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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Reduction of odours from pig manure using *Pseudomonas sp*. isolated from traditional fermented soybeans in Korea

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Abstract

Odours from animal manure have been of a significant problem in environmental pollutions. Various ways of treating livestock manure have been applied in commercial sectors. Many species of microorganisms are being used but their efficiencies for odour reduction have not been much satisfactory. In this study, *Pseudomonas sp.* is screened from the fermented soybeans, which is one of the major ingredients of traditional Korea food. Among livestock manures, pig manure is a major source of environmental pollutions more than any other livestock manure. The optimum conditions of culture medium for microorganisms were also studied and found glucose (anhydride) 1.2% (w/v) plus yeast extract 0.6% (v/v). The cultivation of the microorganisms of interest in this study was maximized when cultured for 72 hrs at 28°C, showing pH of 0.6. The effect of *Pseudomonas sp.* on reduction of NH_3 from pig manure was found highly significant. The strain of *Pseudomonas sp.* used was JGJ-1. The characteristics of JGJ-1 were identified by its physiological characteristics as well as its effect on odour reduction from pig manure.

Keywords: odour; pig manure; *Pseudomonas sp*.

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Introduction

As the national gross national product (GNP) increases along with changes of diet patterns, a large consumption of animal meat creates a scale-up production system of livestock industry in Korea. As a consequence, the animal manure treatment has become a hot issue. The untreated animal manure causes a serious environmental pollution by inappropriate dumping of the manure into river, ocean, and lands (Miyazaki et al., 2005). To reduce the odours from animal manure, rather than any of chemical agents, selection and screening of the effective microorganisms are of most importance (Schenk et al., 1979; Williams et al., 1989). The main odour chemicals from animal manure are mainly ammonia, volatile fatty acids, hydrogen sulphide, dimethyl sulphide, methyl-mercaptane, and many others. The solutions for odour problems are not just the costly expenses but more seriously are the pollution during the fermentation process of the manure (Kinya et al., 2005). Even during the collection and transportation of pig manure, the pathogenic problems occur during the transportation vehicles from farms to farms, causing a rapid spread of infectious diseases. The treatment of pig manure must be cautiously handled. Most of countries have no longer allowed the fill-up land since 2005 (Kuo et al., 2005). The odours from animal manure also create sensitive responses of mental and physiological stresses, headache, hard-breathing and various allergic reactions (Burgess et al., 2001; Selena et al., 2005). Some researchers claimed that no safe solutions had been made yet for the odour reduction of animal manure in Korea (Kim et al., 2008). The methods for solving the odour problems are physical, chemical and biological treatments (Kinya et al., 2005). The physical treatment to reduce odour from animal manure is the use of water, activated carbons, zeolite and many other natural

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minerals. The physical treatment is relatively expensive compared to other methods. The chemical method can be divided into the masking and the neutralization methods. However, the chemical treatment needs an excessive consumption of chemicals which could create the secondary contaminations and costly operation drawbacks (Burgess et al., 2001). Therefore, the biological method for odour removal is highly needed for environmental conservation (Burgess et al., 2001). In this study, a microorganism of *Pseudomonas sp.* was isolated and tested for the reduction of odours generated from pig manure.

Materials and Methods

Collection of microorganism

The pig manure was sampled from a local pig farm. The microorganism was isolated and screened from the pig manure after 1g of the pig manure was cultured with 10 ml nutrient broth (Difco, USA) in the shaking incubator and then, they were spread on the agar plate from which one single colony was harvested.

Isolation of Pseudomonas sp. (JGJ-1) and test for odour reduction

Strains of *Pseudomonas* JGJ-1were isolated from the pig manure. The effect of the *Pseudomonas* JGJ-1was tested. The single colony of the microorganism was treated in 5g of pig manure mixed with 10 ml water. It was cultured in the shaking incubation for 7 days at 28 °C. Then, the gaseous odour was measured by Gastech® (Japan).

Conditions for mass production of microorganisms

The microorganisms were cultured in the commercial microorganism fermentor (CNS Co, Korea) and tested for the effectiveness of pH condition, amount of air supply, agitation speed, and types of carbon and nitrogen sources.

Gas chromatography (GC) analysis for NH_3-N

For the microorganisms with effects on the odour reduction, NH_3-N concentrations were analyzed. For the GC analysis, each of 25ul of sample was used. The column used was IonPac AS12A, carrier gas was 12.5 mM Na_2CO_3 + 17.5 mM $NaHCO_3$, flow rate was 1.2 ml/min, and gas detection device used was the suppressed conductivity of Detector SRS Current 100 mA, which followed the Korea standard method for drinkable water quality test.

Results

Isolation of strain JGJ-1 for odour reduction in pig manure

Table 1 shows the list of strains isolated from a local pig farm in Korea. A total of 34 strains were isolated from the pig manure and among them, 12 strains were *Pseudomonas sp.*, 8 strains were *Corynebacterium*. They were mainly Gram negative and mostly resided in intestines, and excreted in manure. In Table 2, various *Pseudomonas sp.* were listed but the *Pseudomonas sp.*, JGJ-1 only was significantly effective for odour reduction.

Physiological characteristics of Pseudomonas sp. JGJ-1

Physiological characteristics of *Pseudomonas sp.* JGJ-1 were shown in Table 3. The strain survived at temp of 41°C, differing from the characteristics of the standard strain of *Peudomonasstutzeri,* producing arginine dehydrolase.

Conditions for mass production of microorganisms

Rather than lab oriented experiment purpose, the mass production conditions of microorganisms to reduce odours of animal manure were studied and shown in Table 4**.** The cost reduction is essential for commercial purpose of productions and the medium and culture conditions should be optimized in commercial production systems.

Analysis of different strains for NH_3-N reduction in pig manure

Three different groups of microorganisms were tested for their effectiveness on odour reduction. The tested groups are 1) Control 2) Treatment 1 (Bacillus sp), 3) *Pseudomonas sp.* JGJ-1. The changes of NH_3-N contents were observed as shown in Table 5. The treatment of *Pseudomonas sp.* JGJ-1 reduced NH_3-N level by more than 90%, which was also more effective than the *Bacillus sp* that is mostly used in commercial farms.

Discussion

The collection and treatment process generates a large amount of odour components. The general usage of treated pig manure was composting and recycling of fertilizers, animal feeds and aerobic methane gas production (Lee et al., 1989**)**. However, during the process of collecting and transporting pig manure, odours generated are becoming the main issue of complaints. Thus, more effective and hygienic method of odour removal development is urgently needed. The chemical and physical methods for reducing odours are highly costly and the secondary emerging problems are of environmental contamination (Cho et al., 1992). Biological treatment processes are eco-friendly and cost–efficient. In this study, an effective biological method was studied by using the strains that were screened for recycling of pig manure (Kuo et al., 2005; Ujjaini et al., 2005). The strain selected and screened was *Pseudomonas sp.* JGJ-1. This microorganism produces a variety of metabolites such as organic acids and alcohol, which increases palatability and storage period of animal feed

Table 1: Dominant members of strains found in pig manure

Bacterial genus	Species
Pseudomonas	12
Corynebacterium	9
Halomonas	7
Planococcus	3
Arthrobacter	3
Bacillus	2
A total of 6 strains	34 species

Table 2: Isolation and identification of microorganisms for odour reduction

Genus	Species	Effect of odour reduction
Pseudomonas	JGJ-1	+
Pseudomonas	*fragi*	-
Pseudomonas	*alcaligenes*	-
Pseudomonas	*pseudoalcaligenes*	-

Table 3: Physiological characteristics of *Pseudomonas*JGJ-1

Characteristics	*Peudomonasstutzeri*	*Pseudomonas*JGJ-1
Gelatin liquefaction	-	-
HydrolysisEsculin	-	-
$NO_3 \rightarrow NO_2$	+	+
Growth at 4°C	NR	NR
Growth at 41°C	V	+
Hydrolysis of Starch	+	+
Formation ofLipase	-	-
Formation of Arginine dehydrolase	-	+

*NR = no reaction; V = variable reactions; "+" = positive; " -" = negative

Table 4: Optimal medium composition and culture conditions

Laboratory Condition		Mass production culture	
Soluble starch	2.0%	Glucose(anhydride)	1.2%
Yeast extract(First grade)	1.2%	Yeast extract(Edible)	0.6%
Temperature(□)	30	Temperature(□)	28
Agitation(rpm)	200	Agitation(rpm)	180
Aeration(L/Min)	0.5	Aeration(L/Min)	0.1

Table 5: Analysis of NH_3-N Concentration

Treatment	Concentration (ng/kg-ppt)
Control 1(Before treatment)	600.12
Treatment 1(*Bacillus* sp.)	102.12
Treatment 2(*Pseudomonas* JGJ-1)	24.48

additives. The experiment was repeated to confirm the effects of the odour removal from the strain selected in this study (ISO 6326-4, 1994). And also, by analysis, ammonia and hydrogen sulphide were analyzed and the most significant effect was found by the strain JGJ-1. The volatile fatty acids were the main sources of odours in animal manure, which especially has been stored in anaerobic conditions (Burgess et al., 2001; Yun et al., 2003). Schaefer et al. (1974) also reported that the major odour sources from pig manure comprised of p-cresol (64%), phenol (26%), n-butyric acid (8.4%), skatole (1.4%), indole (0.44%), diacetyl (0.02%) and etc. Yasuhara et al. (1984) reported odours from pig manure were mixture of 2-methylpropanoic acid, butanoic acid, 3-methylbutanoic acid, pentanoicacid, pcresol, indole, 3-methylindole, dimethylsulphide, dimethyl-disulfide, butanol, 3-methylbutanol and others. In conclusion, the *Pseudomonas sp.* isolated from the traditional Korea fermented soybeans had significant effects on reduction of odours from pig manure.

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