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# Survival of Clostridium difficile spore in manure compost

*Clostridium difficile* is a gram-positive spore-forming anaerobic bacterium that causes antibiotic-associated diarrhea and pseudomembranous colitis in humans. *C. difficile* can be isolated not only from patients but also from several animal species (such as pigs and cows), which are considered to be reservoirs of *C. difficile*. The hypervirulent ribotype 078 has been reported as the predominant ribotype in livestock animals worldwide. In Japan, most livestock waste is treated by composting to obtain manure compost. Composting is one of the natural processes capable of stabilizing livestock waste. Proper composting effectively destroys pathogens by the high temperatures (>60°C) achieved through the metabolic heat generated by microorganisms in manure compost. However, *C. difficile* spores are highly resistant to extremes of temperature, desiccation, and various chemicals and disinfectants.

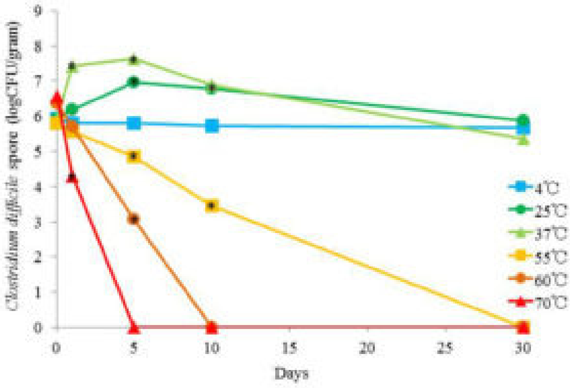


Fig. 1. Log counts of Clostridium difficile feces in pig manure at several temperatures.
Asterisks indicate statistically significant differences in log counts of C. difficile per gram of feces compared with day 0. Data were analyzed using one way analyses of variances (p < 0.05).

To clarify the survival ability of *C. difficile* spores in pig feces in the form of manure compost, *C. difficile* spores were artificially inoculated into pig feces and incubated at several temperatures. *C. difficile* spores survived in the feces incubated at temperatures below 37°C for over 30 days (Fig. 1). However, spore numbers gradually decreased at thermophilic temperatures (over 55°C) ($p$<0.05). The vegetative form of *C. difficile* is more sensitive to heat treatment than the spore form. The high temperatures generated during composting are known to reduce the number of viable pathogenic bacteria. Therefore, ensuring the composting of manure and its exposure to the resulting elevated temperatures can reduce the occurrence of pathogenic bacteria, including both the vegetative and spore forms of *C. difficile*.

Next, to clarify the distribution of *C. difficile* in field manure compost, we isolated and characterized *C. difficile* from the final products of manure compost in 14 pig farms. *C. difficile* was detected in 36 % (5/14) of manure compost samples. From 5 *C. difficile*-positive manure compost samples, we obtained 11 isolates. Of these 11 strains, 82% were toxigenic. The most prevalent ribotype in manure compost was ribotype 078, which is well established in livestock animals, and which has also emerged as a significant pathogen associated with the majority of community-acquired *C. difficile* infection cases.

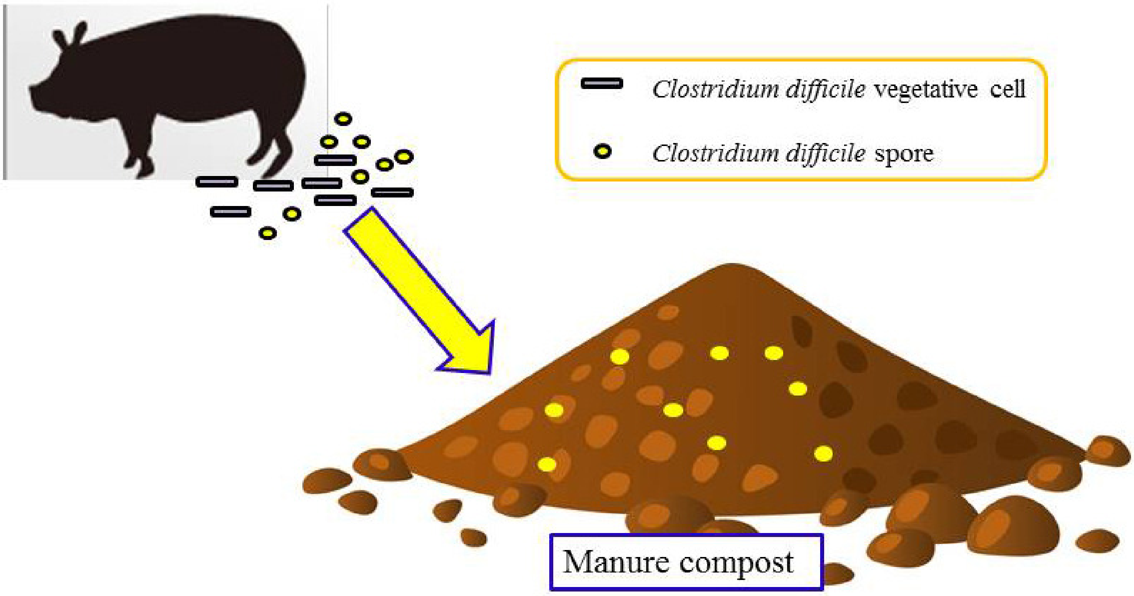


Fig. 2. Clostridium difficile spores were survived in field manure compost.

Although proper composting would reduce the abundance of *C. difficile* by thermophilic temperature, *C. difficile* spores occasionally survive after composting. In addition, composting may not always reach temperatures sufficiently high to kill pathogenic bacteria, because conditions might not always be optimal in terms of initial feedstock (e.g., too dry or too wet) or weather (low ambient air temperature). And livestock wastes are not treated by composting and are directly applied to agricultural land in some countries. In the present study, we show that manure compost derived from pig farms might contain *C. difficile*, including hypervirulent strains (Fig. 2). Spreading fresh manure or incompletely composted manure to land presents a higher risk of *C. difficile* transfer to the food chain. Therefore, the survival of *C. difficile* in fully processed manure compost is an important aspect that should not be ignored.

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## Publication

Survival and prevalence of Clostridium difficile in manure compost derived from pigs.
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