Deodorization of Pig Feces by Fungal Application

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ABSTRACT : This study was carried out to screen yeasts effective in reducing odor from pig feces. Three isolates from soil and compost sources were selected to treat pig feces. On the basis of morphological and biochemical characteristics, one isolate from compost was *Candida rugosa*, and two isolates from soil were *Candida rugosa* and *Candida maris*. These isolates showed deodorizing activity by reducing the concentration of NH_3 and R-NH_2. Volatile fatty acids (VFA) are the specific malodorous compounds of pig feces, and the *Candida maris* from soil showed a 100% reduction of butyric, *iso*-butyric, and *iso*-valeric acid in 10% pig slurry medium. However, the *Candida rugosa* from compost showed a 100% reduction of butyric and *iso*-butyric acid while that from soil showed a 100% reduction of propionic, butyric acid and *iso*-valeric acid in the medium. Also, these yeasts were effective in reducing NH_4-N, soluble-N, and biological oxygen demand (BOD). ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 9 : 1286-1290)***

Key Words : *Candida rugosa*, *Candida maris*, Volatile Fatty Acids, Pig Feces

INTRODUCTION

Livestock waste can be used to improve the quality of soil and provide nutrients for the growth of plants, and reduce the cost of agricultural crop production. This is achieved by composting, which enhances modification of the physiochemistry of soil. However, only the livestock waste produced in smallholder livestock farms is efficiently recycled. Large farms have serious problems with malodors. Efficient techniques to solve the problem have not been developed despite many research and development efforts. The malodor compounds from farms mostly come from production, removal, and composting of animal waste. The problem is increased where stocking density is high (Yun and Lee, 1992). Malodorous compounds are produced during degradation of cellulose, nutrients, and sulfuric-organic compounds. The malodorous gases produced also pollute the environment in the farm area. Pig manure is one of the most unpleasant gas-producing agents. Its main components are nitric organic compounds (nitrite, amine, ammonia), sulfuric organic compounds (methyl mercaptan, ethyl sulfide) and volatile fatty acids (Doring, 1977; Babyish and Stickly, 1978; Kazunari et al., 2003). Their production is linked to the amino acid degradation pathway. Recently, physical, chemical, and biological methods were studied and applied to reduce malodors from such compounds. Removal of malodors from pig slurry was performed by reducing the amount of nitrogen compounds in pig feces (Higaki, 1970) with the help of useful microorganisms (Oho, 1991). Malodorous utilizing microbes showed different activity depending on the condition of the pig waste. Effective microbes for reducing malodors are *Corynebacterium spp*. (Ohta and Sato, 1985), *Micrococcus spp*., *Flavobacterium spp*. (Oho, 1994), *Bacillus spp*., *Staphylococcus spp*., *Pseudomonas spp*., *Streptomyces spp*. (Ohta and Ikeda, 1978), *Actinomycetes spp*. (Danaka, 1978), *Mucor spp*., *Copinus spp*. and *Helminthosporium spp*. (Danaka, 1976). Yeast was reported to be capable of reducing malodors of chicken feces (Tanaka et al., 1977), and to increase crude fiber digestibility at ileum, caecum, and colon (Samarasinghe, 2004). Studies on the use of microbes for reducing malodors of pig slurry are underway but more knowledge is required. This investigation was undertaken to obtain information on malodorous compound utilizing yeasts and their characteristics.

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MATERIALS AND METHODS

Screening of microbes utilizing malodorous compounds

Ten humus soil and fifteen grower manure samples obtained from Kyunggi province of Korea were inoculated on malt extract medium and grown at 28°C for 3 days (Dindal, 1990). Shiny spherical white or yellowish white colonies were isolated and tested (Rural Development Association, 1988). Isolates were inoculated on 10% pig slurry medium mixed with 1.5% agar with pH adjusted to 7.2, and incubated at 28°C for 3 days. Microbes showing fast growth rates were selected for treatments of malodorous compounds (Ohta and Sato, 1985).

Identification of the yeasts

Physiological, biological, and biochemical characteristics of the microbes were investigated according to Kreger-Van Rij (1984). Growth on SDA medium and

Table 1. Instrumental conditions of gas chromatography for the determination of volatile fatty acids

	Condition
Column	Polyethylene glycol 1,500 (30 m×0.32 mm×0.05 μm, HP-innowax, USA)
Detector	FID
Column temperature	35-120°C (Raised at 4°C/min)
Inject temperature	140°C
Detector temperature	140°C
Flow rate (carrier)	H_2 40 ml/min
	Air 1,000 ml/min
	N_2 25 ml/min

Corn meal-Tween 80 agar medium was also examined (Barnett and Yarrow, 1983; Olga, 1986; Davise, 1995).

Growth curve of the yeasts

500 ml of 10% pig slurry medium was inoculated, and a sample taken each day was counted on the malt extract agar (Difco Co.) after 3 days incubation at 28°C.

pH measurement

10 ml of 10% pig slurry medium with microbes inoculated was removed, and pH of the medium was measured with a pH meter (Model 125, Corning Co., USA).

Measurement of malodorous compounds

Assay for utilization of malodorous compounds : The concentrations of ammonia, amine, H_2S, CO_2 and methyl mercaptan in 10% pig slurry medium were measured by using a gas detector (Gasteck Co., Japan).

Assay for volatile fatty acids : 10 g of the culture medium was centrifuged at 10,000 rpm for 20 min, and the supernatant extracted. It was centrifuged at 10,000 rpm for 2 min after addition of 5 ml of 2 N HCl. It was poured into a separator funnel and 3 ml of the upper phase used. Three ml of chilled ethyl ether was added and incubated at -20°C for 4 h. Then it was methylated with diazomethane for the analysis of volatile fatty acids (Metcalfe and Schmidt, 1961). Table 1 shows the conditions for gas chromatography.

Measurement of BOD_5

BOD_5 was measured by standard methods for the examination of water and wastewater (Greenberg, 1992). For each test bottle meeting the 2.0 mg/L minimum DO depletion and the 1.0 mg/L residual DO, calculate BOD_5 as follows;

$$BOD_5, mg/L=(D_1-D_2)/P$$

Where D_1=DO of diluted sample immediately after preparation, mg/L,

Table 2. Biological and physiological characteristics of the isolated yeasts

Characteristics	Strain		
	SY-1	CY-1	SY-2
I. Morphology			
Color	White	White	White
Shape	Yeast-like	Yeast-like	Yeast-like
Growth at 37°C	+	+	+
II. Utilization of			
Glucose	+	+	+
Galactose	+	+	-
Lactose	-	+	-
Sucrose	-	+	+
Maltose	-	+	+
Cellobiose	-	-	-
α-Methyl-D-Glucose	-	+	-
Xylose	-	+	+
Arabinose	-	-	-
Trehalose	-	+	-
Melezitose	-	-	-
Raffinose	-	-	+
N-Acetyl-D-Glucosamins	+	-	-
Xylitol	-	+	+
Ducitol	-	-	-
Adonitol	+	+	+
Palatinose	-	-	-
Glycerol	+	+	+
Sorbitol	+	+	+
Erythritol	+	+	+
Melibiose	-	-	-
Inositol	-	-	-
0.1% Cycloheximide	-	-	-
Potassium nitrate	-	-	-
2-Keto-D-Gluconate	-	-	-
Urea	-	-	-
III. Identification			
Genus	*Candida rugosa*	*Candida rugosa*	*Candida maris*

D_2=DO of diluted sample after 5 d incubation at 20°C, mg/L,

P=decimal volumetric fraction of sample used.

Measurement of soluble-N and NH_4-N

Soluble-N and NH_4-N were measured according to the AOAC method (AOAC, 1980).

RESULTS AND DISCUSSION

Screening of yeast

Thirty isolates were obtained by spreading soil and manure sources on malt extract agar medium. They were streaked on 10% pig slurry medium and incubated at 28°C for 72 h. Fast growing colonies were considered to be malodorous compounds utilizing microbes. Two isolates from the soil source (SY-1 and SY-2) and 1 isolate from the manure (CY-1) were selected.

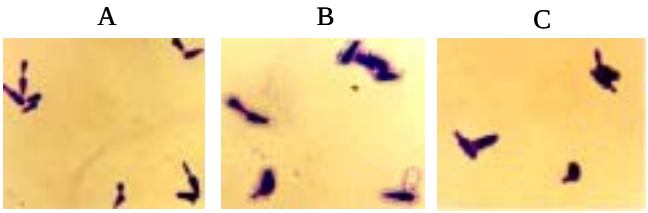

Figure 1. Microscopy of the isolated strains (at ×1,000 magnification) (A: SY-1, B: CY-1, C: SY-2).

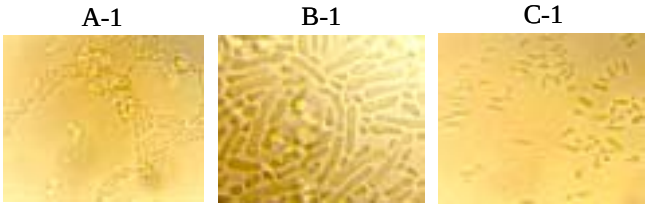

Figure 2. Phase contrast microscopy of the isolated strains on Corn meal-Tween 80 agar (at ×1,000 magnification) (A-1: SY-1, B-1: CY-1, C-1: SY-2).

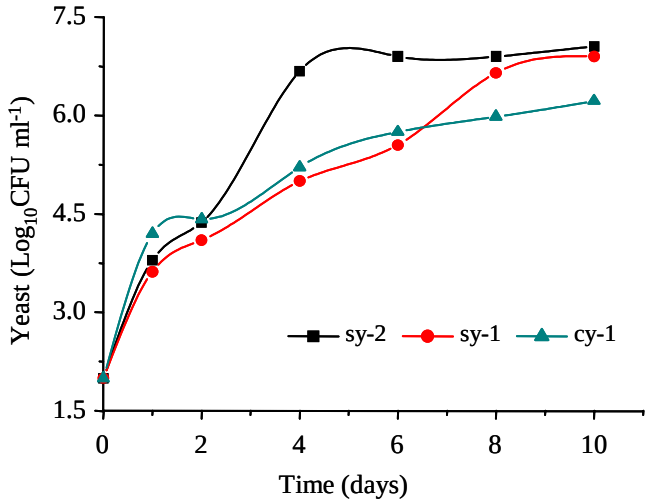

Figure 3. Viable counts of the cultures after yeast application to 10% pig.

Identification of the yeasts

Three isolates (SY-1, SY-2 and CY-1) were tested for their physiological characteristics. After growing on the malt extract agar medium, morphology of three isolates were either oval or long cocci shaped. The results of biochemical tests are shown in Table 2. SY-1 utilized glucose, galactose, glucosamins, adonitol, sorbitol and glycerol, and was able to grow at 37°C. It was *Candida rugosa* (Figures 1 and 2). CY-1 utilized galactose, glucose and adonitol, and was *Candida rugosa* (Figures 1 and 2). SY-2 utilized glucose, sucrose, maltose, xylose, xylitol, and raffinose, and was *Candida maris* (Figures 1 and 2). All three isolates were hemolysis negative.

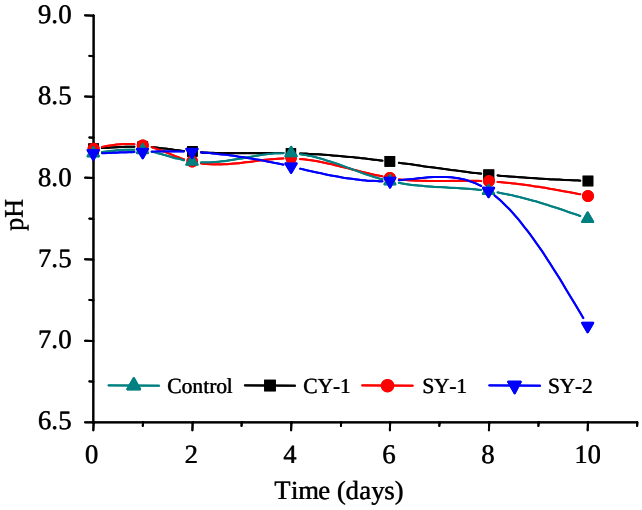

Figure 4. pH of the cultures after yeast application to 10% pig slurry.

Growth curve of the yeasts

Each of the three isolates of yeast was inoculated on 10% pig slurry medium with the initial concentration of 10^2 CFU/ml and incubated at 28°C to observe their growth (Figure 3). SY-2 showed rapid increase after the second day of incubation, and reached to the concentration of 10^7 CFU/ml. However, SY-1 and CY-1 showed gradual increase during the incubation, and reached to the concentration of 10^7 CFU/ml and 10^6 CFU/ml, respectively.

pH

Figure 4 shows the change of pH during the growth of yeast. Initial pH ranged from 8.15-8.18 and was similar to the control group. pH of SY-2 culture showed a sharp decrease after the eighth day of incubation, and reached pH 7.09 on the tenth day of incubation.

Malodorous compounds

Gas detector : Three of the selected isolates of the yeast were inoculated on liquified 10% pig slurry medium and grown for 72 h at 30°C. Samples were taken from the culture medium and the gas detector was used to measure the concentrations of ammonia, amine, H_2S, CO_2 and methyl mercaptan. Distinct characteristics of the three isolates were CO_2 production and reduction of ammonia and amine. Production of H_2S and methyl mercaptan was not detected at all; probably because they produced under the level of detection of the gas detector. Among the selected yeasts, there was not much difference in utilizing ammonia and amine species (Table 3), and this is because all three isolates were *Candida spp.*.

Gas chromatography : Volatile fatty acids such as acetic acid, propionic acid, *iso*-butyric acid, butyric acid and *iso*-valeric acid in the pig slurry medium were measured (Table 4). Compared to the control group, SY-1 showed a 9.5%

Table 3. Concentration of malodorants according to deodrant microorganisms in the 10% pig slurry (Unit: ppm)

Strain	Malodorants				
	NH_3 (<30)	$R.NH_2$ (5-100)	CO_2 (300-1,000)	H_2S	CH_2SH
Control	2.5	6±1.5	1,400±62	ND	ND
SY-1	<2	5±1.2	5,000±150	ND	ND
CY-1	<2	4±1.3	5,000±180	ND	ND
SY-2	<2	5±1.2	4,900±120	ND	ND

SY: soil yeast, CY: compost yeast, ND: not detectable.

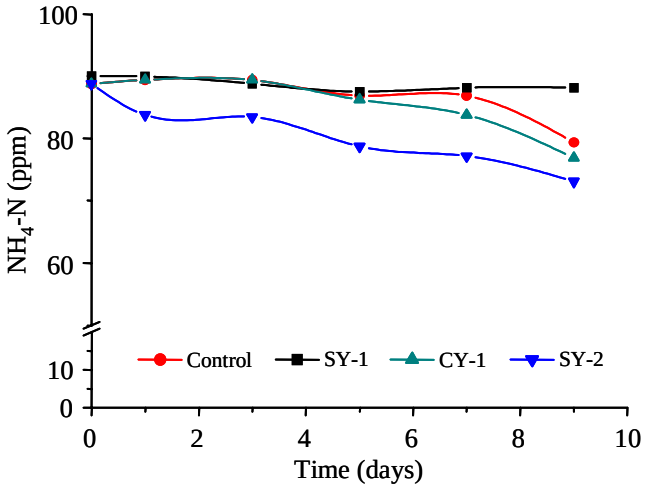

Figure 5. NH_4-N concentration of the cultures after yeast application to 10% pig slurry.

reduction in acetic acid, 100% reduction in propionic acid, 46.1% reduction in *iso*-butyric acid, 100% reduction in butyric acid and 100% reduction in *iso*-valeric acid. CY-1 showed a reduction of 54.0, 74.5, 100, and 78.3%, respectively. SY-2 showed a reduction of 34.2% in acetic acid, 87.4% in propionic acid, and 100% in *iso*-butyric acid, butyric acid, and *iso*-valeric acid.

The major malodor producing compounds in livestock waste are volatile fatty acids such as n-butyric acid, *iso*-butyric acid, n-valeric acid and *iso*-valeric acid (Hamano et al., 1972). These compounds are produced during aerobic degradation of organic compounds. Similar results were obtained as the report that aerobic microbes can produce these volatile fatty acids, and oxidize them to produce CO_2 that is odorless (Higaki, 1970; Ohta and Ikeda, 1978).

Malodorous compounds such as ammonia, sulfuric compounds, indole species and volatile fatty acids are produced by degradation of organic matter, and they can not

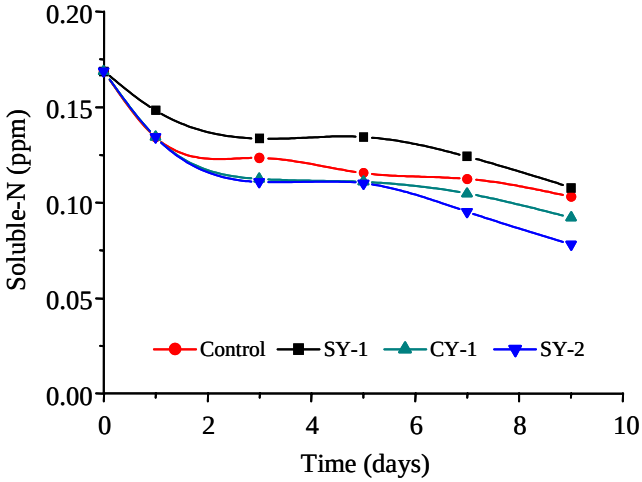

Figure 6. Soluble-N concentration of the cultures after yeast application to 10% pig slurry.

only produce unpleasant odors in the agricultural environment but they also can harm humans and animals. To reduce such compounds using aerobic microbes would enhance the degradation rate of organic materials (Danaka, 1976; Yun and Ohta, 1997).

Chemical analysis

Changes in the concentrations of nitrogen in ammonia, soluble nitrogen, and BOD were measured during the incubation. Figure 5 shows the change in concentration of the nitrogen in ammonia. Gradual decrease of nitrogen in ammonia was found, and SY-2 showed an 8.8% reduction.

Figure 6 shows the change of the soluble nitrogen concentration and again there was a gradual decrease on the ninth day of incubation; CY-1 and SY-2 showed a 14.3% and 23.8% reduction rate respectively. However, in case of SY-1, it showed a 4.8% decrease in the concentration of soluble nitrogen compared to the control.

Figure 7 shows the change of BOD during the incubation. SY-1 and CY-1 had a 15.2% and 4.0% higher BOD value after ninth day of incubation, while SY-2 had a 83.8% lower compared to the control.

When microbes utilizing malodorous compounds are used, depending on their characteristics and conditions given, rates and substrates for utilization by microbes could vary (Ohta and Kuwada, 1988). In this experiment, it is believed that as yeasts grow, they utilize the fatty acids in the pig slurry medium resulting in a reduction of the concentration of the volatile fatty acids compared to the

Table 4. Utilization of volatile fatty acid by deodorant yeasts in the 10% pig slurry medium (Unit: mM)

Sample	VFA				
	Acetic acid	Propionic acid	*Iso*-butyric acid	Butyric acid	*Iso*-valeric acid
Control	6.68±1.320	3.09±0.019	0.09±0.004	0.03±0.001	1.27±0.002
SY-1	6.05±1.280	0	0.05±0.001	0	0
CY-1	3.07±1.041	0.79±0.015	0	0	28±0.001
SY-2	4.40±0.180	39±0.005	0	0	0

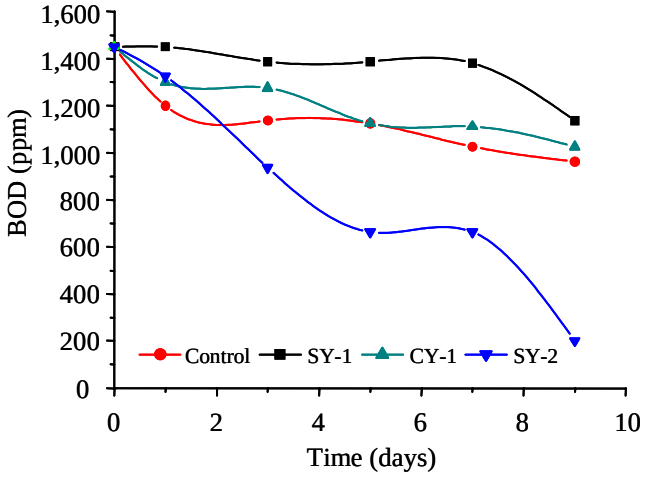

Figure 7. BOD of the cultures after yeast application to 10% pig slurry.

control group. Thus using aerobic microbes, malodorous compounds can be degraded to odorless compounds. In this experiment, among all the yeasts tested, SY-2 showed a greater reduction in the concentrations of nitrogen in ammonia, soluble nitrogen and BOD. Further investigation is necessary to determine other conditions for the yeasts to enhance the reduction of odor in livestock waste.

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