Comparative study of aerobic microorganisms in compost

Doncean Ancaţa 1,*  Şumălan Renata1, Şumălan R.1

1Banat University of Agricultural Sciences and Veterinary Medicine Timisoara, Faculty of Horticulture and Forestry, 119 Calea Aradului, 300645 Timisoara, Romania

*Corresponding author: E-mail: donceananacula@yahoo.com

Abstract Composting is the degradation of organic materials through the activities of diverse microorganisms. This research examined microbial community dynamics, population levels and identification of bacteria, fungi and actinomycetes through the mature compost and immature compost. Incubating the microbial media at 24 °C we determine the number of microbial colonies with actinomycetes, bacteria and fungi and the frequency of occurrence for each sample of compost. In this study we observe the differences between mature compost and immature compost, that we need a highest number of actinomycetes in immature compost to reach the maturity then bacteria.

Composting has been defined as intense microbial activity leading to decomposition of most biodegradable materials (9; 1). The level of stability and maturity of composts is an essential aspect of compost quality. Microbial diversity is a prerequisite for a satisfactory composting process (2). The most important all these microbial diversity in compost is the aerobic microorganisms. They are the most diverse of all organisms. Compost piles have more than one type of bacteria and there population will vary depending on the temperature of the compost pile (8). A large variety of mesophilic, thermotolerant and thermophilic aerobic microorganisms (e.g. bacteria, actinomycetes, yeasts, and fungi) are involved in the composting process (2). Actinomycetes are a heterogeneous group of filamentous bacteria resembling fungi (5; 3; 4; 6).

The objectives of this research were to identify aerobic microorganisms, study microbial community dynamics and populations levels between mature compost and immature compost.

Materials and Methods

We used in this study two samples of composts: mature and immature compost which we compare the number of microorganisms, community dynamics and population level from it.

Due to the large number of microorganisms present in compost, it was necessary to get a sample dilution of the dispersant medium. To determine the total number of microorganisms in these two types of compost was used as dispersant 0,1% sodium pyrophosphate solution (7).

Each dilution mixture of compost and sodium pyrophosphate solution was shaken for 1 min at 200 rev/min and allowed to sit for ~10 min to unravel aggregates. Serial dilutions of 10^2–10^7 were prepared by sequentially transferring 1 ml samples onto test tubes containing 9 ml of sterile 0.1% sodium pyrophosphate. We used the technique of direct isolation of fungi, bacteria and actinomycetes on specific medium. The culture media used for fungi was Czapek's growth medium with 0.0075% streptomycin and 0.025% chloramphenicol; for bacteria was Topping growth media and for actinomycetes was Berezova growth media. Subsamples (100 µl) at selected dilutions were pipetted onto Petri plates. We worked in 2 replicates for each compost type and culture media. All plates were incubated at 24 °C for 7 days. After 2 days we determined the number of microbial colonies with bacteria and the frequency of occurrence, and after other 2 days we repeat the counting operation. In the 7th day we determined the number of microbial colonies with fungi and actinomycetes and the frequency of occurrence of their on the plates media. To identify microbial species have been established morphological types culture highest frequency of occurrence on the boards. We used Papacostea method for determinate the macroscopic morphological characters of microbial colonies for bacteria and fungi. Actinomycetes identification was done by phenotypic study (3). Microscopic examination was done to determine the bacteria Gram character and detailed observation of fungi and actinomycetes fructification.

Results and Discussions

After 4 days of incubating the microorganisms on culture medium at 24 °C, readings were made to determine the number of microbial colonies with bacteria and calculation of frequency of occurrence for bacterial species for each sample of compost. For
determination of the number of microbial colonies with actinomycetes and fungi we made readings after 7 days of incubating media at 24 °C. The number of microorganisms was presented as cfu/g of dry weight of compost. In figure number 1 and 2 it’s represented an average number of microorganisms from both replicates from each type of compost: mature and immature compost.

Figure 1 presents the results for microbial loads of mature compost. Here the number of actinomycetes was $111.35 \times 10^7$ cfu/ gr dry compost, which was the biggest. The second amount was the bacteria with $60.35 \times 10^7$ cfu/ gr. dry compost and the last amount was determinate in fungi case with $17x \times 10^4$ cfu / gr. dry compost.

Figure 2 presents the results of immature compost. Here the number of bacteria was the $35.7 \times 10^7$ cfu/ gr. which was the highest in this samples. The second amount was the actinomycetes with $29.925 \times 10^7$ cfu/ gr. and the last amount was fungi with $28.875 \times 10^4$ cfu / gr dry compost.

From these results we can see that the number of actinomycetes and bacteria is highest in the mature compost then in the immature compost. However, numerical dominance is apparent in the case of actinomycetes in mature compost and bacteria in immature compost. This difference is because the simple organic substances in mature compost were sold, predominantly compounds only partially humificate available just to actinomycetes. We can’t say the same thing about fungi which the number is
highest in immature compost then in the mature compost. In immature compost celolozolitic compounds degradation processes are dominant, a process due to fungal activity.

In the next figure we can see the differences of actinomycetes population between the mature compost (left) and immature compost (right).

The colonies identified in figure 3 are *Streptomyces olivochromogenes* and *Streptomyces griseoruber*. We can see in this figure that number of actinomycetes in the mature compost is higher then in the immature compost but their diameter is smaller. Macroscopic examination confirmed the existence of *Streptomyces olivochromogenes*, as predominant. The predominant in immature compost is *Streptomyces griseoruber* with colonies with larger diameter and more prominent and blue-green color.

In mature compost predominant is *Arthrobacter globiformis*, and in the immature compost predominant are *B. megaterium* and *Arthrobacter oxidans*. In both composts we meet: *Arthrobacter globiforme, Arthrobacter oxidans, B. sphericus var. flavus.*

In table number 1 we presented the cultural characters of bacteria that were identified in both samples of composts.
### Table 1

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Frequency of occurrences (%)</th>
<th>Macroscopic aspects</th>
<th>Gram Character</th>
<th>Microscopic aspects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Arthrobacter globiformis</strong></td>
<td>55</td>
<td>convex colonies, flat, white, smooth, glossy, opaque, with the narrow transparency</td>
<td>G+</td>
<td>fragmented bacilli in cocoide elements, with diameter 0.3 – 0.6 μ arranged in networks</td>
</tr>
<tr>
<td><strong>Arthrobacter oxidans</strong></td>
<td>2</td>
<td>convex colonies, flat, glossy, smooth, gelatinous, highly pigmented yellow citron, without structure in the transmitted light and low iridescent in direct light in marginal zone</td>
<td>G+</td>
<td>Spherical gram variable elements with a diameter of 1-3 μ</td>
</tr>
<tr>
<td><strong>B. sphaericus var. flavus</strong></td>
<td>8</td>
<td>large colonies, yellow, with small white dots, circular, opaque, convex</td>
<td>G+</td>
<td>sub terminal sporangia, free spores are not perfectly spherical and is smooth</td>
</tr>
<tr>
<td><strong>Flavobacterium</strong></td>
<td>10</td>
<td>large colonies, rhizoidal border, yellow color, transparent</td>
<td>G-</td>
<td>unsporulate bacillus, large and narrow, with filaments</td>
</tr>
<tr>
<td><strong>Bacillus megatherium</strong></td>
<td>0</td>
<td>large colonies, light cream colour, with the cutaneous surface lobed border</td>
<td>G+</td>
<td>large bacillus with chains arrangement with spore with central arrangement</td>
</tr>
<tr>
<td><strong>Pseudomonas putida</strong></td>
<td>0</td>
<td>fast-growing colonies, oblate, smooth surface, translucent, cream tan colour</td>
<td>G-</td>
<td>little bacillus, straight and curved with pointed ends</td>
</tr>
<tr>
<td>* Unidentified</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Unidentified

From this table we can observe the high population of bacteria from both composts being Gram positive.

In mature compost the colonies we identified: Flavobacterium sp (10%), Arthrobacter globiforme (55%), Arthrobacter oxidans (2%), B. sphaericus var. flavus (8%).

In immature compost we identified these colonies: Pseudomonas putida (18%), B. megatherium (25%), Arthrobacter globiforme (15%), Arthrobacter oxidans (23%) and B. sphaericus var. flavus (9%).

The colonies of fungi, figure 5, we identified in mature compost are: Aspergillus niger, Mortierella minutissima van Thieghem, Chaetomium sp, Humicola grisea. In the immature compost the colonies we identified are Aspergillus niger, Mortierella minutissima van Thieghem. A high frequency of occurrences in the case of fungi was determined to: Aspergillus niger for immature compost and Mortierella minutissima, for mature compost.
In table number 2 we described cultural characters of these two types of fungi we identified in both composts.

### Table 2

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Frequency of occurrences (%)</th>
<th>Aspects</th>
<th>Microscopic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mature compost</td>
<td>Immature compost</td>
<td></td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>22</td>
<td>32</td>
<td>flooded colonies, textured surface and with features air conidia, black conidia with septal hyphae, conidia made of unseptated hyphae showing terminal columnella, sterigm and chains of conidia</td>
</tr>
<tr>
<td>Mortierella minutissima van Thieghem</td>
<td>48</td>
<td>16</td>
<td>high sporangia of about 100 μ, sporangia with a diameter of about 40 μ containing until 20 spherical spores with a diameter of 8-10 μ</td>
</tr>
</tbody>
</table>

### Conclusions

Microbial biodiversity of compost is dependent on the nature and composition of organic substances predominantly used in the initial material composition heap.

During the composting process the ratio between microbial groups involved in decomposition is changing. In immature compost numerically predominant are bacteria and fungi. Gram positive bacterial species such as Bacillus megaterium and Arthrobacter oxidans were identified with a high frequency of occurrence on the boards. The abundance of complex organic substances, nehumificate in immature compost determine the existence of high number of fungi compared to the number of it in the version of mature compost. Fungal diversity is different in these two types of compost. Among the fungi Mortierella minutissima with a high frequency of occurrence on the plates in case of mature compost can be considered an indicator species of compost maturity. Our future research will confirm this. Biological stability of compost is given the predominance of actinomycetes in microflora composition and the benefit of these microorganisms in microbial antagonism against fungi but also against bacteria (4). It appears that Streptomyces olivochromogenes is the characteristic species in mature compost while Streptomyces griseoruber is specific in immature compost.
References