SELECTION OF CELLULOLYTIC BACTERIA FOUND IN MASK WASTE FROM WANA TIRTA MANGROVE

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ABSTRACT

The amount of mask waste has increased significantly due to the COVID-19 pandemic. Mask waste can be found everywhere, likewise in Wana Tirta Mangrove. Its existence in the mangrove ecosystem, making mask waste, has the potential for cellulolytic bacteria. The purpose of this study is to determine the cellulase enzyme activity, characteristics, and genus of purified cellulolytic bacteria found in mask waste from Wana Tirta Mangrove. The method used in this study was qualitative research. The samples for this research were bacterial isolates from mask waste, which had previously been isolated from the Wana Tirta Mangrove. Samples were grown on CMC media to determine isolates that were included in cellulolytic bacteria. The cellulase activity of cellulolytic bacteria was tested qualitatively and quantitatively. Morphological, physiological and biochemical tests were carried out to obtain phenetic characters which were then used as the basis for determining the genus of cellulolytic bacteria. The results showed that 10 isolates were cellulolytic bacteria. The highest activity of cellulase enzyme qualitatively was produced by isolate 72 with a value of 0.466 mm, while the highest activity of cellulase enzyme quantitatively was produced by isolate 84 with a value of 0.931 U/ml. The dendrogram result showed 4 genera of cellulolytic bacteria, including Pseudomonas, Alcaligenes, Cellulomonas, and Aeromonas.

Keywords: cellulase enzyme activity; cellulolytic bacteria; mask waste; Wana Tirta Mangrove

Introduction

Mask use is a mandatory procedure during the COVID-19 pandemic (Atmojo et al., 2020). The Indonesian Ministry of Health, through Decree KMKHK.01.07/MENKES/413/2020, established guidelines for the prevention and control of COVID-19 in the community, one of which is wearing masks when outside the home (Firdayanti *et al*., 2020). Government policies regarding mask use have led to a drastic increase in the number of mask users. In Indonesia, the amount of disposable mask waste from the start of the pandemic to mid-2021 reached 21,768 tons (Hamdani & Haryanto, 2021).

Mask waste can be found in the environment everywhere, including the Wana Tirta Mangrove. Wana Tirta Mangrove is a mangrove conservation area located in Jangkaran Village, Temon District, Kulon Progo Regency, Yogyakarta Special Region (Rombe *et al*., 2021). The mask waste found around Wana Tirta Mangrove is suspected to be caused by humans carelessly disposing of it or because the waste was carried by river currents.

Mangrove ecosystems harbour a variety of germplasm, one of which is cellulolytic bacteria (Batubara *et al*., 2022). Mask waste found in the Wana Tirta Mangrove may contain cellulolytic bacteria, especially if the masks were partially or completely buried in mud contaminated with mangrove litter. The potential for cellulolytic bacteria in the mangrove ecosystem is due to the rich organic matter produced by cellulolytic bacteria, which is the result of leaf litter, decaying wood, and mud. The decomposition process of mangrove litter, which involves the breakdown of cellulose components by cellulase enzymes, is carried out by microorganisms, particularly cellulolytic bacteria (Khulud et al., 2020).

Cellulase is a complex enzyme consisting of three types of enzymes: endoglucanase, exoglucanase, and β-glucosidase, which work together to hydrolyse insoluble cellulose into glucose (Puspitasari &

Ibrahim, 2020). Several bacteria, such as those from the genera Acetobacter, Bacillus, Cellulomonas, Cythopaga, Sarcina, and Vibrio, are known to produce cellulase enzymes (Bandi *et al.*, 2018). Cellulase enzymes from cellulolytic bacteria have been widely used in various industries. In the fisheries sector, cellulase enzymes are used to process organic waste from shrimp ponds and waste from seaweed processing (Khulud et al., 2020). Cellulase has also been used in the pulp and paper industry for fiber modification and colour removal. In textiles, cellulase is used as a biopolishing agent for fabrics and as a detergent for softness and brightness.

The widespread use of cellulase enzymes in various fields necessitates further study of cellulase enzyme sources to meet the growing demand for these enzymes. The potential of mask waste from the mangrove ecosystem, suspected to be a source of cellulase, necessitates the selection and identification of cellulolytic bacteria, with the hope that the bacteria found can become a new source of cellulase enzymes. Therefore, this study was conducted to determine the cellulase enzyme activity, characteristics, and genera of cellulolytic bacteria found in mask waste from the Wana Tirta Mangrove.

Methods

This research employed a qualitative method, yielding descriptive data. The study was conducted in the Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Yogyakarta State University (YSU) from January to July 2023. The samples were bacterial isolates found in mask waste from the Wana Tirta Mangrove, which had previously undergone isolation. This study consisted of six stages: bacterial isolate purification, cellulolytic bacterial selection, qualitative cellulase enzyme activity testing, quantitative cellulase enzyme activity testing, phenotypic characterisation of the cellulolytic bacterial isolates, and identification of the cellulolytic bacterial genus.

Tools and Materials

The tools used in this study included an autoclave, incubator, laminar air flow meter, Genesys 10s UV-Vis spectrophotometer, oven, analytical balance, hot plate stirrer, shaker, centrifuge, vortex, test tubes, cotton swabs, test tube racks, loop needles, puncture needles, Erlenmeyer flasks, Bunsen burners, beakers, large Petri dishes, small Petri dishes, Durham tubes, Eppendorf tubes, micropipettes, microtips, spatulas, graduated cylinders, volumetric flasks, droppers, microscopes, slides and cover slips, thermometers, universal pH paper, vernier calipers, stand and clamps, UC bottles, vials, refrigerators, smartphone cameras, and the Multi-Variate Statistical Package (MVSP) 3.1 application.

The materials used in this study include bacterial isolates found in mask waste from Mangrove Wana Tirta, Nutrient Agar (NA) media, Nutrient Broth (NB) media, Carboxymethyl Cellulose (CMC) media, Sulfide Indole Motility (SIM) media, gelatin media, Lysine Decarboxylase (LIA) media, Simmons Citrate Agar (SCA) media, Methyl Red-Voges Proskauer (MR-VP) media, Bacteriological Agar (Agar No. 1) media, yeast extract, starch powder, carbohydrate powder (glucose, sucrose, fructose, maltose, galactose, mannitol, lactose), 3,5-Dinitrosalicylic Acid (DNS) solution, 40% KNa-Tartrate solution, Congo red solution, methyl red solution, 3% KOH solution, NaCl powder, HCl solution, NaOH solution, 40% KOH solution, alpha naphthol solution, phenol red solution, Lugol solution iodine, Ehrlich solution, xylene solution, Gram stain-kit, safranin solution, malachite green solution, 3% H2O2 solution, 70% alcohol, distilled water, and spirits.

The research method includes, in detail, tools and materials, characterisation instruments, procedures, data analysis techniques, and other things related to the research method. Published methods or procedures are simply stated with bibliographic citations, while modifications to the methods or procedures are explained in full.

Working Procedure

Purification of Bacterial Isolates

Bacterial isolates were inoculated into petri dishes containing NA media using the streak plate method, then incubated at room temperature for approximately 24 hours. The morphological appearance of the growing bacterial colonies was observed to determine the possibility of multiple

bacterial colonies growing in the petri dish. Separate bacterial colonies that exhibited distinct morphological characteristics were picked using a loop needle and inoculated onto NA agar slants. The growing bacterial colonies were Gram-stained, and the resulting slides were observed under a microscope to determine colony purity. The pure bacterial isolates on the agar slants were then used as stock cultures.

Qualitative Test of Cellulolytic Bacterial Cellulase Enzyme Activity

This test was performed using the Congo red method. Congo red solution (1 mg/ml) was poured onto the culture and allowed to incubate for 15 minutes. The solution was then discarded and rinsed with 1 M NaCl three times. This washing process aims to remove Congo red that is not bound to polysaccharides. Next, incubation is carried out at 37°C for 48 hours to complete the formation of a clear zone, and then the clear zone formed is observed. Bacterial isolates capable of decomposing CMC are indicated by the formation of a clear zone around the colony after being tested with the Congo red method. The cellulase activity index can be determined by measuring the ratio of the diameter of the clear zone to the diameter of the colony. The cellulolytic index is calculated using the following equation:

$$\left(SI = \frac{X1 - X2}{X2}\right)$$

Description:
IS = Cellulase activity index
X1 = Clear zone diameter (mm)
X2 = Colony diameter (mm)

Quantitative Test of Cellulolytic Bacterial Cellulase Enzyme Activity

a. Preparation of a Glucose Standard Curve

First, glucose solutions were prepared at concentrations of 10, 20, 30, 40, and 50 ppm. One ml of each concentration was taken and placed in a test tube. One ml of DNS solution was added and homogenised. The mouth of the tube was closed and heated in boiling water for 5-15 minutes until the solution turned reddish-brown. One ml of 40% KNa-Tartrate solution was added. The test tube was cooled, and distilled water was added to a volume of 10 ml and homogenised. Absorbance was measured using a spectrophotometer at a wavelength of 540 nm.

b. Production of Crude Cellulase Enzyme Extract

Two tubes of the isolate were incubated in 40 ml of CMC Broth and incubated on a shaker at 125 rpm for 18 hours. The inoculum was then taken as 5 ml of CMC Broth medium and incubated on a shaker for 24 hours. The crude enzyme extract was obtained by centrifuging the culture at 2000 rpm for 18 minutes. The supernatant obtained was the crude cellulase enzyme extract and was used to determine enzyme activity.

c. Measurement of Crude Cellulase Enzyme Extract Activity Using the DNS Method

1 ml of crude cellulase enzyme extract was mixed with 1 ml of substrate (1% CMC) and incubated for 40 minutes. 1 ml of the mixed solution was taken, added to 1 ml of DNS solution, and then homogenised. The mouth of the test tube was closed and heated in a boiling water bath for 5-15 minutes until the solution turned reddish-brown. 1 ml of 40% KNa-Tartrate solution was added. The test tube was cooled, and distilled water was added to the volume to 10 ml and homogenised. Absorbance was measured with a spectrophotometer at a wavelength of 540 nm. Cellulase activity was determined by converting the absorbance value obtained from the standard glucose concentration, then calculated using the formula:

$$\left(AE = \frac{C}{BM\ Glukosa\ \times\ t} \times \frac{H}{E}\right)$$

Description:
AE : Enzyme activity
C : Glucose concentration
BM : Glucose molecular weight (180 g/mol)
T : Incubation time (minutes)
H : Total enzyme-substrate volume (ml)
E : Enzyme volume (ml)

Characterisation of Cellulolytic Bacterial Isolates

Characterisation was conducted to determine the phenotypic characteristics of each cellulolytic bacterial isolate through various tests. These tests examined morphological, physiological, and biochemical characteristics. These tests included morphological testing, motility testing, Gram staining, endospore staining, O2 requirement testing, pH tolerance testing (5, 7, 9), temperature tolerance testing (4°C, 27°C, 37°C), NaCl tolerance testing (0.5%, 0.8%, and 1.5%), catalase testing, citrate testing, H2S testing, indole testing, methyl red testing, Voges-Proskauer testing, gelatin hydrolysis testing, starch hydrolysis testing, lysine decarboxylase testing, and carbohydrate fermentation testing (glucose, sucrose, fructose, galactose, maltose, lactose, and mannitol).

Genus Identification of Cellulolytic Bacterial Isolates

Bacterial identification was conducted using Bergey's Manual of Systematic Bacteriology and Bergey's Manual of Determinative Bacteriology. The identification process was based on the morphological, physiological, and biochemical characteristics obtained. This data was then analysed using the MVSP 3.1 application.

Data Analysis Techniques

Data from cellulase enzyme activity tests of cellulolytic bacterial isolates were indicated by the formation of a clear zone after Congo red staining and were analysed descriptively. Genus identification was performed using phenetic characteristics data processed with the MVSP 3.1 application. Similarity values between isolates were determined using the Simple Matching Coefficient, while the clustering (UPGMA) algorithm was used to present the results in the form of a dendrogram (Salaki et al., 2010).

Results and Discussion

Bacterial Isolate Purification

Purification results showed that 10 selected bacterial isolates were identified, numbered 66, 71A, 71B, 72, 75A, 75B, 80, 82, 84, and 168B. The bacterial colonies were purified using the streak plate method in quadrants on NA media to obtain pure bacteria or separate colonies. This is in accordance with the statement by Prihanto et al. (Prihanto et al., 2018), who stated that the quadrant streak method aims to obtain a pure culture without contamination from other undesirable microorganisms. The isolates on the plates were then transferred to agar slants. This was done to preserve the bacterial isolates for long-term use.

Selection of Cellulolytic Bacteria

From the inoculation process of 10 selected bacterial isolates, all bacterial isolates were able to grow and colonise on CMC agar. This indicates that all ten selected bacterial isolates belong to the cellulolytic bacteria group.

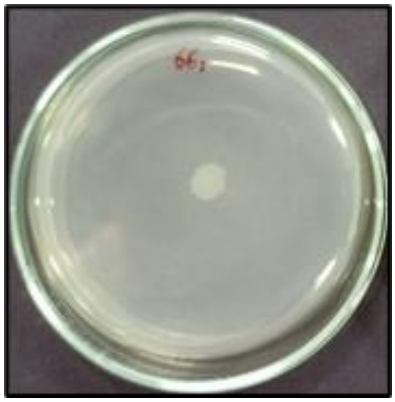

Figure 1. Bacteria growing on CMC agar

Bacteria are categorised as cellulolytic bacteria because they have the ability to grow on CMC media, a selective medium for cellulose-degrading bacteria. Cellulose is a linear polysaccharide consisting of glucose units linked by β-1,4 glycosidic bonds. Bacteria capable of producing cellulase can degrade cellulose. Cellulase enzymes are capable of degrading cellulose by breaking β-1,4 glycosidic bonds, producing oligosaccharides derived from cellulose and glucose (Murtiyaningsih & Hazmi, 2017). The CMC (Carboxy Methyl Cellulase) substrate in selective bacterial growth agar media can be degraded by the cellulase enzyme (CMCase). If bacteria are able to grow on CMC agar media, it indicates that the bacteria are cellulolytic.

Qualitative Test of Cellulase Enzyme Activity in Cellulolytic Bacteria

Qualitative cellulase enzyme activity tests indicate a positive result when a clear zone forms on the CMC agar media after the addition of Congo red solution. The principle of Congo red staining is that the dye will diffuse into the agar medium and will only be absorbed by long-chain polysaccharides containing β-D-glucan bonds, resulting from cellulolytic activity. The larger the resulting clear zone, the higher the solubility of an enzyme (Alkahfi *et al*., 2021). Congo red will bind specifically to polysaccharides containing β-1,4 glycosidic bonds, which are the polysaccharides contained in the test medium, namely CMC. The red colour indicates the remaining cellulose that has not been hydrolysed, resulting in the formation of cellulose-Congo red (Rori *et al*., 2019).

The results of qualitative cellulase enzyme activity tests showed that all ten bacterial isolates were able to form clear zones on CMC agar media after being tested using the Congo Red method. The diameter of the bacterial isolate colonies that grew ranged from 1.1 to 12.3 mm. Meanwhile, the diameter of the clear zones ranged from 1.9 to 13.3 mm. The cellulolytic index values of the ten bacterial isolates are shown in Table 1.

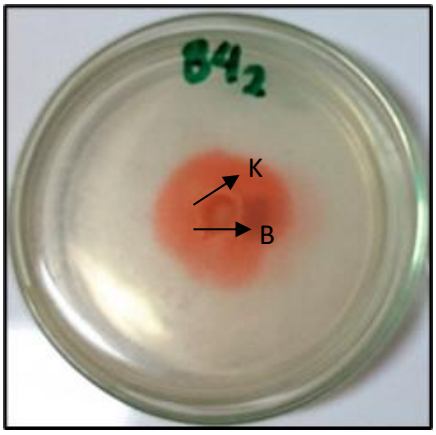

Figure 2. Results of cellulase enzyme activity test using the Congo red method (description: K = colony diameter; B = clear zone diameter)

Table 1. Bacterial Cellulolytic Index

No.	Isolate	CI (mm)	Category
1.	66	0.067	Low
2.	71A	0.348	Low
3.	71B	0.314	Low
4.	72	0.466	Low
5.	75A	0.091	Low
6.	75B	0.047	Low
7.	80	0.069	Low
8.	82	0.409	Low
9.	84	0.103	Low
10.	168B	0.463	Low

The cellulolytic index ranged from 0.067 mm for isolate number 66 to 0.466 mm for isolate number 72. The cellulolytic index indicates the ability of a bacterial isolate to degrade cellulose present in CMC agar media (Hidayatulloh et al., 2022). Fitri et al. (2021) categorised the cellulolytic index as follows: low (≤ 1), medium (1–2), and high (≥ 2). The higher the cellulolytic index, the higher the resulting cellulolytic activity (Nugraha et al., 2014). Table 1 shows the cellulolytic index for each bacterial isolate, and all of them fell into the low category. The low cellulolytic index indicates that all ten isolates had low cellulolytic activity.

Quantitative Test of Enzyme Activity of Cellulolytic Bacterial Isolates

Quantitative testing of cellulase enzyme activity began with the creation of a glucose standard curve to determine reducing sugar levels. Based on the curve above, a linear regression equation of y = 0.0011x + 0.0904 was obtained with an R^2 value of 0.8147. This equation was used to calculate the concentration of reducing glucose produced by enzyme activity, thus determining the cellulase enzyme activity. The obtained concentration was then substituted into the enzyme activity calculation formula to obtain enzyme activity units.

Quantitative cellulase enzyme activity testing was performed using the DNS method based on estimating the amount of glucose (reducing sugar) resulting from cellulose hydrolysis. DNS is an aromatic compound that reacts with reducing sugars to form 3-amino-5-dinitrosalicylic acid and strongly absorbs electromagnetic radiation at a wavelength of 540 nm. The formation of reducing sugars is closely related to enzyme activity. The higher the enzyme activity, the greater the amount of reducing sugar produced, which translates into a higher absorbance value measured on a spectrophotometer (Talantan et al., 2018).

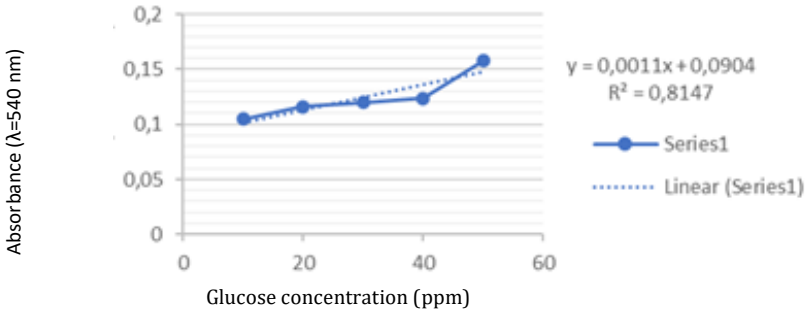

Figure 3. Glucose standard curve

The reaction between reducing sugars and DNS is a redox reaction in which the aldehyde group of the sugar is oxidised to the carboxyl group. Meanwhile, DNS, as an oxidising agent, is reduced to form 3-amino and 5-nitrosalicylic acids. This reaction occurs under alkaline conditions and at high temperatures of around 90-100°C. If reducing sugars are present in the sample, the initially yellow

DNS solution will react with the reducing sugars, producing a reddish-orange colour. The darker the DNS colour, the greater the amount of reduced sugar (Puspitasari & Ibrahim, 2020).

Table 2. Cellulase Enzyme Activity

No.	Isolate	Enzyme activity (U/ml)
1.	66	0.572
2.	71A	0.295
3.	71B	0.255
4.	72	0.291
5.	75A	0.388
6.	75B	0.332
7.	80	0.387
8.	82	0.299
9.	84	0.931
10.	168B	0.600

Enzyme production results can be determined through the enzyme activity values produced by the ten bacterial isolates tested. Cellulolytic activity, expressed in units per millilitre (U/ml), is defined as the activity of a bacterial isolate that produces glucose, as a cellulose monomer, per minute. One unit of cellulase activity is defined as the amount of enzyme that produces 1 µmol of glucose per minute under the measurement conditions. Cellulase activity is proportional to the level of reducing sugar produced; the higher the enzyme activity, the higher the reducing sugar production (Murtiyaningsih & Hazmi, 2017). The highest cellulase enzyme activity was found in isolate 84 at 0.931 U/ml, while the lowest enzyme activity was found in isolate 71B at 0.255 U/ml. Differences in activity values among bacterial isolates are influenced by the genes of each isolate and the carbon source used (Bandi, 2018).

Characterisation of Cellulolytic Bacterial Isolates

The phenetic characteristics obtained from testing the cellulolytic bacterial isolates were then tabulated, as shown in Table 3. The phenetic characteristics obtained from testing the morphological, physiological, and biochemical characteristics of the ten cellulolytic bacterial isolates will be used as the basis for the identification process of the cellulolytic bacterial isolate genus.

Table 3. Tabulation of Phenetic Character Data

No.	Characters	Isolate									
		66	71A	71B	72	75A	75B	80	82	84	168B
	Morphology										
1	Coccus cell shape	−	+	+	+	−	−	−	+	−	+
2	Bacillus cell shape	+	−	−	−	+	+	+	−	+	−
3	Spiral cell shape	−	−	−	−	−	−	−	−	−	−
4	Positive Gram	+	−	−	−	+	+	+	+	+	−
5	Negative Gram	−	+	+	+	−	−	−	−	−	+
6	Circular colony shape	+	+	+	+	+	+	+	+	+	−
7	Irregular colony shape	−	−	−	−	−	−	−	−	−	+
8	Filamentous colony shape	−	−	−	−	−	−	−	−	−	−
9	Spindle-shaped colony	−	−	−	−	−	−	−	−	−	−
10	Rhizoid colony shape	−	−	−	−	−	−	−	−	−	−
11	Entire colony margin	+	−	+	+	+	−	+	+	+	−
12	Curled colony margin	−	−	−	−	−	−	−	−	−	−
13	Undulate colony margin	−	+	−	−	−	+	−	−	−	+

14	Lobate colony margin	−	−	−	−	−	−	−	−	−	−
15	Rhizoid colony margin	−	−	−	−	−	−	−	−	−	−
16	Filamentous colony margin	−	−	−	−	−	−	−	−	−	−
17	Raised colony elevation	+	−	−	+	+	+	+	−	+	−
18	Flat colony elevation	−	+	+	−	−	−	−	−	−	+
19	Convex colony elevation	−	−	−	−	−	−	−	+	−	−
20	Pulvinate colony elevation	−	−	−	−	−	−	−	−	−	−
21	Umbonate colony elevation	−	−	−	−	−	−	−	−	−	−
22	Punctiform colony size	−	−	−	−	−	−	−	−	−	−
23	Small colony size	−	−	−	−	−	−	−	−	−	−
24	Moderate colony size	−	−	−	−	−	−	−	−	−	−
25	Large colony size	+	+	+	+	+	+	+	+	−	+
26	Glistening colony appearance	−	−	−	−	−	−	−	+	−	−
27	Dull colony appearance	+	+	+	+	+	+	+	−	−	+
28	Transparent colony optical property	−	+	+	+	−	−	−	−	−	+
29	Translucent colony optical property	−	−	−	−	−	−	−	−	−	−
30	Opaque colony optical property	−	−	−	−	+	+	+	+	−	−
31	Butyrous colony texture	−	−	−	−	−	−	−	−	−	−
32	Rough colony texture	+	−	−	−	−	−	−	−	−	−
33	Smooth colony texture	+	+	+	+	+	+	+	−	+	+
34	Mucoid colony texture	−	−	−	−	−	−	−	+	−	−
35	Dry colony texture	−	−	−	−	−	−	−	−	−	−
36	White colony color	+	+	+	+	+	+	+	−	−	+
37	Yellow colony color	−	−	−	−	−	−	−	+	−	−
38	Endospore	−	−	−	+	+	+	+	+	+	−
39	Motility	+	+	+	+	+	+	+	+	+	−
	Physiology										
40	Grows at temperature 4°C	+	+	+	+	+	+	−	+	+	+
41	Grows at temperature 27°C	+	+	+	+	+	+	+	+	+	+
42	Grows at temperature 37°C	+	+	+	+	+	+	+	+	+	+
43	Grows at pH 5	−	+	+	+	+	+	+	+	−	+
44	Grows at pH 7	+	+	+	+	+	+	+	+	+	+
45	Grows at pH 9	+	+	+	+	+	+	+	+	+	+
46	Grows at 0,5% NaCl	+	+	+	+	+	+	+	+	+	+
47	Grows at 0,8% NaCl	+	+	+	+	+	+	+	+	+	+
48	Grows at 1,5% NaCl	+	+	+	+	+	+	+	+	+	+
49	Aerobe	+	−	−	−	+	+	+	−	+	−
50	Facultative anaerobe	−	+	+	−	−	−	−	−	−	+
51	Anaerobe	−	−	−	+	−	−	−	+	−	−
52	Microaerophile	−	−	−	−	−	−	−	−	−	−
	Biochemistry										

53	Indol Production	+	−	−	+	−	+	−	−	+	−
54	*Red methyl*	−	+	+	+	+	+	+	+	−	−
55	*Voges Proskauer*	−	−	−	−	−	−	−	−	−	−
56	Citrate production (Simmons)	−	−	−	+	−	−	−	−	−	+
57	H2S production	−	+	+	+	−	−	−	−	−	−
58	Lysine decarboxylase	+	−	−	−	−	−	−	+	+	+
59	Gelatin hydrolysis	+	−	−	−	−	+	−	−	−	−
60	Katalase Production	+	+	+	+	+	+	+	+	+	+
61	KOH 3%	−	+	+	+	−	−	−	−	−	+
62	Cellulase enzyme production	+	+	+	+	+	+	+	+	+	+
63	Amylase enzyme production	−	−	−	−	−	−	−	−	−	−
64	Acid production from glucose	−	+	+	+	+	+	+	+	−	−
65	Acid production from sucrose	−	−	−	−	−	−	−	−	−	−
66	Acid production from fructose	−	+	+	+	−	−	−	−	−	−
67	Acid production from lactose	−	+	+	+	+	+	+	+	−	−
68	Acid production from maltose	−	+	+	+	−	−	−	−	−	−
69	Acid production from galactose	−	−	−	+	−	−	−	−	−	−
70	Acid production from mannitol	−	−	−	+	−	−	−	−	−	−
71	Gas production from glucose	−	+	+	+	−	−	−	−	−	−
72	Gas production from sucrose	−	−	−	−	−	−	−	−	−	−
73	Gas production from fructose	−	+	+	+	−	−	−	−	−	−
74	Gas production from lactose	−	−	−	−	−	−	−	−	−	−
75	Gas production from maltose	−	−	−	+	−	−	−	−	−	−
76	Gas production from galactose	−	−	−	+	−	−	−	−	−	−
77	Gas production from mannitol	−	−	−	+	−	−	−	−	−	−

Identification of Genus of Cellulolytic Bacterial Isolates

The identification process was carried out by comparing the bacteria to be identified with previously identified bacteria. The method used was phenetic numerical taxonomy, which organises bacteria into groups (taxons) based on quantitative similarities based on their phenetic characteristics. The reference genera used in this study were Pseudomonas, Alcaligenes, Cellulomonas, and Aeromonas. The phenetic characteristics of the cellulolytic bacterial isolates and the reference genera were analysed using the MVSP 3.1 program, resulting in a dendrogram.

The dendrogram in Figure 4 shows the kinship relationship between the bacterial isolates obtained with the suspected reference genera. The greater the similarity of an isolate, the closer the kinship relationship (Imtiyaz et al., 2023). According to the taxonomic concept, microbial isolates are grouped into the same type if they have a similarity index of 70% (Missa et al., 2022). The dendrogram results show two clusters with a similarity level of more than 0.7 (70%). Cluster 1 has a similarity index of 0.771 (77.1%) with 4 bacteria in it, while cluster 2 has a similarity index of 0.732 (73.2%) with 9 bacteria. The ten cellulolytic bacterial isolates, along with the four reference genera, are combined into one cluster at a similarity value of 0.631 (63.1%).

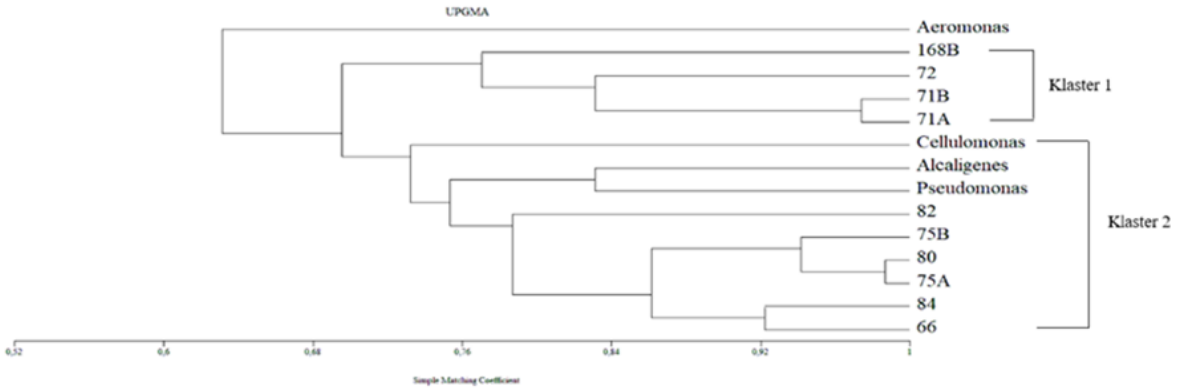

Figure 4. Dendogram results

Table 4. Simple Matching Coefficient similarity matrix

Isolate	Reference Genera			
	Pseudomonas	**Alcaligenes**	**Cellulomonas**	**Aeromonas**
66	79.2%	77.9%	76.6%	64.9%
71A	62.3%	68.8%	70.1%	63.6%
71B	59.7%	71.4%	72.7%	66.2%
72	57.1%	61%	57.1%	74%
75A	74%	77.9%	76.6%	59.7%
75B	76.6%	72.7%	74%	59.7%
80	72.7%	79.2%	75.3%	58.4%
82	72.7%	71.4%	72.7%	58.4%
84	74%	75.3%	71.4%	59.7%
168B	74%	77.9%	71.4%	57.1%

Based on the similarity index values obtained, it is known that there are 3 isolates of cellulolytic bacteria with a similarity index of >70% to the reference genus Pseudomonas, including isolates 66, 75B, and 82. There are 4 isolates of cellulolytic bacteria with a similarity index of >70% to the reference genus Alcaligenes, namely isolates 75A, 80, 84, and 168B. There are 3 isolates of cellulolytic bacteria with a similarity index of >70% to the reference genus Cellulomonas, including isolates 71A, 71B, and 82. There is 1 isolate of cellulolytic bacteria with a similarity index of >70% to the reference genus Aeromonas, namely isolate 72. The similarity index of isolate 82 to the genera Pseudomonas and Celulomonas has the same value, namely 72.7%. This indicates that isolate 82 has a close kinship relationship with both genera.

Conclusion

The selection of cellulolytic bacteria on the ten bacterial isolates resulted in all isolates being classified as cellulolytic bacteria. The cellulase enzyme activity values of the ten isolates varied. The highest qualitative cellulase enzyme activity value was shown by isolate 72 at 0.466 mm, while the highest quantitative cellulase enzyme activity value was shown by isolate 84 at 0.931 U/ml. There were four genera of cellulolytic bacteria that were successfully suspected as cellulolytic bacteria found in mask waste from Mangrove Wana Tirta, including Pseudomonas, Alcaligenes, Cellulomonas, and Aeromonas.

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