Detergent-Tolerant Heterotrophic Bacteria Consortium Strain Decomposer to Improve Environmental Health

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Abstract. The increasing population and level of life in big cities in Indonesia has resulted in higher pollution of domestic wastewater in recent years. Efforts are urgently needed to overcome them, among others, with indigenous heterotrophic bacteria in the form of a consortium. The method used in this study was carried out in several stages, namely isolating and characterizing heterotrophic bacteria from Malang city domestic wastewater, testing the resistance of bacteria to various types of detergents, testing antagonism of various pathogens, and identifying strains up to the level of strains. The conclusion of this study was found that there were 37 isolates that had tolerance to various detergents with a concentration of 25% to 95%, were antagonistic to *Salmonella typhi, Shigella dysenteriae, Vibrio cholerae*, and *Escherichia coli*, and found 4 bacterial strains that had great potential as decomposers. Waste. These findings, consortium strains of heterotrophic bacteria consisting of *Bacillus cereus strain BQAR-01, Bacillus thuringiensis strain MSS-2, Bacillus cereus strain JDA-1*, and *Bacillus sp. B31(2008)* can be used in the development of environmental health as a decomposer of domestic wastewater.

Keywords: heterotrophic bacteria consortium strain · deterjent tolerant · decomposer · environmental health

1 Introduction

The increase in population in Indonesia, especially in big cities and the increase in public consumption has resulted in an increase in the volume, type and characteristics of waste. The increasing number of population and standard of living means that the number of water users is increasing, especially the use of clean water. An increase in the number of water users has resulted in a decrease in the quantity and quality of clean water, especially in big cities such as Jakarta, Bandung, Surabaya and Malang. This condition causes environmental sanitation facilities and infrastructure to not meet health requirements and the amount of domestic waste entering water bodies also increases [15, 20, 26].

Malang City's environmental conditions can affect the volume, type, and characteristics of waste compared to other cities, including the characteristics of microbes present in its domestic wastewater. The more domestic (household) waste that enters water

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bodies, it causes various infectious diseases to spread easily. Domestic wastewater is a source of microbial contaminants that cause various diseases and has the potential to become a source of disease transmission by water-borne pathogens. As a consequence, this domestic wastewater must be processed and treated in advance with environmentally safe management methods and techniques with the aim of not causing negative impacts on human health and the environment [16, 17, 23].

There are many types of hazardous chemicals in domestic wastewater, so special handling is required before being discharged into the environment. The effectiveness of this treatment depends on changes in biochemical processes produced by various kinds of microbes and environmental factors. Domestic wastewater, apart from containing residues that are easy to decompose, still contains a lot of substances that are not easy to decompose. Substances that are not easily decomposed, for example residual germ killers, residual insecticides, residual detergents and pesticides. Some of these substances can kill natural decomposing microbes that should be present in wastewater, resulting in an imbalance in the number of pollutants with natural decomposing microbes. One of the efforts to handle domestic wastewater microbiologically is by utilizing native (indigenous) microbial isolates that have the potential to decompose the waste [1, 2, 4, 5, 9, 10, 13, 28].

Innovation is needed to overcome the obstacles mentioned above, in order to produce an environmentally friendly microbial consortium formula. Efforts to deal with pollution are environmentally friendly, meaning that after a microbial consortium is given to decompose a waste into the environment, it does not cause additional consequences with the presence of residual substances. As an effort to solve problems that are appropriate and effective for the benefit of the community in microbiological processing of domestic wastewater [6, 8, 14, 24, 27].

2 Method

2.1 Procedure Isolating and Characterizing Heterotrophic Bacteria from Domestic Wastewater

This research uses a descriptive research type that is carried out with a survey approach or method, observation method, and laboratory method. The survey method was used to collect data on domestic wastewater research subjects by taking domestic wastewater from the city of Malang. Observational and laboratory methods were used to collect data on the type and number of isolates of heterotrophic bacteria originating from domestic wastewater. The method used to obtain microbial isolates is to isolate them from the domestic wastewater. The variables measured were the characteristics of heterotrophic bacteria by looking at the shape of the colony, the color of the colony, the surface of the colony, the edge of the colony, the nature of Gram, the type of respiration that was different from each microbe [3, 7, 11].

The steps for isolating heterotrophic bacteria from domestic wastewater from Malang City are by (a) Dissolving the nutrient agar medium in a water bath, (b) Cooling the medium to a temperature of ± 50 °C, (c) Pouring the nutrient agar medium into a petri dish. Aseptically sterile, and allowed to cool and solid, (d) Take 0.1 mL of suspension of material from domestic wastewater aseptically. Then make scratches on the surface of

the agar. At the beginning of streaks there will be dense growth after incubation making it difficult to isolate. At the end of the stroke, colonies will grow which are separate and can be isolated, (e) Turn the labeled and rewrapped Petri dish upside down. The Petri dish is turned upside down with the aim of preventing the occurrence of water droplets on the surface of the agar from condensation, (f) After it is incubated, separate colonies will appear. Each separate colony may come from 1 bacterial cell, (g) Choose from each type of colony only one colony which is one type of bacterial isolate, (h) Take aseptically with the loop of the desired colony and suspend it in sterile water, (i) Checking with Gram staining, (j) Transferring each type of isolation into nutrient medium so that it is slanted, (k) Incubating at the appropriate temperature for 24 – 48 h, (l) Retesting the pure culture with Gram staining, (m) If there is only one type of bacteria in each test tube, it means that the isolation has been successful, (n) To make sure the isolated colonies are back to ensure the purity of the culture, (o) The above steps are repeated 3 times. [9, 12, 22, 29].

2.2 Tolerance Potential Test of Heterotrophic Bacterial Isolates on Various Types and Concentrations of Detergent Products

The research design for isolate tolerance to detergent uses an experimental research type carried out using a laboratory method approach. Laboratory methods were carried out to test the tolerance potential of heterotrophic bacterial isolates against detergents. This tolerance test used a Factorial Completely Randomized Design (CRD) with 3 replications. The types of detergent products used are cleaning products, clothes washing agents, household washing products, and house cleaning products. Detergent concentrations used were 25%, 50%, 75% and 95%.

The research instruments consisted of incubators, ent kast, colony counters, water baths, petri dishes, oses, test tubes, ovens, cooler boxes, microscopes, glass objects, cover glasses, spirit lamps. The media used are Czapek Doc agar, Nutrient agar, lactose broth. The research locations were carried out in the Microbiology sub-laboratory, the Biotechnology laboratory at the University of Muhammadiyah Malang, and the Microbiology laboratory at the Faculty of Medicine, Brawijaya University, Malang. The tolerance power of heterotrophic bacterial isolates to various types and concentrations of detergents was analyzed by Anova followed by Duncan's advanced test at 5% significance.

2.3 Antagonistic Testing of Heterotrophic Bacterial Isolate Against Pathogen Bacteria

This study employed a full random design with one factor and three cycles. The study materials were the heterotrophic bacterial isolate taken from domestic wastewater and the pathogen bacteria. The sample was taken from the heterotrophic bacterial isolate treated with the Mac Farland 0.5 solution with the density of 1.5×10^8 cell/mL. The location of the study was in the microbiology sub-laboratory and the Biotechnology laboratory, University of Muhammadiyah Malang.

Antagonistic testing was conducted using the paper disk method [3, 5]. The pathogen bacteria used as the testing agents were the *Salmonella typhi, Shigella dysenteriae*, *Vibrio*

cholerae, and *E. coli* taken from the medical faculty laboratory, Airlangga University, Surabaya. The bacteria were cultured in the agar nutrient and were incubated in the room temperature. Then, they were suspended in the solution of physiological salt until the population density reached 10^6 CFUl/mL. Each of 100 μL suspense heterotrophic bacteria isolate was 156 cultured using the NA (oxoid) medium in the Petri dish. The agar nutrient medium consisted of 3 g of beef extract, 5 g of pepton, 1,000 mL of aquades, and 1,5–2,0% agar. Next, the sterile paper disk consisting of 100 μL pathogen bacteria of different variety was put aseptically inside the bacteria culture on the Petri dish. The testing bacteria cultures were then incubated in the temperature of 37 °C for 48 h. The antagonistic quality of the bacteria was visible from the inhibition zone around the paper disk.

2.4 The Stage of Identifying Superior Heterotrophic Bacterial Isolates

The identification stage is the next step after the detergent tolerance test and the antagonistic test against pathogens. Identification was carried out on 37 isolates of heterotrophic bacteria initially described based on their detergent tolerance character followed by antagonistic tests against pathogens. Complete identification was carried out using MicrobactTM GNB 12A/B/E, 24E Identification Kits. Physiological characters measured included lysine decarboxylase, ornithine decarboxylase, H2S production, glucose fermentation, mannitol, xylose, ONPG (beta-galactosidase), indole production, urea hydrolysis, Voges-Proskauer (VP) reaction, use of citrate, tryptophan deaminase (TDA), gelatin melting, malonate inhibition, inositol fermentation, sorbitol, rhamnose, sucrose, lactose, arabinose, adonitol, raffinose, salicin, and arginine dihydroxylase. Identification with Microbact 12A/E -24E. The results of the characterization were then referred to using the identification and determination reference book Bergey's Manual of Determinative Bacteriology [22].

DNA isolation was carried out using the procedure from the DNA Isolation Kit using user guide by life technologies (PureLinkR 'Genomic DNA Kits For purification of genomic DNA, Catalog Numbers K1820–01, K1820–02, K1821–04, Document Part Number 25–1012, Publication Number MAN0000601 Revision 2.0'). The heterotrophic bacterial samples were then stored at −200 °C until used. Measurement of DNA Purity and Concentration. Measurement of the purity and concentration of isolated DNA using a Biorad UV spectrophotometer machine. Primers are designed based on the 16sRNA gene found in the NCBI database (National Center for Biotechnology Information). Primers are designed using Oligo Analyzer software version 1.0.2., Oligo Explorer 1.1.0. And BLAST (Basic Local Alignment Search Tool) which can be accessed online at NCBI. Primers that have been designed were tested with the Oligo Analyzer software version 1.0.2. Including% GC content, Tm (time melting), hairpin or loop test, dimmer test and multiplex test between forward primers and reverse primers. Forward gene primers (5'- AGAGTTGATCATGGCTCAG-3 ') and reverse primers (5'-

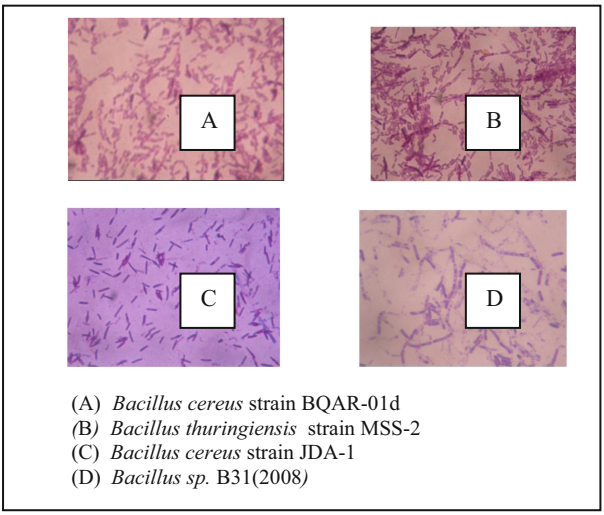
TACGGCTACCTTGTTACGA-3'). The amplification of genotyping of 16sRNA gene polymorphisms was carried out using the Polymerase Chain Reaction or PCR technique using a thermalcycler machine. The amplicon sample resulted from PCR 16sRNA gene which had been partially used for confirmation of electrophoresis was inserted into a tube with a maximum volume of 1.5 ml. The sample was added with sodium acetate (Merck) as much as 0.1% of the total volume of the amplicon sample in the tube to be purified. Next, cold absolute ethanol is added twice the volume of the sodium acetate solution and the amplicon sample. Then spin down using a centrifuge and stored at a temperature of −20 °C for 60 min. DNA sequencing or sequencing is carried out to determine the DNA sequence that undergoes polymorphism. Sequencing was performed using purified DNA samples from PCR results. In confirmation of DNA amplification by agarose gel electrophoresis, the thickest bands were selected as samples for sequencing. Sequencing of DNA nucleotides encoding 16S rRNA. DNA amplifiers encoding pure 16S rRNA from each detergent tolerant isolate were sequenced using Bigdye V.3.1. The sequencing process of pure 16S rRNA for each bacterial isolate was carried out according to the ABI PRIMS 310 genetic analyzer procedure using a sequencer machine. The sequencing results are in the form of files consisting of an electropherogram and a tex-file of DNA nucleotide sequences. DNA sequence data encoding 16S rRNA from each detergent tolerant bacterial isolate was aligned with data from reference strains downloaded from the gene-bank http://www.blast.ncbi.nlm.nih.gov/Bast.cgi and http://www.ncbi.nlm.nih.gov/Taxonomy [22].

3 Results

Morphological and cytological characters of 37 heterotrophic bacteria found from domestic wastewater from Malang City are described in Table 1.

Table 1 shows the documented characters of colony form, colony margin, colony elevation, colony color, colony consistency, Gram reaction, motility, spore formation, and cell shape of the microbes. The data is then compared with the differences and similarities in the characters. Different characters are then given different codes. Documented characters are morphological and cytological characters. The results of the isolation, identification, and characterization of microbes succeeded in obtaining 37 isolates of heterotrophic bacteria from liquid domestic waste in the city of Malang.

Tolerance potential test of heterotrophic bacteria isolates against treatments of various types and concentrations of detergent products. The results achieved by research on the tolerance of heterotrophic bacterial isolates to the type and concentration of detergents are presented in Table 2. Summary of the antagonistic power of isolates of heterotrophic bacteria against pathogens consisting of *Salmonella typhi, Shigella dysenteriae, Vibrio cholerae,* and *Escherichia coli* is presented in Table 3. Molecular identification results can be seen in Table 4. The four superior heterotrophic bacterial strains that have the potential to decompose wastewater as shown in Fig. 1.



(A) *Bacillus cereus* strain BQAR-01d
(B) *Bacillus thuringiensis* strain MSS-2
(C) *Bacillus cereus* strain JDA-1
(D) *Bacillus sp.* B31(2008)

Fig. 1. The four superior heterotrophic bacterial strains that have the potential to decompose wastewater

Table 1. Characteristics and morphological characters and cytology of isolates of heterotrophic bacteria from domestic wastewater

No	**Morphological and cytological features**									Code isolates
	Colony Form	Edge colony from above	Colony surface	Color of the colonies	Colony softness	Gram stain	Motility	Presence of spore	Cell type	
1	smooth	choppy	embossed flat	beige	hard and dry	positive	motile	spores exist	basil	A9
2	smooth	choppy	embossed flat	beige	hard and dry	positive	motile	spores exist	basil	A8
3	smooth	jagged	embossed flat	beige	hard and dry	positive	motile	spores exist	basil	A7
4	smooth	choppy	embossed flat	beige	hard and dry	positive	motile	spores exist	basil	A6
5	smooth	choppy	embossed flat	beige	hard and dry	positive	motile	spores exist	basil	A5
6	smooth	choppy	embossed flat	beige	hard and dry	positive	motile	spores exist	basil	A4
7	smooth	choppy	embossed flat	beige	hard and dry	positive	motile	spores exist	basil	A3
8	smooth	choppy	embossed flat	beige	hard and dry	positive	motile	spores exist	basil	A2
9	smooth	jagged	embossed flat	beige	hard and dry	positive	motile	spores exist	basil	A1
10	smooth	jagged	embossed flat	beige	hard and dry	positive	motile	spores exist	basil	A11
11	smooth	jagged	embossed flat	beige	soft	positive	motile	spores exist	basil	A11

(*continued*)

Table 1. (*continued*)

No	Morphological and cytological features									Code isolates
	Colony Form	Edge colony from above	Colony surface	Color of the colonies	Colony softness	Gram stain	Motility	Presence of spore	Cell type	
12	smooth	jagged	embossed flat	beige	hard	positive	motile	spores exist	basil	A10
13	smooth	jagged	embossed flat	beige	hard	positive	motile	spores exist	basil	A16
14	smooth	jagged	embossed flat	beige	hard	positive	motile	spores exist	basil	A15
15	smooth	jagged	embossed flat	beige	soft	positive	motile	spores exist	basil	A14
16	smooth	jagged	embossed flat	beige	hard	positive	motile	spores exist	basil	A13
17	smooth	jagged	embossed flat	beige	hard	positive	motile	spores exist	basil	C1
18	smooth	intact	convex	beige	hard	positive	motile	spores exist	basil	C2
19	smooth	jagged	flat	beige	hard	positive	motile	spores exist	basil	C3
20	elips	intact	convex	beige	hard	positive	motile	spores exist	basil	C4
21	smooth	jagged	embossed flat	beige	hard	positive	motile	spores exist	basil	C5
22	elips	intact	embossed flat	beige	hard	positive	motile	spores exist	basil	C6
23	smooth	choppy	embossed flat	beige	hard	positive	motile	spores exist	basil	C7
24	elips	choppy	convex	beige	hard	positive	motile	spores exist	basil	C8
25	smooth	choppy	embossed flat	beige	hard	positive	motile	spores exist	basil	C9
26	smooth	choppy	embossed flat	beige	soft	positive	motile	spores exist	basil	C10
27	smooth	intact	embossed flat	beige	soft	positive	motile	spores exist	basil	C11
28	smooth	choppy	embossed flat	beige	hard	positive	motile	spores exist	basil	C13

(*continued*)

Table 1. (*continued*)

No	Morphological and cytological features									Code isolates
	Colony Form	Edge colony from above	Colony surface	Color of the colonies	Colony softness	Gram stain	Motility	Presence of spore	Cell type	
29	smooth	choppy	embossed flat	beige	hard	positive	motile	spores exist	basil	C12
30	smooth	choppy	embossed flat	beige	soft	positive	motile	spores exist	basil	C16
31	elips	choppy	embossed flat	beige	hard	positive	motile	spores exist	basil	C15
32	smooth	jagged	embossed flat	beige	hard	positive	motile	spores exist	basil	C14
33	smooth	jagged	flat	beige	hard	positive	motile	spores exist	basil	C17
34	elips	intact	convex	beige	hard	positive	motile	spores exist	basil	C18
35	elips	intact	convex	beige	hard	positive	motile	spores exist	basil	C19
36	smooth	jagged	embossed flat	beige	soft	positive	motile	spores exist	basil	C20
37	smooth	jagged	embossed flat	beige	hard	positive	motile	spores exist	basil	C21

Table 2. Tolerance of various types and concentrations of detergent products to isolates of heterotrophic bacteria (cm)

No	Isolate	Inhibition zone diameter (cm)			Average
		repetition 1	repetition 2	repetition 3	
1	A1	1,9639	1,4695	1,8687	1,7674 ± 0,26
2	A2	1,9625	1,4661	1,8759	1,7682 ± 0,27
3	A3	0,1857	0,1406	0,4291	0,2518 ± 0,16
4	A4	0,1784	0,1144	0,3144	0,2024 ± 0,10
5	A5	1,6794	1,1679	1,5724	1,4732 ± 0,27
6	A6	1,7556	1,2538	1,6550	1,5548 ± 0,27
7	A7	1,7216	1,2228	1,6209	1,5218 ± 0,26
8	A8	1,9610	1,4641	1,8611	1,7621 ± 0,26
9	A9	1,9644	1,4559	1,8388	1,7530 ± 0,26
10	A10	2,3484	1,8575	2,2575	2,1545 ± 0,26
11	A11	1,5456	1,0381	1,4428	1,3422 ± 0,27
12	A12	1,8613	1,3546	1,7554	1,6571 ± 0,27
13	A13	0,1844	0,0975	0,3021	0,1947 ± 0,10
14	A14	0,4432	0,1453	0,5384	0,3756 ± 0,21

(*continued*)

Table 2. (*continued*)

No	Isolate	Inhibition zone diameter (cm)			Average
		repetition 1	repetition 2	repetition 3	
15	A15	0,1884	0,1364	0,4031	0,2426 ± 0,14
16	A16	0,1769	0,1222	0,3214	0,2068 ± 0,10
17	C1	1,0617	0,5711	0,9731	0,8686 ± 0,26
18	C2	1,5335	1,0348	1,4543	1,3409 ± 0,27
19	C3	1,8286	1,3265	1,7280	1,6277 ± 0,27
20	C4	0,4872	0,1878	0,4871	0,3874 ± 0,17
21	C5	0,6229	0,2187	0,5177	0,4531 ± 0,21
22	C6	1,1225	0,7936	1,0703	0,9955 ± 0,26
23	C7	1,5072	1,0256	1,4157	1,3162 ± 0,26
24	C8	0,5853	0,2978	0,4986	0,4606 ± 0,15
25	C9	0,2456	0,1594	0,4388	0,2813 ± 0,14
26	C10	1,0799	0,6559	0,9840	0,9066 ± 0,15
27	C11	1,4503	0,9859	1,3628	1,2663 ± 0,25
28	C12	1,8792	1,3968	1,7819	1,6860 ± 0,26
29	C13	2,1284	1,6325	2,0222	1,9277 ± 0,26
30	C14	1,7456	1,2567	1,6462	1,5495 ± 0,26
31	C15	0,2174	0,1409	0,4285	0,2623 ± 0,15
32	C16	1,7136	1,2178	1,6145	1,5153 ± 0,26
33	C17	1,9591	1,4728	1,8646	1,7655 ± 0,26
34	C18	0,6904	0,2918	0,5913	0,5245 ± 0,21
35	C19	2,0259	1,5119	1,9238	1,8205 ± 0,27
36	C20	0,3298	0,1603	0,5300	0,3400 ± 0,19
37	C21	2,0081	1,5100	1,9129	1,8103 ± 0,26

Table 3. Antagonistic power of isolates of heterotrophic bacteria from domestic wastewater to pathogens (cm)

No	Isolate	The average of inhibition zone diameter against pathogen (cm)			
		Salmonella typhi	*Shigella dysenteriae*	*Vibrio cholerae*	*Escherichia coli*
1	A1	0,44 ± 0,11 bcdefgh	0,37 ± 0,09 cdefghijk	0,72 ± 0,13 jkl	0,38 ± 0,08 defgh
2	A2	0,74 ± 0,08 jklmn	0,50 ± 0,07 klmn	0,21 ± 0,13 a	0,40 ± 0,12 defghij
3	A3	1,03 ± 0,25 o	0,47 ± 0,13 ijklm	0,55 ± 0,09 hijk	0,51 ± 0,13 fghijkl
4	A4	0,77 ± 0,16 klmn	0,77 ± 0,19 o	0,79 ± 0,13 l	0,65 ± 0,10 klmn
5	A5	0,58 ± 0,06 efghijk	0,20 ± 0,09 abcd	0,40 ± 0,13 abcdefgh	0,33 ± 0,21 bcdefg
6	A6	0,45 ± 0,10 cdefghi	0,36 ± 0,04 cdefghijk	0,23 ± 0,12 abcd	0,27 ± 0,14 abcde
7	A7	0,33 ± 0,07 abcd	0,49 ± 0,08 jklmn	0,56 ± 0,08 hijk	0,31 ± 0,08 bcdefg
8	A8	0,38 ± 0,10 bcdef	0,35 ± 0,07 cdefghijk	0,44 ± 0,09 defgh	0,23 ± 0,08 abcd
9	A9	0,31 ± 0,08 abc	0,32 ± 0,12 bcdefghijk	0,55 ± 0,13 hijk	0,47 ± 0,07 efghijk
10	A10	0,37 ± 0,12 bcde	0,37 ± 0,10 cdefghijk	0,37 ± 0,09 abcdefgh	0,36 ± 0,06 cdefg
11	A11	0,34 ± 0,08 bcd	0,39 ± 0,10 efghijkl	0,52 ± 0,08 abcd	0,59 ± 0,06 hijklm
12	A12	0,40 ± 0,05 bcdefg	0,36 ± 0,03 cdefghijk	0,28 ± 0,10 abcdef	0,40 ± 0,03 defghij
13	A13	0,85 ± 0,12 mno	0,97 ± 0,02 p	1,18 ± 0,14 m	0,70 ± 0,14 lmn
14	A14	0,45 ± 0,12cdefgh	0,35 ± 0,18 cdefghijk	0,74 ± 0,10 lm	0,47 ± 0,08 efghijk
15	A15	0,88 ± 0,05 no	0,63 ± 0,14 mno	1,29 ± 0,12 m	0,77 ± 0,11 mno
16	A16	0,51 ± 0,17 cdefghi	0,61 ± 0,08 mno	0,80 ± 0,07 l	0,32 ± 0,10 bcdefg
17	C1	0,45 ± 0,07 cdefghi	0,22 ± 0,03 abcdef	0,34 ± 0,09 abcdefgh	0,44 ± 0,09 defghijk
18	C2	0,49 ± 0,12 cdefghi	0,20 ± 0,08 abcde	0,71 ± 0,08 jkl	0,33 ± 0,10 bcdefg
19	C3	0,23 ± 0,07 ab	0,30 ± 0,06 bcdefghij	0,46 ± 0,08 efghi	0,34 ± 0,16 cdefg
20	C4	0,31 ± 0,06 abc	0,15 ± 0,06 ab	0,33 ± 0,14 abcdefg	0,30 ± 0,05 bcdef
21	C5	0,59 ± 0,13 fghijk	0,24 ± 0,12 abcdefgh	0,43 ± 0,13 cdefgh	0,52 ± 0,09 ghijkl
22	C6	0,57 ± 0,10 efghijk	0,29 ± 0,07 bcdefghi	0,26 ± 0,11 abcdef	0,50 ± 0,10 fghijkl
23	C7	0,61 ± 0,09 ghijkl	0,23 ± 0,08 abcdefg	0,36 ± 0,09 abcdefgh	0,24 ± 0,08 abcd
24	C8	0,54 ± 0,11defghij	0,42 ± 0,12 ghijkl	0,24 ± 0,11 abcde	0,45 ± 0,11 defghijk
25	C9	0,42 ± 0,05 bcdefg	0,17 ± 0,03 abc	0,31 ± 0,10 abcdefg	0,23 ± 0,15 abcd
26	C10	0,47 ± 0,07 cdefghi	0,41 ± 0,06 fghijkl	0,31 ± 0,10 abcdefg	0,23 ± 0,14 abcd
27	C11	0,57 ± 0,10 efghijk	0,25 ± 0,08 abcdefgh	0,47 ± 0,12 abcd	0,25 ± 0,11 abcde
28	C12	0,31 ± 0,08 abc	0,41 ± 0,10 fghijkl	0,22 ± 0,10 abc	0,15 ± 0,05 abc
29	C13	0,85 ± 0,11 mno	0,58 ± 0,09 lmn	0,35 ± 0,13 abcdefgh	0,39 ± 0,16 defghi
30	C14	0,65 ± 0,10hijklm	0,31 ± 0,10 bcdefghijk	0,43 ± 0,11 bcdefgh	0,12 ± 0,02 ab
31	C15	0,81 ± 0,14 lmn	0,66 ± 0,09 no	1,09 ± 0,10 m	0,80 ± 0,16 no
32	C16	0,59 ± 0,08 efghijk	0,37 ± 0,07 defghijk	0,46 ± 0,08 efghi	0,12 ± 0,10 ab
33	C17	0,13 ± 0,06 a	0,23 ± 0,10 abcdefg	0,21 ± 0,14 ab	0,70 ± 0,06 lmn
34	C18	0,66 ± 0,12 ijklm	0,43 ± 0,08 hijkl	0,24 ± 0,12 abcd	0,60 ± 0,14 ijklmn
35	C19	0,52 ± 0,13 cdefghi	0,26 ± 0,15 abcdefgh	0,25 ± 0,10 abcdef	0,61 ± 0,12 jklmn
36	C20	0,75 ± 0,09 jklmn	0,76 ± 0,12 o	0,65 ± 0,09 ijkl	0,94 ± 0,17 o
37	C21	0,34 ± 0,17 bcd	0,08 ± 0,07 a	0,40 ± 0,11 abcdefgh	0,07 ± 0,05 a

Table 4. Molecularly identified heterotrophic bacterial strains from 37 detergent tolerant isolates from domestic wastewater

No	Isolate Code	Strain Name
1	A3, A13, A16	*Bacillus cereus* strain BQAR-01d
2	C11, A15, C20	*Bacillus thuringiensis* strain MSS-2
3	C15	*Bacillus cereus* strain JDA-1
4	A1, A6, A7, A14, C1, C5, C7, C9, C13, C17	*Bacillus sp.* B31 (2008),
5	A2, A4, A11, C10, C14	*Aneurinibacillus sp.* YR247
6	A5, A8, A9, A10, A12, A16, C3, C21	*Bacillus sp.* Gut03
7	C8	*Enterococcus sp.* 79w3
8	C2, C4, C18, C19	*Bacillus sp.* Z-3
9	C6	*Uncultured Bacillus sp.* Clone C6A08

4 Discussion

Heterotrophic bacterial isolates found from septic tank waste, sewer waste, and used bath water waste from the city of Malang were of different types. In general, 37 isolates were found. Some of the same types of isolates were obtained from the three types of waste, namely septic tank waste, sewer waste, and used bath water waste. The ability to exist of the same isolates in different types of waste shows the ability of these bacterial isolates to live with different environmental conditions and show high resistance (tolerance) to the environment in various types of domestic wastewater [15, 16, 19].

In general, the 37 isolates of heterotrophic bacteria were tolerant to various types and concentrations of detergents. This is in line with technological developments, now the product forms of detergents are becoming diverse. The forms of these products are (a) personal cleaning products, as self-cleaning products, such as shampoo, hand washing soap, (b) laundry, as a washing agent which is the most popular product in society, (c) diswashing products, as household cleaners for both manual and dishwasher use, and (d) household cleaners, as house cleaners, for example cleaning floors, cleaning porcelain, plastic, glass, and others. The definition of detergent includes a wide range of uses, not just laundry soap. In connection with the environmental conditions of domestic wastewater with a composition that is quite high in detergent, one of the requirements for the ability of indigenous bacterial isolates from domestic wastewater is to be tolerant of detergents in a wide range of water, including their surfactants. The results showed that 15 isolates of heterotrophic bacteria were more tolerant to detergents than the other isolates [9, 11, 16, 18, 21].

The results of the calculation of the Manova test showed T Hotelling isolates (11.36) $>$ T Hotelling tables with a p value $= 0.000$ at a significance level of 0.05 or with a p value $= 0.000$ which means there is a significant difference in antagonistic power to pathogens by measuring the diameter of the pathogen inhibition zone on 37 isolates of heterotrophic bacteria that have been isolated from domestic wastewater in Malang City. The next step is to select from each pathogen parameter, the heterotrophic bacterial isolates that have

the greatest antagonistic power against the four pathogens. The antagonistic potential against pathogens can be seen from the largest average diameter of the inhibition zone. The ability to kill pathogens in waste is a requirement for safe and environmentally friendly decomposers [8, 21, 25, 30, 31].

Based on molecular analysis of ARN 16 S, it was concluded that 4 heterotrophic bacterial strains had been identified which had superior properties compared to the other strains. In general, the consortium that has the potential to be a decomposer consists of the genus *Bacillus* which has the property of being able to form spores so that when used as a decomposer it is highly resistant to various environmental factors. This bacterial consortium can be used to improve environmental health. [2, 7, 9, 11, 16, 18, 21, 22, 27].

5 Conclusion

The results of this present research shows conclusion of this study was found that there were 37 isolates that had tolerance to various detergents with a concentration of 25% to 95%, were antagonistic to *Salmonella typhi, Shigella dysenteriae, Vibrio cholerae*, and *Escherichia coli*, and found 4 bacterial strains that had great potential as decomposers waste. These findings, consortium strains of heterotrophic bacteria consisting of *Bacillus cereus strain BQAR-01, Bacillus thuringiensis strain MSS-2, Bacillus cereus strain JDA-1,* and *Bacillus sp. B31(2008)* can be used in the development of environmental health as a safe and environmentally friendly decomposition of domestic wastewater.

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