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Mini Review

Peningkatan Produksi Padi Berkelanjutan Pada Lahan Rawa Pasang Surut (*Increasing of Sustainable Rice Production on Swampland*) **Nurita, Isdijanto Ar-Riza**

Penelitian

Pengaruh Perbedaan Suhu Fermentasi Moromi Terhadap Sifat Kimia Dan Mikroflora Moromi Kecap Koro Pedang (*Canavalia ensiformis L.*) (*Effect of Different Temperature of Moromi Fermentation on Chemical and Microflora Characteristics of Jack Bean Sauce (Canavalia ensiformis L.)*) **Beti Cahyaning Astuti**

Kajian Proses Produksi Pulp Dan Kertas Ramah Lingkungan Dari Sabut Kelapa (*Study on the Production of Environmental Friendly Pulp and Paper from Coconut Husk*) **Khaswar Syamsu, Han Roliadi, Krishna Purnawan Candra, Akbar Jamaluddin Arsyad**

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Telp 0541-749159
e-mail: jtpunmul@gmail.com

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ISOLATION OF CELLULOLYTIC MICROBIALS FROM SEVERAL LOCATIONS WERE ASSOCIATED WITH THE PALM OIL INDUSTRY

Isolasi Mikroba Selulolitik dari Beberapa Lokasi Industri Minyak Sawit

Hamka Nurkaya

Samarinda State Polytechnic for Agriculture, Jl. Samratulangi-Kampus Poltanesa, Kotak Pos 192, Samarinda 75131, E-mail: hamka_nurkaya@yahoo.com

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ABSTRACT

Cellulose is the main constituent component of photosynthesis in the plant biomass composed of fibrous and woody material such as straw, weeds, grass, leaves, stems and branches of plants which could be easily degraded by microbial such as bacteria, fungi and actinomycetes. With the utilization of twelve (12) samples for isolation of microbials collected from several locations associated with the palm oil industry which were obtained as many fifty eight (58) isolates of microbial and then classified to be the thirty nine (39) isolates are bacteria, ten (10) isolates of actinomycetes and nine (9) isolates of fungi. All isolates of microbial isolated were identified by colony properties of microorganisms, Gram stain and slide culture technique.

Keywords: cellulose, bacteria, fungi, actinomycetes.

BACKGROUND

Cellulose is the main constituent component of photosynthesis in the plant biomass composed of fibrous and woody material such as straw, weeds, grass, leaves, stems and branches of plants (Sutedja *et al.*, 1991; Jarvis, 2003; and Zhang *et al.*, 2004). As plant biomass, cellulose often found in the biomass lignocellulosic materials such as biomass agricultural, forestry, and agro-industrial wastes that are abundant, renewable and inexpensive energy sources. Cellulose can be degraded easily and quickly just by specific organisms such as bacteria, fungi, actinomycetes, and the lower animals. Experts classify cellulose decomposers organisms are aerobic bacterial, myxobacteria, anaerobic bacteria, thermophilic group, actinomycetes, filamentous fungi, mushroom, protozoa and insects. Success in breaking down cellulose depends on the nature or circumstances of cellulose degrading microorganisms and environmental factors such as humidity, aeration, temperature, and nitrogen availability of

adequate and nutritional elements (Sutedja *et al.*, 1991).

In this study, All samples used for the isolation of cellulolytic microbials (soil, leaf bamboo, rod of palm tree, etc.) were collected from several places associated with the palm oil industry. Aim of this study was to isolate and identify the cellulolytic microorganisms from samples were collected at the locations associated with the oil palm plantation and industry.

MATERIALS AND METHODS

Raw materials

All of the samples for the isolation of cellulolytic microbials (soil, leaf bamboo, rod of palm tree, seed of the palm tree, fiber of the oil palm empty fruit bunch, etc.) were collected from the palm oil plantation and palm mill area in Petchabury Province, Thailand.

Source of microbials

Samples for isolation of microbials used in this study collected from several places associated with the oil palm Industry. Sample properties also recorded.

Isolation of microbials

Liquid carboxymethyl cellulose medium (CMC medium) consist of 1 g KH_2PO_4 , 0.5 g NaCl , 0.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01 g $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 0.5 g $(\text{NH}_4)_2\text{SO}_4$ and 10 g CMC in 1 liter of distilled water was prepared and sterilized at 121°C for 15 min, which was used to enrich microorganism from sample. Each sample of 1 g was added into 100 mL sterilized CMC medium and incubated at room temperature ($30\text{-}32^\circ\text{C}$) under shaking condition (150 rpm) for 10 days. After incubation, microorganism; bacteria, fungi and actinomycetes were collected by a streak plate technique on CMC agar (1.5 % agar was added into the CMC medium). Cultured petridishes were incubated at room temperature ($30\text{-}32^\circ\text{C}$) for 3 days. After incubation, colonized microorganisms were subsequently transferred on fresh CMC agar and incubated for 3 days to obtain pure cultures. All strains were kept in a deep freezer at -80°C and used for the future experimentation.

Pre-primary identification and characterization of cellulolytic microbials

Pre-primary identification was done to separate and classify selected strains into the member of bacteria, actinomycetes and fungi.

Colony properties of microorganism

Colony properties of microorganism were observed based on a single colony of microbes grown on a CMC medium. Cultures were incubated for 3-5 days at room temperature and then the shape, color, surface, internal structure and the structure of the edge of the colony were also observed. Based on colony properties, bacteria, actinomycetes and fungi were also separated. Cell morphologies and spore formulation of selected isolates were also determined.

Slide culture technique for fungi

In order to accurately identify fungus, it is essential to observe the precise arrangement of the conidiophores (conidial ontogeny). A simple modification of fungal slide culture technique was used to observe conidiophores of selected fungal isolates (Riddle, 1950). A method is described by Riddle (1950) and conidiophores picture was recorded and used to identify the genus of fungal isolates.

Gram staining technique

The Gram stain is the most important and universally used stained technique in the bacteriology laboratory. It is used to distinguish between gram-positive and gram-negative bacteria, which have distinct and consistent differences in their cell walls. Gram staining of bacteria is used Gram method and consequently, Gram-positive and Gram-negative bacteria were classified.

RESULT AND DISCUSSION

Samples for isolation of cellulolytic microbials

The samples that are used in this research for isolation of microbials were collected from the palm oil plantation and palm oil mill areas in Pechabury Province, Thailand. The samples were shown in Table 1.

The twelve (12) samples were selected from the palm oil plantation and palm oil mill areas such as soil, a rod palm tree, immature bunch, seed of the oil palm empty fruit bunch, shell, fiber of the oil palm empty fruit bunch which they contain normal flora that can decompose cellulosic material; cellulolytic microorganism. According to Smruti *et al.* (1995) reported the cellulase producing microbes have been isolated from lignocellulosic materials such as decaying wood, forest residues and decomposed leaf in soil.

Table 1. Types, sources, and the characteristic of samples, which were selected from the palm oil plantation and palm oil mill areas.

Samples	Type of samples	Sources	Sample Characteristic	Figure
1	Soil	Plantation field	Brown color, and hard structure	
2	Leaf bamboo mix with gravel and soil	Plantation field	Dried leaves and brown color	
3	Root bamboo	Plantation field	Yellow color and easily broken	
4	Rod palm tree	Plantation field	Decomposed, dark color, easily broken, and fiber	
5	Stalk leaf palm tree	Plantation field	Stalk leaf decomposed, soil attaches at the stalk, brown color, easily broken, and no hard structure	
6	Leaf and Oil	Plantation field	Leaf decomposed and dark color	
7	Shell, fiber and seed of processing palm oil	Palm oil mill	Dark and brown color, decomposed and white spot color	
8	Pasta palm oil processed	Palm oil mill	Smooth, clear brown color, residue pressed processing oil and oily	
9	Seed (kernel) and fiber	Palm oil mill	Dark color and decomposed	
10	Immature Bunche	Palm oil mill	Dark and brown color and white spot color	
11	Residues pressed processing palm oil	Palm oil mill	Smelt, oily, easy broken, solid structure and brown color	
12	Shell, immature bunch and fiber	Palm oil mill	After boiling process, gray color and white spot color	

Isolation of microbials

There were fifty eight (58) isolates of microbials isolated from the samples

asso-ciated with oil palm industry. Based on colony formation and cell morphology (under microscope), those microorga-

nisms obtained from screening was separated into a member of bacteria, actinomycetes and fungi. Thirty nine isolates (39) of microorganisms belonged to member of bacteria, while ten isolates (10) was classified to be a member of actinomy-cetes. The rest isolates (nine isolates) are fungi.

According to Heck *et al.*, (2002) and Semedo *et al.*, (2000), reported many of cellulolytic aerobic and anaerobic bacteria could be isolated from decomposed soil and the lignocellulosic of residues (leaf, stalks, and stems of garden plants) for biotechnological applications.

Table 2. The fifty eight (58) isolates of microorganisms obtained from plant and soil samples associated with palm oil industry

Samples	Type of Microorganisms	Amount of Isolates	Isolate codes
1	Actinomycetes (A)	1	1.2.A
	Bacteria (B)	3	1.1.B, 1.3.B, 1.4.B
	Fungi (F)	2	1.5.F, 1.6.F
2	Actinomycetes (A)	1	2.3.A
	Bacteria (B)	4	2.1.B, 2.2.B, 2.4.B, 2.5.B
	Fungi (F)	N.D	-
3	Actinomycetes (A)	1	3.1.A
	Bacteria (B)	4	3.2.B, 3.3.B, 3.4.B, 3.5.B
	Fungi (F)	N.D	-
4	Actinomycetes (A)	1	4.2.A
	Bacteria (B)	4	4.1.B, 4.3.B, 4.4.B
	Fungi (F)	1	4.5.F
5	Actinomycetes (A)	1	5.1.A
	Bacteria (B)	3	5.2.B, 5.3.B, 5.4.B
	Fungi (F)	N.D	-
6	Actinomycetes (A)	1	6.2.A
	Bacteria (B)	3	6.1.B, 6.3.B, 6.4.B
	Fungi (F)	N.D	-
7	Actinomycetes (A)	N.D	-
	Bacteria (B)	3	7.1.B, 7.2.B, 7.3.B
	Fungi (F)	1	7.4.F
8	Actinomycetes (A)	1	8.3.A
	Bacteria (B)	3	8.1.B, 8.2.B, 8.4.B
	Fungi (F)	2	8.5.F, 8.6.F
9	Actinomycetes (A)	N.D	-
	Bacteria (B)	4	9.1.B, 9.2.B, 9.3.B, 9.4.B
	Fungi (F)	N.D	-
10	Actinomycetes (A)	1	10.1.A
	Bacteria (B)	4	10.2.B, 10.3.B, 10.4.B, 0.5.B
	Fungi (F)	1	10.6.F
11	Actinomycetes (A)	1	11.1.A
	Bacteria (B)	1	11.2.B
	Fungi (F)	1	11.3.F
12	Actinomycetes (A)	1	12.3.A
	Bacteria (B)	2	12.1.B, 12.2.B
	Fungi (F)	2	12.4.F, 12.5.F

Colony properties of microbials

Table 3, showed cell and colony morphological studies of bacterial isolates. Under a light microscope, bacteria were clearly separated from fungi and actinomycetes. Colony morphological studies of bacteria can help at bacterial genus classification. Various factors

influence the shape and color of the colonized bacterial colony such as type of bacterial culture medium, pH, temperature and condition (aerobic, anaerobic, anoxic etc.) for incubation. In this experiment, all microorganisms were cultured on CMC agar.

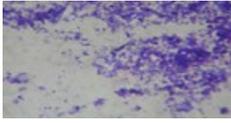
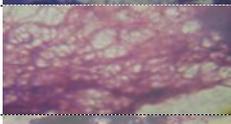
Table 3. Cell, colony morphology and Gram staining of bacterial isolates

Isolates	Gram Reaction	Cell Morphology	Colony Morphology
1.1.B	Positive	Curved rod	White, circular, opaque with low convex and entire edge
1.3.B	Positive	Coccus	White, circular, opaque, with low convex and entire edge
1.4.B	Negative	Rod	White-Cream, irregular, opaque, with convex and entire edge
2.1.B	Negative	Rod	White-Cream, irregular, opaque, with low convex and undulate edge
2.2.B	Negative	Rod	White-Cream, circular, translucent, with convex and entire edge
2.4.B	Negative	Rod	White-Cream, irregular, opaque, with convex and entire edge
2.5.B	Negative	Rod	White-Cream, irregular, opaque, with low convex and undulate edge
3.2.B	Positive	Curved rod	White, circular, translucent, with low convex and undulate edge
3.3.B	Positive	Rod	White-yellow, circular, translucent, with low convex and entire edge
3.4.B	Positive	Rod	White-Cream, irregular, opaque, with convex and entire edge
3.5.B	Negative	Coccus	White-Cream, irregular, opaque, with convex and undulate edge
4.1.B	Positive	Coccus	White-Cream, circular, translucent, with convex and entire edge
4.3.B	Negative	Rod	White, irregular, translucent, with convex and undulate edge
4.4.B	Positive	Curved rod	White-yellow, irregular, opaque, with convex and entire edge
5.2.B	Negative	Coccus	White-yellow, irregular, opaque, with low convex and undulate edge
5.3.B	Negative	Rod	White-yellow, irregular, translucent, with low convex and undulate edge
5.4.B	Positive	Curved rod	White-yellow, irregular, opaque, with convex and undulate edge
6.1.B	Negative	Rod	White-yellow, circular, opaque, with convex and entire edge
6.3.B	Positive	Curved rod	White-Cream, irregular, translucent, with convex and undulate edge
6.4.B	Negative	Rod	White-Cream, irregular, opaque, with low convex and undulate edge
7.1.B	Negative	Curved rod	White-Cream, irregular, opaque, with convex and entire edge
7.2.B	Negative	Curved rod	White-yellow, irregular, translucent, with convex and entire edge
7.3.B	Negative	Rod	White-Cream, irregular, opaque, with low convex and entire edge
8.1.B	Positive	Coccus	White-Cream, irregular, translucent, with low convex and entire edge
8.2.B	Negative	Curved rod	White-Cream, circular, translucent, with convex and entire edge
8.4.B	Negative	Curved rod	White-Cream, irregular, opaque, with low convex and entire edge
9.1.B	Positive	Curved rod	White-Cream, irregular, translucent, with low convex and undulate edge
9.2.B	Positive	Coccus	White-Cream, irregular, opaque, with convex and undulate edge
9.3.B	Negative	Curved rod	White-Cream, irregular, opaque, with low convex and undulate edge
9.4.B	Positive	Coccus	White-Cream, irregular, translucent, with convex and undulate edge
10.2.B	Positive	Rod	White, Amoeboid, translucent, with low convex and undulate edge
10.3.B	Negative	Rod	White-yellow, irregular, translucent, with convex and undulate edge
10.4.B	Positive	Coccus	White-Cream, irregular, translucent, with convex and undulate edge
10.5.B	Positive	Coccus	White-Cream, irregular, opaque, with convex and undulate edge
11.1.B	Positive	Curved rod	White-yellow, irregular, translucent, with convex and entire edge
12.1.B	Positive	Curved rod	White-Cream, irregular, opaque, with convex and entire edge
12.2.B	Negative	Curved rod	White-Cream, irregular, opaque, with convex and undulate edge

There were fifty five (58) microbials isolates obtained from plant and soil samples associated with oil palm industry. Cell morphology and colony morphology of bacterial isolates such as shape, the margins or edges of the colony, the colony's color, colony and Gram cell wall were explained to support pre-primary identification of bacterial isolates. Under a light microscope, various shapes of bacterial cell were observed such as rod, curved rod, and coccus. There were 2 major types of bacterial colony shapes which could be seen after cultured on

CMC agar including circular and irregular form. The color or the pigment of the colony of all bacteria were white color (opaque or translucent) and also present of cream or yellow colonies. The shape of the colony of bacteria is some circular or some irregular. The edge of bacterial colony such as low convex with entire edge and convex with undulate edge was also determined. With Gram stain technique, there were nineteen (19) isolates and twenty (20) isolates classified into a member of Gram-negative and Gram-positive bacteria, respectively.

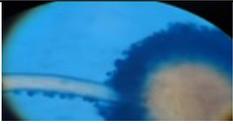
Table 4. Cell, colony morphology and Gram staining of actinomycetes isolates

Isolates	Gram Reaction	Cell Morphology	Colony Morphology	Figure
1.2.A	Positive (+)	Filamentous	White, circular, opaque, with low convex and entire edge	
2.3.A	Positive (+)	Filamentous	White, circular, opaque, with low convex and undulate edge	
3.1.A	Positive (+)	Filamentous	White-cream, irregular, opaque, with low convex and undulate edge	
4.2.A	Positive (+)	Filamentous	White-green, circular, opaque, with low convex and undulate edge	
5.1.A	Positive (+)	Filamentous	White, irregular, opaque, with low convex and undulate edge	
6.1.A	Positive (+)	Filamentous	White, irregular, opaque, with low convex and undulate edge	
8.3.A	Positive (+)	Filamentous	White, irregular, opaque, with low convex and entire edge	
10.1.A	Positive (+)	Filamentous	White, circular, opaque, with low convex and entire edge	
11.2.A	Positive (+)	Filamentous	White, irregular, opaque, with low convex and entire edge	
12.3.A	Positive (+)	Filamentous	White, circular, opaque, with low convex and undulate edge	

There were ten (10) isolates of actinomycetes which could be isolated from the samples associated with oil palm industry. Colonies of actinomycetes were white color, opaque with low convex and irregular in shape (table 4). Under microscope and Gram-stain technique, cell morphology of actinomycetes was

observed. A filamentous property and Gram stain positives were seen under light microscope (1,000×). With specific property of actinomycetes cell, all selected isolates were determined and confirmed to be actinomycetes. Characteristic of the colony and cell of actinomycetes represented in Table 4.

Table 5. Cell and colony morphology of fungal isolates

Isolate	Genus	Colony Color	Morphology	Figure
1.5.F	<i>Aspergillus</i> spp.	Black	Septate hyphae. Typical radiate conidial head	
1.6.F	<i>Aspergillus</i> spp.	Green	Septate hyphae. Typical columnar, uniseriate conidial head	
7.4.F	<i>Aspergillus</i> spp.	Green	Septate hyphae. Typical columnar, uniseriate conidial head	
8.5.F	<i>Aspergillus</i> spp.	Green	Septate hyphae. Typical columnar, uniseriate conidial head	
8.6.F	<i>Aspergillus</i> spp.	Green	Septate hyphae. Typical columnar, uniseriate conidial head	
10.6.F	<i>Aspergillus</i> spp.	Green	Septate hyphae. Typical columnar, uniseriate conidial head	
11.3.F	<i>Aspergillus</i> spp.	Green	Septate hyphae. Typical columnar, uniseriate conidial head	
12.4.F	<i>Aspergillus</i> spp.	Green	Septate hyphae. Typical columnar, uniseriate conidial head	

Fungi were important cellulose microorganism associated with cellulose cycle. Fungi are known as the best source of cellulase enzyme and their applications in biological technology such as

saccharification by fungal cellulase were reported. Table 5. shows the colony and cell morphological studies of fungal isolates which were isolated from the samples associated with the oil palm

industry. Based on colony morphology, fungal isolates were selected. Pre-primary identification was done by investigation of arrangement of conidia under a light microscope (Lactophenol cotton blue as staining dye). All fungal isolates presented conidiospore as a special characteristic of the genus *Aspergillus*. Therefore, nine (9) isolates of fungi were classified as member of the genus *Aspergillus*. Table 5, represents cell and colony morphology of fungal isolates.

CONCLUSION

The twelve (12) samples selected from several locations associated with the palm oil industry which were used to isolate and identified microbials and as many fifty eight (58) isolates of microbials were obtained from the locations at the palm oil industry areas. The thirty nine (39) isolates were classified as bacteria, ten (10) isolates of actinomycetes, and nine (9) isolates of fungi. All isolates of microbial isolated were identified by colony properties of microbial, Gram stain and slide culture technique.

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Pembahasan. Berisi interpretasi dari hasil penelitian yang diperoleh dan dikaitkan dengan hasil-hasil penelitian yang pernah dilaporkan (publikasi).

Ucapan Terima Kasih. Digunakan untuk me-

nyebutkan sumber dana penelitian dan untuk memberikan penghargaan kepada beberapa institusi atau orang yang membantu dalam pelaksanaan penelitian dan atau penulisan laporan.

Daftar Pustaka. Daftar Pustaka ditulis memakai sistem nama tahun dan disusun secara abjad. Beberapa contoh penulisan sumber acuan:

Jurnal

Wang SS, Chiang WC, Zhao BL, Zheng X, Kim IH (1991) Experimental analysis and computer simulation of starch-water interaction. *J Food Sci* 56: 121-129.

Buku

Charley H, Weaver C (1998) *Food a Scientific Approach*. Prentice-Hall Inc USA

Bab dalam Buku

Gordon J, Davis E (1998) Water migration and food storage stability. Dalam: *Food Storage Stability*. Taub I, Singh R. (eds.), CRC Press LLC.

Abstrak

Rusmana I, Hadioetomo RS (1991) *Bacillus thuringiensis* Berl. dari peternakan ulat sutera dan toksisitasnya. Abstrak Pertemuan Ilmiah Tahunan Perhimpunan Mikrobiologi Indonesia. Bogor 2-3 Des 1991. p. A-26.

Prosiding

Prabowo S, Zuheid N, Haryadi (2002) Aroma nasi: Perubahan setelah disimpan dalam wadah dengan suhu terkontrol. Dalam: *Prosiding Seminar Nasional PATPI*. Malang 30-31 Juli 2002. p. A48.

Skripsi/Tesis/Disertasi

Meliana B (1985) Pengaruh rasio udang dan tapioka terhadap sifat-sifat kerupuk udang. Skripsi Fakultas Teknologi Pertanian UGM Yogyakarta.

Informasi dari Internet

Hansen L (1999) Non-target effects of Bt corn pollen on the Monarch butterfly (Lepidoptera: Danaidae). <http://www.ent.iastate.edu/entsoc/ncb99/prog/abs/D81.html> [21 Agu 1999].

Bagi yang naskahnya dimuat, penulis dikenakan biaya Rp 175.000,00 (seratus tujuh puluh lima ribu rupiah).

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