

Microbiological Evaluation of Antibiotic Resistance and Pathogenicity in Autothermal Thermophilic Aerobic Digestion Treated Swine Manure

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Abstract

In both untreated and conventionally stabilized swine manures antibiotic resistant (AR) microorganisms, *Staphylococcus*-like and *Salmonella*-like microorganisms were detected. Also pathogens with MAR phenotype were detected. Presence of such microorganisms suggest high level of pathogen-related health risk to farmers who may be in direct contact with the manure and its conventionally stabilized product. In contrast, the autothermal thermophilic aerobic digestion (ATAD) treatment have efficiently reduced AR and pathogenicity from the swine manure. When soil was fertilized using swine manure and its stabilized products, despite no detection of MAR-exhibiting pathogen-like microorganisms in fertilized soil, potential pathogen-related health risk could not be ruled out from the fertilized soil since the organic fertilization led to increase in AR and pathogenicity in the soil microbial communities. As conclusion, this microbiological study demonstrated that an ATAD process is applicable in control of pathogen-related health risk in livestock manure.

Key word : antibiotic resistance, autothermal thermophilic aerobic digestion (ATAD), multiple antibiotic resistance (MAR), livestock manure, pathogens, organic fertilizer.

1. Introduction

In agricultural industry livestock manures are anaerobically stabilized and used as alternatives to chemical fertilizers [Division of Sustainable Development, 2000]. These stabilized manures contain high concentrations of antibiotics due to large quantities of antibiotics added to livestock feeds (~0.2% w/w) [Christian *et al.*, 2003; Walsh, 2003]. The high antibiotic concentrations can result in selection of AR microorganisms in the livestock manure and its stabilized products [Walsh, 2003]. AR microorganisms could raise significant health risk by generating AR or even MAR pathogens in livestock manure through mechanisms known as horizontal gene transfer (HGT) [Medigan *et al.*, 2003].

Lagoon fermentation have been conventional manure stabilization methods and it require several months of stabilization period. To reduce this period autothermal thermophilic aerobic digestion (ATAD) was applied. According to our preliminary study ATAD process only required 3 days for livestock manure stabilization, and due to thermophilic condition (60~65°C) developed ATAD is also applicable in control of pathogenicity risk in livestock manure. Therefore the ATAD stabilized swine manure was evaluated to meet the requirements for an organic fertilizer [Samjung Construction Co. LTD, 2005]:

2. Research method

Evaluation of swine manure and its stabilized products

Untreated swine manure (Untreated), swine manure conventionally stabilized in a lagoon for 6 months (Fermented), and ATAD-treated swine manure were sampled. Their AR were evaluated with a laboratory culture technique using solid culture media amended with four antibiotics unique in their bacteriostatic or bactericidal actions.

The samples pathogenicities were evaluated for two human pathogen-like microorganisms, *Staphylococcus* and *Salmonella*, using organism specific plate media. Furthermore, possible presence of zoonotic bacteria with MAR phenotype was evaluated in the swine manure and its stabilized products.

Evaluation of organic fertilized soil

To examine the effects of fertilization on AR and pathogenicity in soil microbial communities, unfertilized soil from a forest was collected and amended with fermented or ATAD-treated swine manure. AR, pathogenicity and MAR in the soil samples were analyzed by adapting the culture methods applied for the evaluation of the liquid sample.

2.1 Results

Antibiotic resistance evaluation

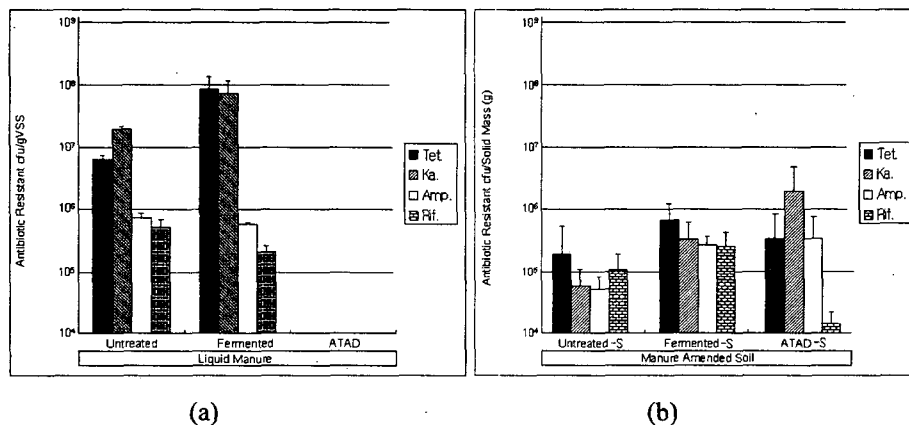


Fig. 2-1 Culturable AR microorganisms in (a) untreated, fermented, ATAD treated manure samples; and in (b) unfertilized soil (Untreated-S) and soil samples amended with fermented (Fermented-S), ATAD treated (ATAD-S) swine manure. The Y-axis error bar indicates one standard deviation from three independent experiments.

Microorganisms resistant to various antibiotics were detected in "Untreated" and "Fermented" (Figure 2-1a). The detection of such microorganisms suggests a possible presence of MAR phenotypes. In contrast, AR microorganisms were not detected in the ATAD-treated swine manure. These findings indicate the improved control of AR microorganisms by ATAD treatment. According to the results for "Untreated" and "Fermented", a greater increase in tetracycline and kanamycin resistant microorganisms was observed compared to that of ampicillin and rifampicin. This might be attributed to the chlortetracycline added to swine feed. When untreated soil was amended with the fermented and ATAD-treated swine manures, increases in AR counts were observed for most of the tested antibiotics (Fig. 2-1b).

Pathogenicity evaluation

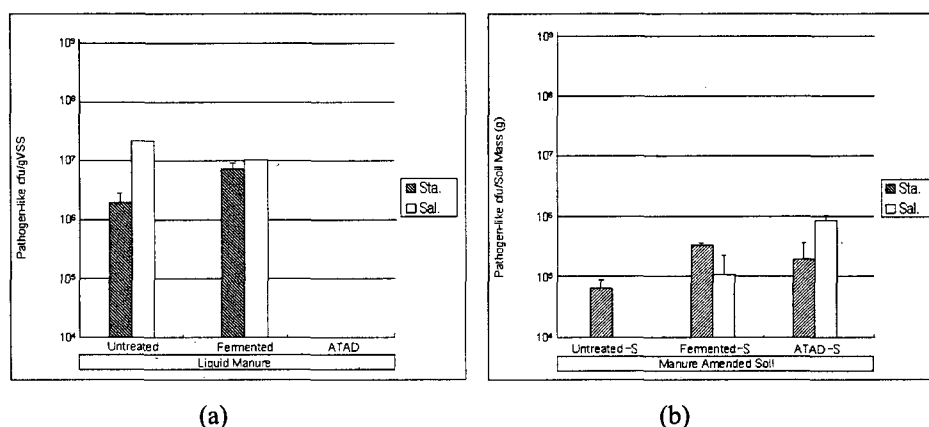


Fig. 2-2 Culturable tested pathogens in (a) untreated, fermented, ATAD treated swine manures; and in (b) unfertilized soil (Untreated-S) and soil amended with fermented (Fermented-S) and ATAD-treated (ATAD-S) swine manures. The Y-axis error bar indicates one standard deviation from three independent experiments.

The total numbers of *Staphylococcus*-like and *Salmonella*-like microorganisms were not significantly reduced in the fermented manure. However in ATAD treated swine manure, *Staphylococcus*-like, *Salmonella*-like microorganisms were not detected (Fig. 2-2a). These results indicate improved control of pathogenicity of swine manure by the ATAD treatment. When the fermented and the ATAD-treated swine manures were amended in soil, increased numbers of *Staphylococcus*-like and *Salmonella*-like microorganisms were observed with respect to the unfertilized soil (Fig 4-4 b).

Examination of MAR phenotype in pathogens

Table 2-1. Detection of MAR phenotype in pathogen-like microorganisms.

Pathogenicity		Antibiotics	Untreated	Fermented	ATAD
Liquid Manure Sample	<i>Staphylococcus</i> -like	Tet+Ka+Amp	3%*	2%	ND#
		Tet+Ka+Rif	5%	5%	ND
	<i>Salmonella</i> -like	Tet+Ka+Amp	4%	4%	ND
		Tet+Ka+Rif	4%	3%	ND
Soil sample	<i>Staphylococcus</i> -like	Tet+Ka+Amp	ND	ND	ND
		Tet+Ka+Rif	ND	ND	ND
	<i>Salmonella</i> -like	Tet+Ka+Amp	ND	ND	ND
		Tet+Ka+Rif	ND	ND	ND

* Percentage of MAR colonies among 80 pathogen - like colonies separated.

ND indicates "non-detected"

MAR phenotypes were detected among the *Staphylococcus*-like and *Salmonella*-like colonies separated from the untreated and the fermented liquid manures (Table 2-1). The detection of MAR phenotype in the pathogen-like microorganisms implies significant pathogen-related health risk to farmers who may be in direct exposure to the swine manure and its fermented product (no tested pathogens were detected in ATAD treated

swine manure therefore there are no MAR pathogens). In all tested of soil samples, no MAR phynotypes were detected in the assays.

3. Conclusion

In both untreated, fermented swine manures AR microorganisms and tested pathogen-like microorganisms were detected. Furthermore pathogens with MAR were present within them. These suggest a high level of pathogen-related health risk to farmers who may come in contact with the manures and its conventionally stabilized product. In contrast the ATAD treatment could efficiently reduce AR and pathogen-like microorganisms from the swine manure.

Despite no detection of MAR-exhibiting pathogen-like microorganisms in the soil samples amended with the stabilized products, potential pathogen-related health risk could not be ruled out since the addition of the stabilized manure led to increase in AR and pathogenicity in the soil microbial communities. As conclusion, this microbiological study demonstrated that an ATAD process is applicable in control of pathogen-related risk in livestock manure.

4. References

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