Biodegradation of Non-lignocellulosic Substances III Effects of hot- and cold-water extractives of sawdust on bacterial multiplication

by

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非リグノセルロース質の微生物分解Ⅲ バクテリアの増殖に対するノコ屑の冷・温水抽出物の影響

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Abstract

The effects of hot- and cold-water extractives from the sawdust of six softwood species used as an artificial soil matrix in the GADE (Garbage Automatic Decomposer-Extinguisher) machine on bacterial growth were examined.

The extractives from karamatsu, sugi, and todomatsu did not inhibit the bacterial growth but rather promoted their growth. The extractives from ezomatsu and hiba did not affect on the bacterial growth so much. On the other hand, the extractives from hinoki intensively inhibited the bacterial growth.

These results suggest that sawdust from softwoods such as karamatsu, sugi and todomatsu are useful as an artificial soil matrix in the GADE machine and the sawdust from hiba and ezomatsu are also usable. On the contrary, mixed use of the sawdust of hinoki with the sawdust of karamatsu would be recommended in order to reduce the influence of the extractives of hinoki on bacterial activities when the operators of the GADE machines will use the sawdust of hinoki.

Keywords : garbage automatic decomposer-extinguisher (GADE), hot-water extract, cold-water extract, bacteria, sawdust, artificial soil matrix

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Introduction

We have developed the GADE (Garbage Automatic Decomposer-Extinguisher) machine to provide a new method for the management of food waste produced in our daily life[1].

In previous studies, we evaluated the efficiency of the GADE machine[2], and described the function of sawdust and bacteria as a bioreactor[3]. It is known that some extractives such as hinokithiol in hinoki inhibit the growth of bacteria and karamatsu contains large amount of taxifolin and arabinogaractan[4]. These facts suggests us to investigate the influence of the extractives from sawdust used as artificial soil matrix on the bacterial activities.

We cultured bacteria on various media with hot- and cold-water extracts obtained from six wood species (ezomatsu, todomatsu, karamatsu, sugi, hinoki and hiba) in different concentrations and examined whether bacteria grow normally on the media containing the extractives.

Materials and methods

Experimental materials

Sawdust samples (40 mesh) were prepared from ezomatsu (*Picea jezoensis* Carr.), todomatsu (*Abies sachalinensis* Masters), karamatsu (*Larix leptolepis* Gordon), sugi (*Cryptomeria japonica* D. Don), hinoki (*Chamaecyparis obtusa* Sieb Zucc.), and hiba (*Thujopsis dolabrate* var. hondae Makino). The sawdust was used after adjusting moisture content to 10-11%.

Preparation of extractives and bacterial growth media

Hot- and cold-water extractive solutions were prepared from 2g samples of sawdust with 100ml of deionized water. The hot- and cold-water extractive solutions in the concentrations of 1, 2, 5, and 10 times the original solution were prepared.

Hot- and cold-water extractives were concentrated by a rotary evaporator to dryness. Weights of the extractives were measured after dehydration of the extractives in a desicator with phosphorous anhydride as drying reagent.

Inoculation of bacteria

The bacteria of the 6 species (two *Norcadia* spp, two *Bacilus* spp, one *Acetobactor* sp, and *Mycobacterium* sp) precultured on agar medium (1% beef extract, 1% peptone, 0.5% NaCl, 1.5 % agar, pH 7.2),

Table 1 Composition of bacterial growth media.

Standard	Control	Hot- and cold-water extracts
NH_4NO_3 (0.1g)	○	○
KH_2PO_4 (0.1g)	○	○
$MgSO_4$ $7H_2O$ (0.05g)	○	○
KCl (0.02g)	○	○
Glucose (1.0g)	×	○
Agar (1.5g)	○	○
Distilled water (100ml) (pH 7.2)	○	× [1]

Note: 1) Hot- and cold-water extractives solutions (original solution, 2, 5, 10 con.) were used instead of distilled water.

were suspended in 4ml of deionized water. The suspension agent was diluted to 1000 times of the original concentration. The diluted solution (150 μ l) was spread on the bacterial growth media (Table 1). After an incubation period of 3-5 days at 30°C, the numbers of the colonies on the media were counted.

Results and discussion

Hot- and cold-water extractives from sawdusts

Table 2 shows the amount of the extractives from of ezomatsu, todomatsu, karamatsu, sugi, hinoki and hiba. The maximum quantity of extracts, among the hot-water extractives, was obtained from ezomatsu. Hinoki yielded the maximum quantity of extracts from the cold-water extractives.

The extractives in the hot-water extract solutions, obtained from the six wood species, were greater in quantity than the extractives obtained from the cold-water solutions (Table 2).

Table 2 Hot-and cold-water extractives obtained from ezomatsu, todomatsu, karamatsu, sugi, hinoki, and hiba (% on dry sawdust).

	Hot-water extractive (%)	Cold-water extractive (%)
ezomatsu	5.6	0.7
todomatsu	1.8	0.8
karamatsu	3.0	1.3
sugi	1.3	0.6
hinoki	2.9	1.9
hiba	1.3	1.0

Colony formation on bacterial multiplication media

Colony numbers on the media containing hot- and cold-water extractives of different concentrations prepared from ezomatsu, todomatsu, karamatsu, sugi, hinoki, and hiba were compared with those of a standard media. The average of colony numbers on the control media were 89. The results are shown in Figs. 1 and 2.

Comparison of colony number between hot- and cold-water extract media

Figs. 1 and 2 show the effect of cold- and hot-water extractives from 6 softwood species on

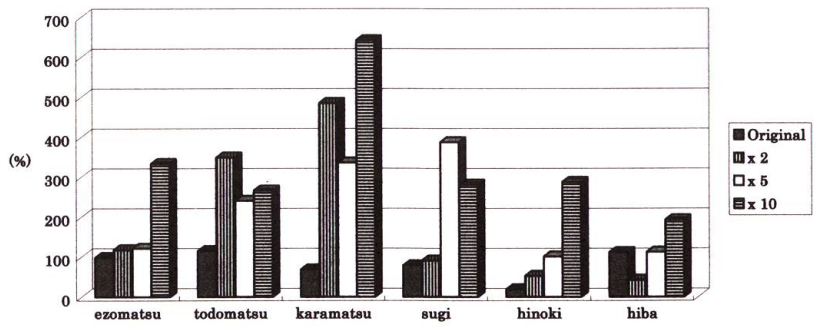

Fig. 1 Effect of cold-water extractives from six softwood species on bacterial growth
Note : Colony number ratio to the controls

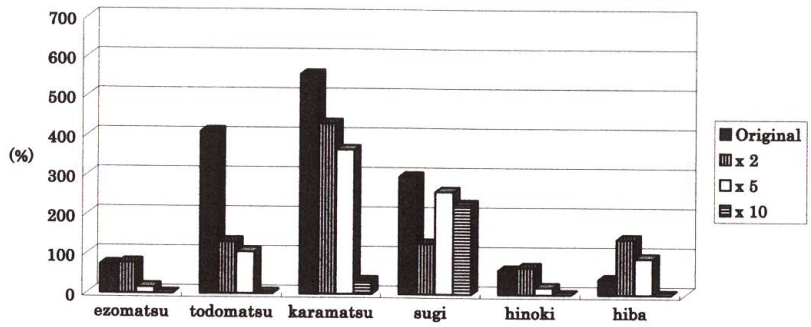

Fig.2 Effect of hot-water extractives from six softwood species on bacterial growth
note : Colony number ratio to the controls

bacterial growth, respectively.

The cold-water extractives promoted the bacterial growth with increasing in concentration, leading to 620 % in the case of karamatsu (Fig. 1). Cold-water extractives seem not to inhibit the bacterial growth.

In contrast, the hot-water extractives showed different tendency from those of the cold-water extractives (Fig. 2). Namely, the colony number ratio on the media containing the hot-water extractives from ezomatsu and hinoki were less than 100 %, showing their inhibitory effect on the bacterial growth. Kinjo and Yaga[5]) have reported the existence of the components which inhibit mycelial growth of basidiomycetes in hinoki. The colony number ratio on the media containing the hot-water extractives of todomatsu and hiba were around 100%, showing no big effect on the bacterial growth. On the other hand, the colony number ratio on the media containing the hot-water extractives of karamatsu, sugi and todomatsu leads to 200-540%, showing their promotive effects on the bacterial growth (Fig. 2).

During the operation of a GADE machine, the moisture content of the sawdust matrix is being kept about 60 %. This means that concentration of the extractives in the free water of the sawdust matrix might be high. Therefore, an attention must be paid to the growth attitude of bacteria on the media containing extarctives in higher concentrations. Furthermore, the temperatures of the matrix in the GADE machine also gradually increase around 40-50 °C. Therefore, another attention must be paid more on the growth attitude of the bacteria on the media containing hot-water extractives than on those containing cold-water extractives.

Differences in the colony number ratios between the media containing hot- and cold-water extractives seem to be caused by differences in the contents and amounts of extractives. It will be an interesting future work to clarify the factors for the promotion and inhibition phenomena of bacterial growth.

In conclusion, it is found that the extractives in the sawdust of the six softwood species influence the bacterial growth somehow. The extractives from the sawdust of karamatsu, sugi and todomatsu promote the bacterial growth and extractives from the sawdust of hinoki inhibit the bacterial growth.

From these results, it is suggested that the sawdusts of karamatsu, sugi and todomatsu are useful material as artificial soil matrix in GADE system, the sawdust of ezomatsu and hiba are usable, and the operators of GADE machine would be recommend to mix the sawdust of hinoki with the sawdust of karamatsu to reduce the inhibitory effect on the bacterial growth when they will use the sawdust of hinoki as artificial soil matrix for GADE system.

Acknowledgments

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要　　旨

生ゴミ分解消滅システム（GADE）中の人工土壌マトリックスとして用いられるノコ屑からの冷・温水抽出物が，バクテリアの増殖にあたえる影響を調べた。

その結果，カラマツ，スギ，トドマツのノコ屑の抽出物は，バクテリアの増殖に好影響を与え，エゾマツおよびヒバのノコ屑の抽出物はバクテリアの増殖に大きな影響はなく，一方，ヒノキのノコ屑の抽出物は，バクテリアの増殖を大きく阻害した。

このことから，カラマツ，スギ，トドマツのノコ屑は，GADE システムの人工土壌マトリックスとして使う際に有効であり，エゾマツおよびヒバは特に大きな支障が無いであろうことが示唆された。一方，阻害効果の大きいヒノキのノコ屑は，そのまま使用せずに，もっとも優良なカラマツのノコ屑と混合して使用することで，バクテリアの増殖阻害効果が軽減されるであろうことが示唆された。

キーワード：生ゴミ自動分解消滅(GADE)システム，冷水抽出物，温水抽出物，バクテリア，ノコ屑，人工土壌マトリックス