Culture media performance on the detection of actinomycetes from composts

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Abstract
In this study we evaluated the performance of six culture media on the detection of actinomycetes that were present in composts of agro-industrial wastes from the Algarve region. The composts were produced with orange wastes, olive pomace and grass clippings, in the proportion of 2:1:1 of volume. Two compost piles were built with different ventilation systems, one with forced ventilation and the other with mechanical turning and natural ventilation.
In order to quantify the population of actinomycetes, different growth media containing antibiotics were tested and one culture medium containing only half of the concentration of the Plate Count Agar (½ PCA) was also tested. The incubation took place at two temperatures, 25º and 55ºC.
Different results were obtained for the enumerations from the culture media. The population of actinomycetes achieved higher values when incubated at 55ºC and when the samples were inoculated in the ½ PCA culture medium.
Thus, the antibiotics showed no beneficial effect in the tested culture media. The results suggest the use of the ½ PCA culture medium as the most adequate in order to count the bacterial populations in these samples. This medium is also the less expensive and the one that showed the fastest bacterial growth.

Introduction

Composting is a complex process where microorganisms had an important role in organic wastes degradation (Day & Shaw, 2005). Different populations of bacteria and fungi are specific for each stage of the composting process (Miller, 1993). Actinomycetes activity is fundamental for degradation of macromolecules such as cellulose, hemicellulose, lignin and chitin (Ryckeboer, 2003). The degradation of these components releases inorganic nutrients which are an important part of humus formation (Epstein, 1997).
The actinomycetes enumeration along the composting process is an important tool for an adequate monitoring of the process.
One of the main difficulties is the lack of a standard methodology for a selective bacterial enumeration in composts.

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However, a standard methodology has not been adopted since few studies have been conducted on their comparative performance in counting actinomycetes from compost samples.
The aim of the present study was to select, among six culture media, the most efficient in isolating and counting actinomycetes from compost samples, through the composting process. [2]

Materials and Methods

Two compost piles were prepared using a mixture of agro-industrial waste with orange waste, olive pomace and grass clippings (2:1:1 v/v), which diverged in the ventilation system. In one of the piles we resorted to forced ventilation (FV) and in the other to mechanical turning with natural ventilation (MV). In FV the fan was triggered by temperatures above 60 °C inside the pile, being the aeration done by air extraction. In MV two turnings took place at 51 and 151 days after the beginning of the process. The latter was accompanied by a mechanic fractionation, for homogenization of the material. At the same time, a turning was made also in the FV. Whenever moist went down to levels approaching 40% we resorted to the wetting of the piles.
Samples were taken periodically and randomly, between March and December, each 2 to 3 weeks, in triplicate to a depth of 30 cm in order to monitor the composting process. The temperature of the compost piles was also registered using several probes (PT100 probes).
Six different culture media were tested to select the one that showed the better performance for the actinomycetes enumeration from compost samples. They were: ½ PCA (Merck, Germany); Actinomycetes Isolation Agar - AIA (Difco, França); AC, ACPP, ACCN and ACPPCN. The last four mentioned culture media were not commercial.
PCA was prepared with half the strength recommended by the manufacturer (½ PCA), before inoculation samples were pasteurized at 80 ºC during 10 m. AIA was prepared as recommended by the manufacturer. Inoculated plates were incubated at 25 and 55 ºC during 24 hours. The other four culture media had as common starch, casein, $CaCO_3$, $MgSO_4.7H_2O$, K_2HPO_4, NaCl, KNO_3, and $FeSO_4.7H_2O$ having the same concentrations in both media. AC medium had no antibiotics added. Penicillin (Sigma, Germany); polymixin (Sigma, Germany), cycloheximide (Sigma, USA) and nistatine (Bristol,UK) were added to the others media. Samples suffered a previous treatment with $CaCO_3$. Inoculated plates were incubated at 25 during 7 days.
All microbiological analyses were done in triplicate.
Precision of the different studied media was obtained by the D^2 Fisher index, applying the Eisenhart & Wilson (1943) formula, expressed as:

$$D^2 = \frac{\left[N\Sigma xi^2 - \left(\Sigma xi^2\right)\right]}{\Sigma xi}$$

Where xi is the count in each plate of the same sample and volume, and N is the number of replicated plates. The number of samples for each media was 18.
Accuracy was evaluated by comparison of the strain counts obtained in each culture medium with those obtained in the reference media (½ PCA) by the following equation (Dionísio & Borrego, 1995):

$$\Sigma = \frac{\left(CFU\ for\ each \frac{media}{mean} CFU\ for\ reference\ media\right)}{number\ of\ samples}$$

To determine the statistical differences between the two composting piles the t-Student test ($p \leq 0.05$) was performed using a SPSS statistic programme (version 15.0, SPSS Inc.).

Results and Discussion

Table 1 shows the results for the actinomycetes grown in the six culture media. Higher actinomycetes populations were observed in ½ PCA at 55 ºC incubation. AC media showed no selective properties. Enumerations in all the culture media supplemented with antibiotics were lower when compared with the ones obtained in ½ PCA strength as showed in Figure 1 and Figure 2. The results for the actinomycetes growth in the culture media ½ PCA and AIA were in agreement with those of Golueke (1972), were by the end of the process a higher number of colonies was observed. This tendency was not observed in culture media with antibiotic. Enumerations obtained from ½ PCA are also in accordance with Day & Shaw (2005), who observed an increase in the actinonomycetes population at the compost maturation stage when nutrients such as cellulose, hemicelluloses and lignin are free for these bacteria growth, an adequate mesophilic temperature occurs (Nakasaki et al., 1985) and the medium is a slightly alkaline, which is important because these decomposers are affected by acidic conditions (Goodfellow & Willians, 1983).

Fisher Index values were higher for samples collected from the two composting piles and inoculated in ½ PCA medium and 55 ºC incubation, being 5.67E+03 cfu.g^{-1} (transformed in ln) in FV and 5.74E+03 cfu.g^{-1} in MV. Media with antibiotics showed a lower Fisher Index, inferior to 2.22E+03 cfu.g^{-1} (

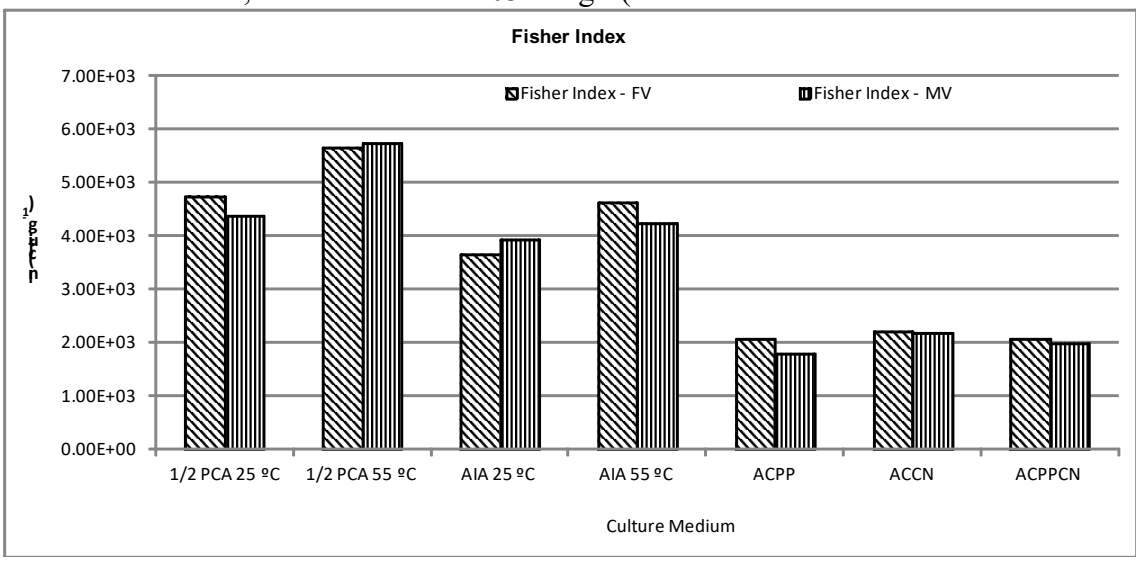

Figure 3).

Nakasaki et al. (1985) suggested that actinomycetes become more active when temperature is closer to or goes up 60 ºC and the levels of nutrients decrease.

Taking ½ PCA medium as the reference one, samples from MV inoculated in AIA media and incubated at 25 ºC showed 90% of precision. In this medium too samples from FV incubated at 55 ºC showed 80% of precision. Samples grown in media supplemented with antibiotics showed a precision value lower than 50% (Figure 4).

The techniques for evaluating the efficiency of the culture medium, direct count, Fisher Index and precision showed that the culture medium ½ PCA is the more adequate to enumerate the actinomycetes population during the composting of organic wastes.
Once two of the studied antibiotics are antifungal, it would be expected that these media were more effective. Nevertheless during the composting process we verified the same tendency, which proves that the medium ½ PCA was more efficient. More studies are needed, using other composting processes or other kinds of agricultural wastes, to clarify this tendency.

Conclusion

The results suggested that the use of the PCA culture medium, with half the strength recommended by the manufacturer (½ PCA), is the most adequate for the actinomycetes enumeration from compost samples.
The antibiotics supplemented to the studied media showed no beneficial effect.
½ PCA strength is the less expensive and the one that showed the fastest actinomycetes growth.

Acknowledgments

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Literature Cited

Day, M., Shaw, K. 2005. Processos Biológicos, químicos, físicos del compostaje. In: Stollella, P. & Kahn, B. (eds.), Utización de compost en sistemas de cultivo hortícola. Ediciones Mundi-Prensa. pp. 17-50.

Dionísio, L.P.C., Borrego, J.J. 1995. Evaluation of media for enumeration of faecal streptococci from natural water samples. Journal of Microbiological Methods 23: 183-203.

Eisenhart, C., Wilson, P.C. 1943. Statistical methods and control in bacteriology. Bacteriol. Rev. 7: 57-72.

Epstein, E. (1997). In: E. Epstein (Ed.), The Science of Composting (pp. 77e106). Boca Raton, Florida: CRC Press LLC.

Golueke, C. G. 1972. Composting: A Study of the Process and its Principles, 110 pp. Emmaus, Pennsylvania, USA: Rodale Press. In: Boulter, J. I., Boland, G. J.& Trevors, J. T. 2000. Compost: A study of the development process and end-product potencial for suppression of turfgrass disease. World Journal of Microbiology & Biotechnology 16: 115-134.

Goodfellow, M. & Willians, S. T. 1983. Ecology of Actinomycetes. Annual Review of Microbiology 37: 189-216. In: Boulter, J. I., Boland, G. J.& Trevors, J. T. 2000. Compost: A study of the development process and end-product potencial for suppression of turfgrass disease. World Journal of Microbiology & Biotechnology 16: 115-134.

Miller, F.C. (1993). In: J.F. Blaine Metting (Ed.), Soil Microbial Ecology – Applications in Agricultural and Environmental Management (pp. 515-539). New York: Marcel Dekker.

Nakasaki, K., Sasaki., Shoda, M. & Kubota, H. 1985a. Change in microbial numbers during thermophilic composting of sewage sludge with referente to CO_2 evolution rate. Applied and Environnmental Microbiology 49, 37-41.

Ryckeboer, J., Mergaert, J., Coosemans, J., Deprins, K., Swings, J. (2003) Microbiological aspects of biowaste during composting in a monitored compost bin. J. Appl. Microbiol. 94, 127-137.

Tables

Table 1: FV and MV actinomycetes population in different culture medium

Culture media	FV	MV
½ PCA 25 ºC	15.8E+00b	14.5 E+00b
½ PCA 55 ºC	18.8 E+00a	18.9 E+00a
AIA 25 ºC	11.9 E+00c	12.7 E+00c
AIA 55 ºC	15.0 E+00b	13.6 E+00bc
ACPP	7.16 E+00d	6.19 E+00d
ACCN	7.42 E+00d	7.26 E+00d
ACPPCN	7.13 E+00d	6.85 E+00d

In the same column, values referred with the same letter showed no statistical differences for $p \leq 0.05$ (Duncan test)

FV, compost of forced ventilation pile; MV, compost of mechanical turning with natural ventilation pile; PCA, plate count agar; AIA, actinomycetes isolation agar; ACPP, soluble starch, casein, penicillin and polymixin; ACCN, soluble starch, casein, cycloheximide and nistatine; ACPPCN, soluble starch, casein, penicillin and polymixin, cycloheximide and nistatine ; 25º C e 55ºC, incubation temperature

Figures

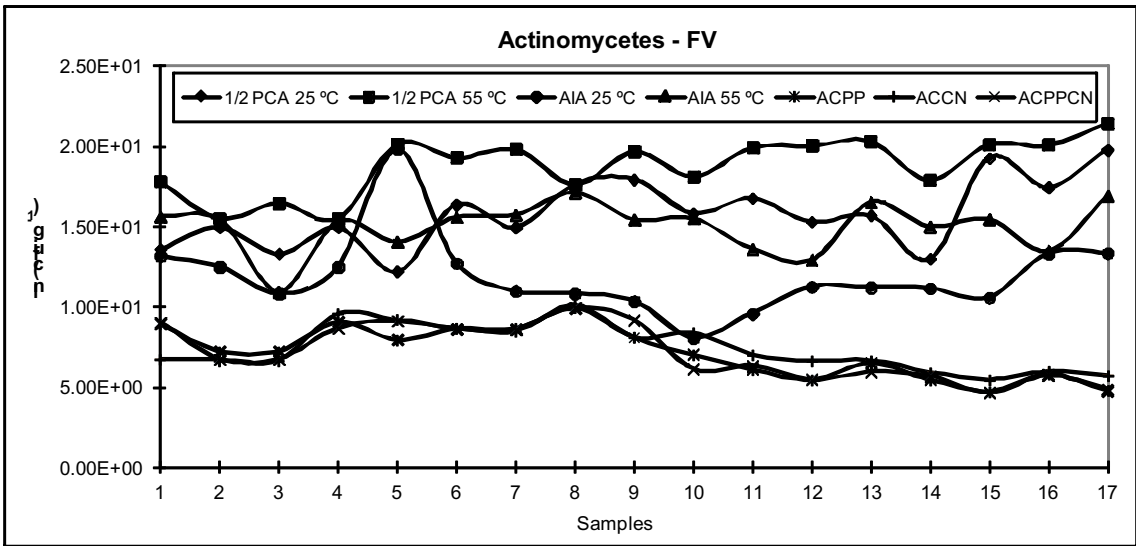

Figure 1: Actinomycetes monitorization in different culture media in FV (forced ventilation pile)

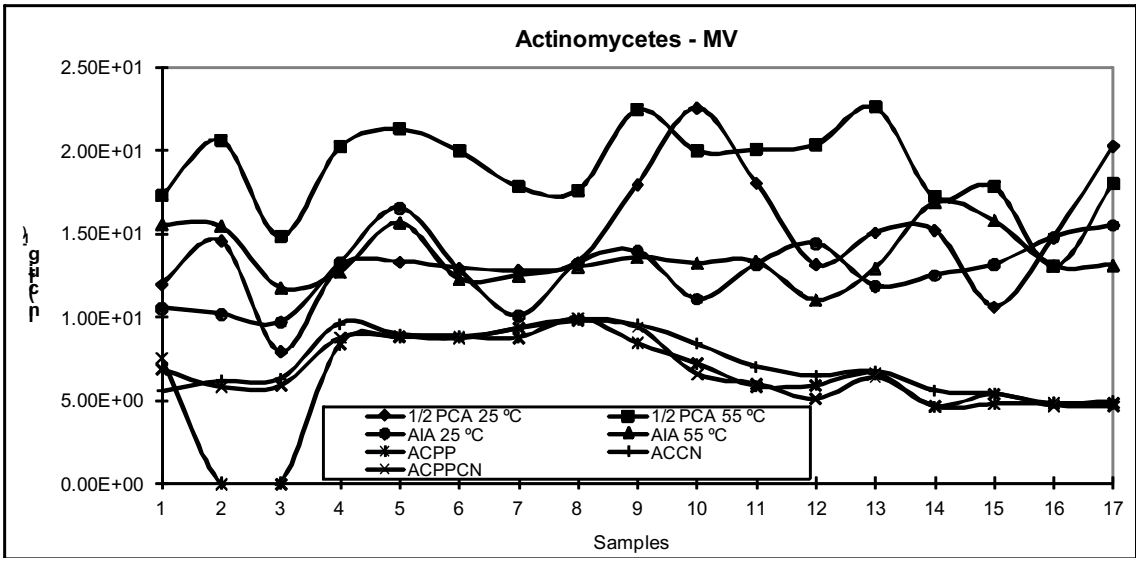

Figure 2: Actinomycetes monitorization in different culture media in MV (mechanical turning with natural ventilation)

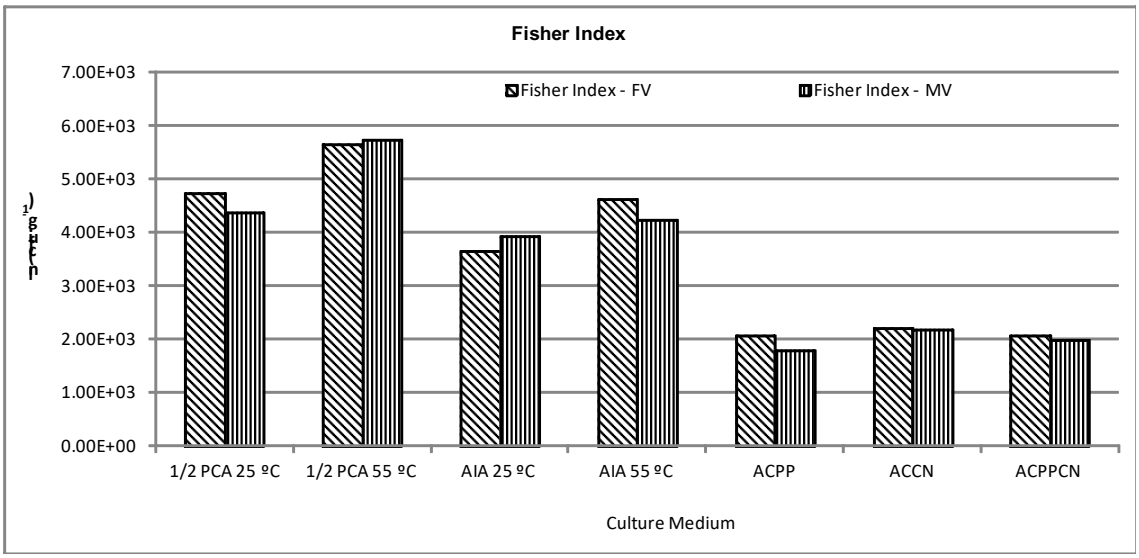

Figure 3: Fisher Index of culture medium in FV (forced ventilation pile) and MV (mechanical turning with natural ventilation pile)

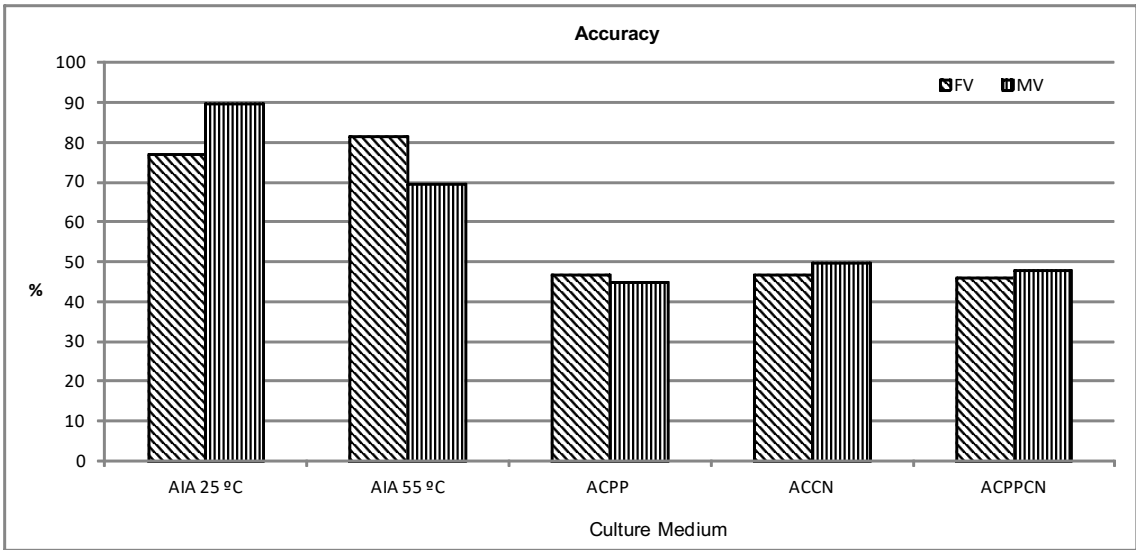

Figure 4: Accuracy of culture medium in FV (forced ventilation pile) and MV (mechanical turning with natural ventilation pile)