

ISOLATION AND IDENTIFICATION OF *PYTHIUM* FROM SOIL

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ABSTRACT

The objectives of the experiment were to isolate and identify the *Pythium* from soil. The experiment used a soil dilution plate and selective medium. The soil was taken from three different areas in Pedurungan district of Semarang City. The first soil was taken from a field planted with *Ipomoea aquatica* and was fertilized with urea. The second soil was taken from a dry field which was uncultivated, and the third soil was from a rice field which has never been fertilized or treated by any chemical. The key of Robertson (1979) and Plaat-Niterink (1981) were used to identify the *Pythium* and then two isolates were chosen.

The results showed that there were sixteen different isolates from the three different soils. In the first, second and third soils were obtained eleven isolates, two isolates and three isolates, respectively. The identification of two isolates chosen showed that isolate number 2 was determined as *Pythium aphanidermatum* Fitzpatrick and isolate number 6 was determined as *Pythium periplocum* Drechsler. These *Pythium* isolates need further elucidation for any fermentative purpose in processing high cellulose feedstuffs.

Keywords : isolation, identification, *Pythium*, soil

INTRODUCTION

Soil fungi are about 100 times less numerous in soils than bacteria but usually have a greater biomass (Gams *et al.*, 1987). Fungi always play the greatest role in the microbial decomposition processes in the soil. Most of them are saprophytes (soil borne fungi) which often occur with increased densities around roots.

Pythium is one of the soil fungi. The genus *Pythium* was established by Pringsheim in 1858, and placed in the family Saprolegniaceae. *Pythium entophyllum* Pringsheim and *Pythium monospermum* Pringsheim were the first described species and *Pythium monospermum* became the type species, while *Pythium entophyllum* was transferred in 1890 by Zopf to the genus *Lagenidium* (Robertson, 1979). De Bary in 1881 and Fisher in 1892 moved *Pythium* and *Lagenidium* to Peronosporaceae (Plaat Niterink, 1981; and Watanabe *et al.*, 1988). Owing to the differences between *Pythium* and other Oomycetes, Schorter in 1897 moved *Pythium* to a new family, Pythiaceae.

Since that time the taxonomy position of the genus has remained unchanged (Hendrix and Campbell, 1973). In the textbook by Alexopoulos and Mims (1979), the complete taxonomy of the genus *Pythium*, is listed as follows: Superkingdom: Eucaryota; Kingdom: Myceteae (fungi); Division: Mastigomycota; Sub Division: Diplomastigomycota; Class: Oomycetes; Order: Peronosporales; Family: Pythiaceae.

Pythium species are ecologically versatile and physiologically unique fungi. They are ubiquitous in soil and aquatic environments, both in the temperate and tropical zones *Pythium* species occurs in almost all types of soils (Stanghellini, 1974 ; Dick and Ali-Stayeh, 1986). As earlier mentioned, the soil fungi are mainly responsible for the decomposition of ligno-cellulosic matters in the upper soil layers. In other words, the activity of soil fungi can produce enzymes. In addition to other soil fungi, *Pythium* can also produce the enzymes. Hendrix and Campbell (1974) reported that most of genus *Pythium* have possess extreme catabolic abilities, such as for cellulose and pectic

degradation. Endo and Colt (1974) also noted that *Pythium* have an ability to produce pectic and cellulolytic enzymes.

Based on the ability, *Pythium* is possible to be used in many fermentative purposes either in temperate zone or in tropical zone. In Animal agriculture practise, cellulase producing fungi e.g. *Trichoderma viride*, *Aspergillus niger* and *Sacharomyces cereviceae*, can be used as an inoculum for fermentation of agriculture by-product (Rahmadi *et al.*, 1997 ; Nuswantara *et al.*, 2001; Christiyanto *et al.*, 2001; Prawirodigdo and Andayani, 2005). Therefore *Pythium* as well as other cellulase producing fungi could also be used as an inoculum for fermentative purpose in processing high cellulolistic feedstuff.

The first step in creating the inoculum of *Pythium* is producing its isolate from the soil. There are some problems in the isolation process of *Pythium* soil. Even *Pythium spp* profusely produce their structures in the soil, but it is difficult to recover it in the medium, that is because *Pythium* grows less than other fungi or bacteria. Because of the difficulties, a special technique is required. According to Dingra and Sinclair (1985) there is one of the most popular method for isolation and enumeration of soil born fungi, that is soil dilution plate. This study was aimed to isolate and identify the *Pythium* from soil using the key of Robertson (1979) and Plaat-Niterink (1981). The methods use selected media, and have been proved to simplify the recovery of *Pythium*.

MATERIALS AND METHODS

Isolation of *Pythium* from Soil

The experiment used the soil dilution plate. The soil samples from three different areas in Pedurangan district Semarang City was used in the study. The first soil was taken from a field planted with *Ipomoea aquatica* and was fertilized with urea. The second soil was taken from a dry field which was uncultivated, and the third soil was from a rice field which has never been fertilizer or treated

by any chemical. Each soil sample (100 gr) was diluted to create four different concentrations from 10^4 to 10^{-1} . One ml from the solution from each concentration was smear across the surface of solidified plate agar selective medium P₁₀ VP. The plates were incubated at 25°C for 24 hours. Soil particles and bacterial contaminant were removed from the plates by washing the agar surface under running water, and then the plates were examined for the presence of *Pythium spp*.

Identification of Two *Pythium* Isolates

From the sixteen isolates, two isolates were chosen for identification. The two isolates chosen were isolate number 2 and 6, because the growth of those isolates. The isolate number 2 grew slower and the isolate number 6 grew faster than others. Both isolates were grown in three different media : potato carrot agar (PCA); potato dextrose agar (PDA), and V-8 juice agar. All plates were placed at 25°C and examined at regular intervals for production of sporangia, oogonia and antheridia. The production of zoospores production was induced by adding water to isolates that was grown on V-8 juice agar. Lactofenol with cotton blue was used to stain for examining reproduction structures. The key of Robertson (1979) and Plaat-Niterink (1981) were used to identify the *Pythium spp*.

RESULTS AND DISCUSSION

It is better to use a selective medium because there is a difficulty in isolating *Pythium spp* (Green and Jensen, 1992) . The selective medium of modified P₁₀ VP from Tsao and Ocana contains antibiotics piramicyn, vancomycin and pentachloronitrobenzene. Hendrix and Campbell (1974) reproted that species of Pythiaceae (including *Pythium*) are insensitive to polyene antifungal antibiotics, such as pentachloronitro benzene. Therefore, the usage of medium could allow *Pythium* to grow better than other fungi, because the antibiotics inhibit growth of other fungi and bacteria than *Pythium* itself.

Table 1. Number of *Pythium* Isolate from Three different Soils

Type of soil	Number of Isolate
Cultivated (planted with <i>Ipomoea Aquatica</i>)	11
Uncultivated	2
Cultivated (without fertilizer and any chemical)	3

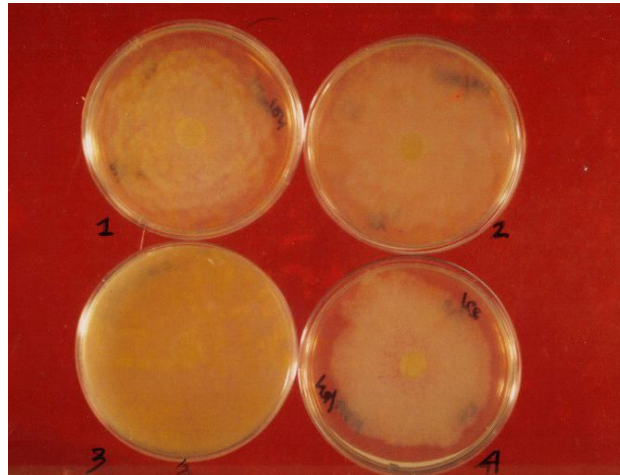


Figure 1. The different growth pattern of *Pythium* isolates. 1. Mixed Chrysanthemum with roset pattern; 2. Chrysanthemum pattern; 3. No special pattern; 4. Unclearly define.

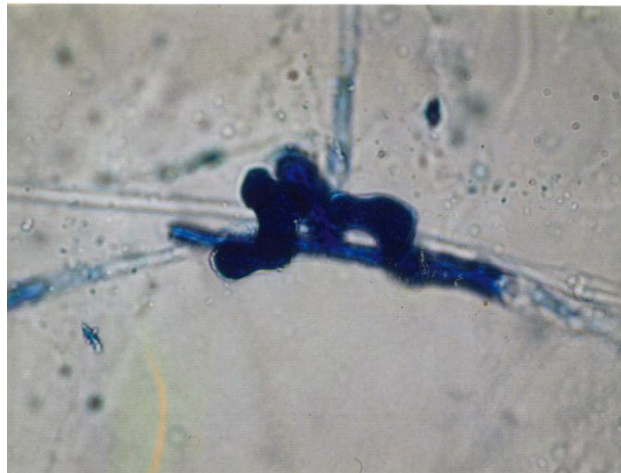


Figure 2. The sporangia of *Pythium aphanidermatum*

The isolation have been done from three samples of soil. From these isolations were obtained sixteen different isolates. In the first, second and third soils were obtained eleven isolates, two isolates and three isolates, respectively (Table 1).

From the three different kinds of soil were isolated a different number of *Pythium spp.* This was possibly due to the different conditions of the soil. The first soil planted with *Ipomoea Aquatica* and was fertilized by urea. Like other cultivated and fertilized soil, in the first soil there were many root exudates, therefore the soil was rich with nutrition for *Pythium*. These condition was favourable to *Pythium* growth, and a condition which was not found in the second and third soils. The second

soil which was uncultivated and dry soil, therefore the soil was unfavourable for *Pythium* growth. Though the third soil was cultivated soil, but the soil never be treated by any fertilizer or chemical. Therefore the soil was not comfortable for the growth of plant, and the condition was unfavourable for *Pythium*. Owing to the number of isolates from the first soil was much higher than in the second and third soils. Plaatsma and Nierink (1981) and Knudsen *et al.* (1992) reported that *Pythium spp.* occurs in almost all types of soils and abundant in cultivated soil than in uncultivated soil.

Water is essential for a life, and fungi in general are particularly dependent upon a moist environment. The soil moisture of the first soil was much higher than those of the second and the third

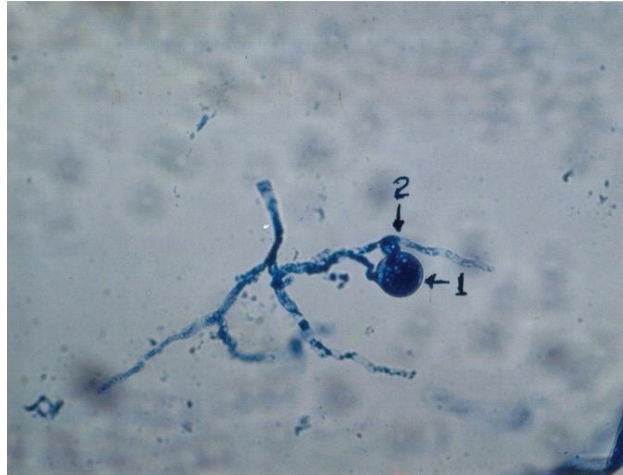


Figure 3. The oogonium (no.1) and antheridium (no.2) of *Pythium aphanidermatum*

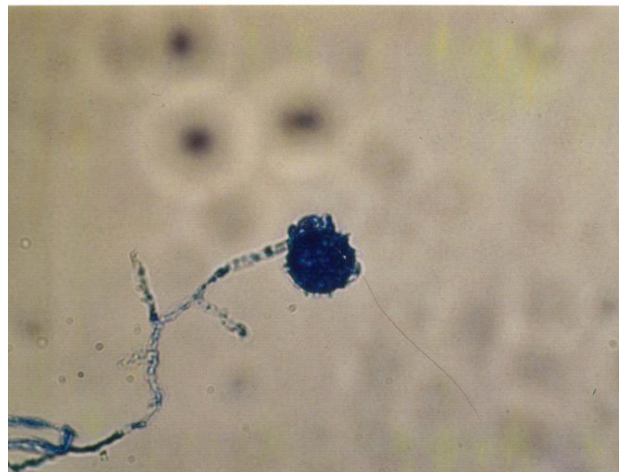


Figure 4. The oogonium of *Pythium periplocum*

soils. Therefore it made the number of isolates from the first soil was much higher than in the second and the third soils. The influence of soil moisture to the growth of fungi. The soil moisture can affect the production of sporangia, development of antheridia, oogonia and development of mycelia of *Pythium*. Mirchetich (1970) found that at a high moisture soil *Pythium spp* can be colonized the dead and roots plants much more than in less moist condition. Agnihotri and Vaartaja (1967) also found from their *in vitro* experiment that sporangial germination was higher when moisture content was increased from 67% to 85%.

In this experiment the isolate number 2 and 6 were decided to be identified. After the two isolates were grown on three different media. their reproductive structures were produced. This was

observed under the microscope using the terms in the keys of Robertson (1979) and Plaat-Niterink (1981). The characteristics of isolate number 2 were as follows : the colonies pattern on potato carrot agar were without special pattern (Figure 1). The main hyphae had 10.08 μm wide, and the sporangia (15.12 μm wide) were formed by swollen hyphae branches (Figure 2). The zoospores were formed at 25°C, and the oogonia terminal, globose, smooth and the diameter was 24.5 μm (Figure 3). The antheridia were mostly intercalary, and sometimes were terminal and 10.08 μm long, 10.08 μm wide, 1 –2 per oogonium (Figure 3). The oospore aplerotic had diameter of 20.16 μm , and the thick wall was 1.5 μm . The daily growth on potato dextrose agar at 25°C was more than 20 mm. The isolate number 2 and its characters was

confirmed to Dr.R. De Cock (Central Bureau Voor Schimmecultures, Holland), and was determined as *Pythium aphanidermatum* Fitzpatrick.

The characteristics of isolate number 6 were as follows : the colonies pattern on potato carrot agar were without special pattern (Figure1). The main hyphae had 10.08µm wide. The zoospores were formed at 20°C, and the oogonium wasterminal and intercalary with blunt spines 2.5 µm long. The diameter of oogonium was 25.2 µm (Figure 4). The antheridia had 1 – 4 per oogonium and was declinous. The oospore was aplerotic with diameter 22 µm, and the thick wall was 1.5µm. The daily growth of the *Pythium* on potato dextrose agar was at 25°C, and was less than 20 mm. The isolate number 6 and its characters was confirmed to Dr.R.A. Samson (Central Bureau Voor Schimmecultures, Holland), and was determined as *Pythium periplocum* Drechsler.

CONCLUSION

A selective medium was used to isolate and identify *Pythium spp.* The two isolates were determined as *Pythium aphanidermatum* Fitzpatrick and *Pythium periplocum* Drechsler. These *Pythium* isolates need further elucidation for any fermentative purpose in processing high cellulosic feedstuffs.

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