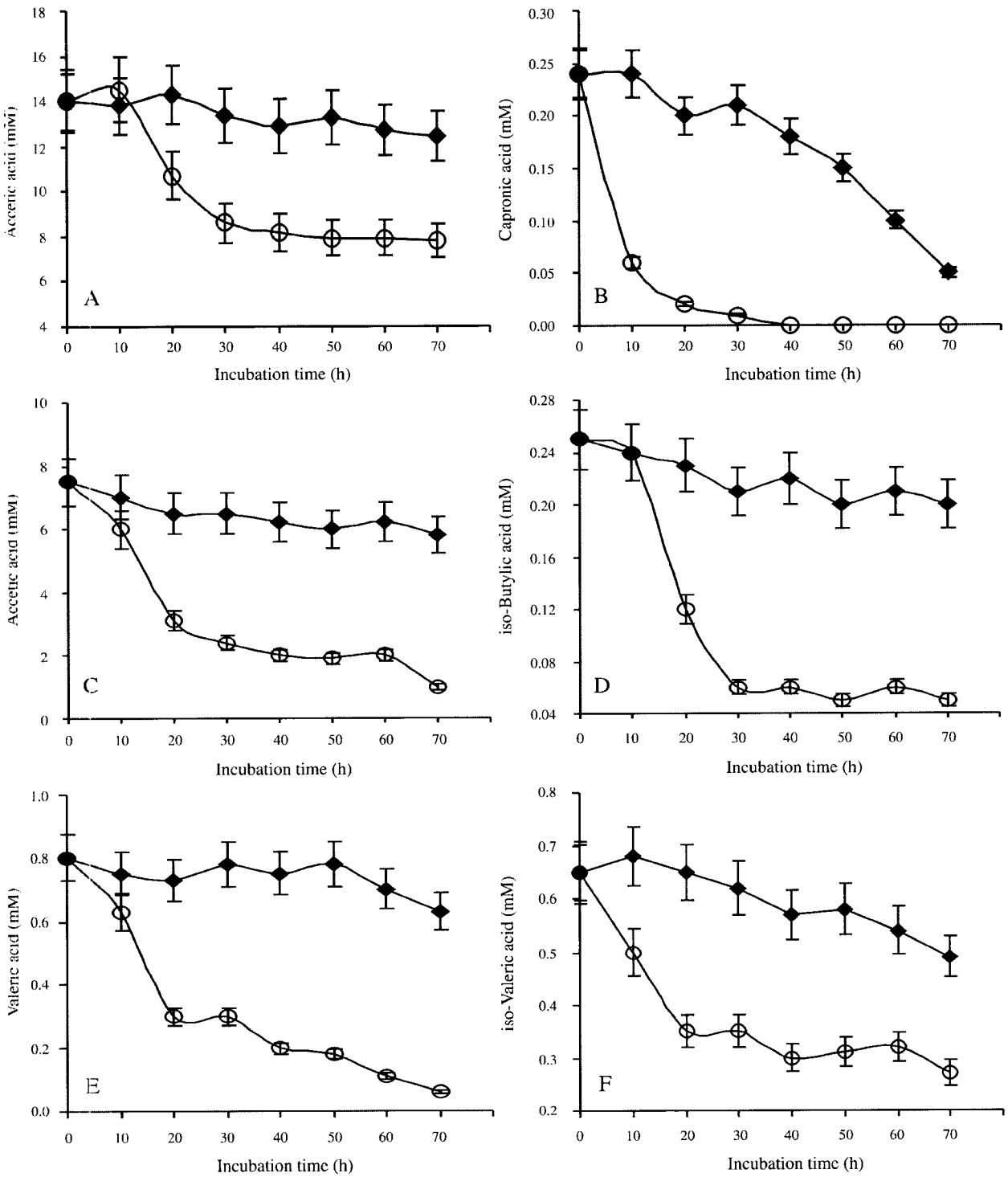

Fig. 5. Change of the volatile fatty acid concentrations by the treatment with isolates KJ-108 and KJ-1033 incubated at 37°C and pH 6 in an anaerobic condition.

The initial cell concentration of 1,000 µl was adjusted to approximately 1.3 O.D. at 660 nm. (A) Acetic acid, (B) Capronic acid, (C) Butylic acid, (D) Isobutylic acid, (E) Valeric acid, and (F) Isovaleric acid. Symbols; (◆), treated with KJ-1033; (○), treated with KJ-108.

concentration was raised up to 250 ppm at the initial period, but the removal efficiency of NH_3 was improved dramatically during the incubation of the isolate KJ-108. Moreover, NH_3 removal efficiency reached a maximum of 98.5% after 50 h of incubation. On the other hand, the concentration of H_2S was not changed. As pointed out previously, the

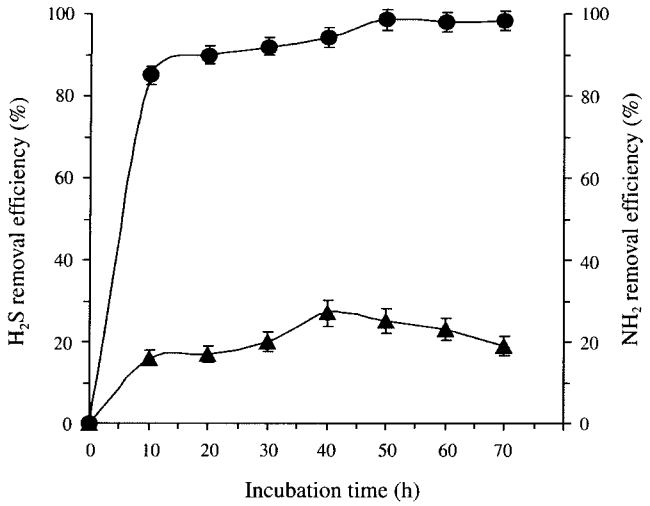

Fig. 6. Removal efficiency of ammonia (NH_3, ●) and hydrogen sulfide (H_2S, ▲) by isolate KJ-108 incubated in fresh pig feces medium at 37°C and pH 6.0 under anaerobic culture conditions.

high NH_3 concentration (>120 ppm) inhibited the H_2S metabolism of *Thiobacillus thioparus* CH11 [4]. Chung *et al.* [6] suggested the presence of NH_3 did not hinder the NH_3 removal, but a high H_2S concentration would result in low removal efficiency. In contrast, adequate concentrations of H_2S would favor the NH_3 removal efficiency. In *Thiobacillus* sp. IW [14], the removal efficiency of NH_3 was reduced when the inlet loading rate of NH_3 was increased above 100 ppm h^{-1}. In the present study on H_2S removal efficiency, as NH_3 existed in a high initial concentration, the decrease of H_2S removal did not appear to be caused by the isolate KJ-108, and there seemed to be some relationship between H_2S removal efficiency and the initial NH_3 concentration. In order to confirm these results, the relationship between NH_3 and H_2S, when treated with KJ-108, and the influence of each gas on the growth of the KJ-108 will be further investigated.

Identification of the Isolate KJ-108 by 16S rDNA Sequence Analysis

The 16S rDNA sequence was analyzed to determine to which species the isolate KJ-108 matched with the highest homology among the *Lactobacillus* species cited in GenBank. The phylogenetic tree constructed using the neighbor-joining method is shown in Fig. 7. The sequence data were aligned to construct a phylogenetic tree. The phylogenetic position of isolate KJ-108 was then compared with certain *Lactobacillus* species and related taxa in a dendrogram. In the phylogenetic tree, isolate KJ-108 was closest to *Lactobacillus pentosus* JCM 1558 and part of a robust monophyletic cluster with *L. plantarum* JCM 1149 and *L. arizonensis* DSM 13273. The level of sequence similarity of isolate KJ-108 within the monophyletic cluster was greater than 97% (Table 5). The sequence of isolate KJ-108 was almost identical with that of *L. pentosus* JCM 1558 with a 100% similarity. Hence, this appears to be the first report that *L. pentosus* KJ-108 has a capability to deodorize the malodorous gases from piggery slurry. This

Table 5. Levels of 16S rDNA similarity for isolate KJ-108, the type strains of some *Lactobacillus* species and representatives of some other related taxa.

Strain	% of Similarity:																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1. Strain KJ-108																			
2. *Lactobacillus pentosus* JCM 1558^T	100																		
3. *Lactobacillus plantarum* JCM 1149^T	99.9	99.9																	
4. *Lactobacillus arizonensis* DSM 13273^T	97.4	97.4	97.3																
5. *Lactobacillus kimchii* KCTC 8903^T	93.0	93.0	92.9	90.6															
6. *Lactobacillus alimentarius* DSM 20249^T	93.2	93.2	93.2	91.0	98.4														
7. *Lactobacillus collinoides* JCM 1123^T	94.1	94.1	94.0	91.9	92.3	92.0													
8. *Lactobacillus brevis* ATCC 14869^T	94.3	94.3	94.2	92.1	92.2	92.2	94.1												
9. *Lactobacillus animalis* DSM 20602^T	91.5	91.5	91.4	88.6	90.1	89.8	90.4	91.6											
10. *Lactobacillus casei* ATCC 334^T	91.8	91.8	91.8	89.5	90.6	90.1	92.4	91.7	91.3										
11. *Lactobacillus reuteri* DSM 20016^T	91.0	91.0	90.9	88.8	89.2	89.6	91.4	91.2	90.2	91.6									
12. *Lactobacillus fermentum* ATCC 14931^T	91.6	91.6	91.7	88.6	90.1	90.1	91.3	91.6	89.8	90.0	93.8								
13. *Lactobacillus acidophilus* ATCC 4356^T	86.9	86.8	86.8	84.8	87.3	87.0	87.9	87.6	87.7	87.6	89.5	88.4							
14. *Lactobacillus delbrueckii* JCM 1002^T	88.3	88.3	88.2	85.9	87.2	86.9	88.5	88.3	87.2	88.4	88.9	88.9	93.8						
15. *Leuconostoc mesenteroides* NCIMB 8023^T	86.5	86.5	86.5	84.8	85.1	84.4	85.7	86.0	86.4	86.9	87.0	87.3	86.6	86.1					
16. *Weissella paramesenteroides* DSM 20288^T	89.4	89.4	89.4	86.8	87.9	88.0	88.9	89.3	89.3	89.5	89.1	88.7	86.2	86.3	90.3				
17. *Lactococcus lactis* ATCC 19435^T	86.8	86.8	86.7	85.9	85.9	85.9	86.1	86.6	86.8	86.3	86.3	85.7	85.0	85.6	83.8	86.0			
18. *Bacillus subtilis* NCDO 1769^T	88.2	88.2	88.2	85.4	87.7	87.6	86.9	87.8	89.5	87.4	86.2	86.9	87.0	87.1	85.0	86.8	85.0		
19. *Clostridium butyricum* ATCC 19398^T	83.0	83.0	83.0	81.1	83.6	83.4	83.0	82.4	84.2	83.5	82.4	82.7	82.9	82.4	80.8	82.5	82.6	84.6	
20. *Escherichia coli*	77.6	77.6	77.6	76.8	77.3	76.4	77.4	76.7	78.7	77.6	77.9	77.5	77.7	77.9	78.4	77.2	77.2	79.4	77.8

^{T}Type strain.

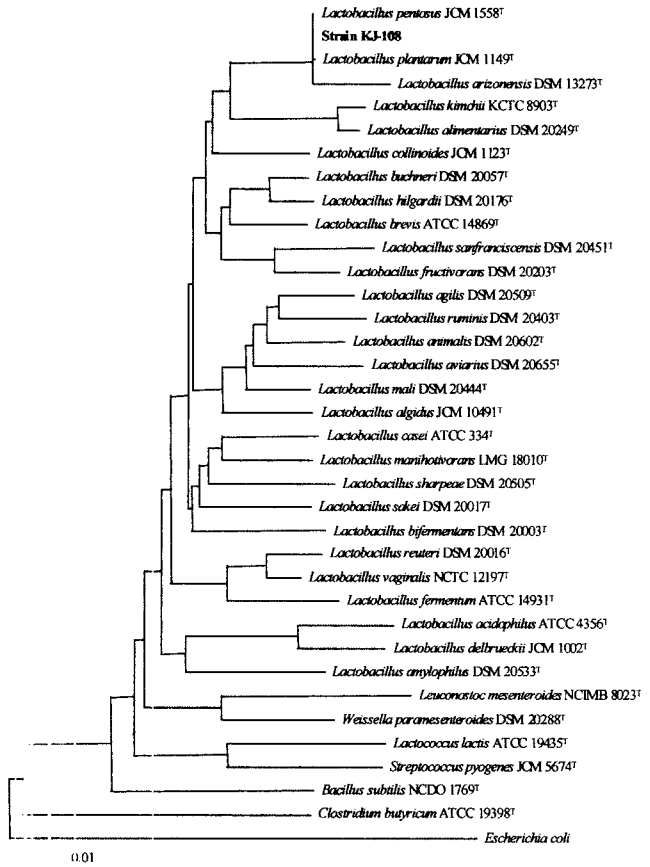

Fig. 7. Phylogenetic tree based on 16S rDNA sequences, showing position of isolate KJ-108, and reference Protobacteria organisms.
The diagram shows the position of isolate KJ-108, the type strains of some *Lactobacillus* species, and representatives from some other related taxa. Scale bar represents 0.01 substitutions per nucleotide position. T denotes type strain.

strain is shown to have an excellent denitrifying ability under anaerobic conditions, as revealed by the nitrate disappearance and nitrogen gas production. Therefore, it is suggested that the strain KJ-108 is suitable for piggery slurry or wastewater treatment in activated sludge systems in the future by means of immobilized cells.

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