Isolation and characterization of extremophile bacteria for hydrolytic enzyme production

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Presented at the 2nd International Conference on Life Science and Biology Education (ICoLIBE) organized by Biology Department, Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Negeri Makassar, 15 November 2022

Introduction

- Extremophiles are organisms that have been adapted to live in extreme environments.
- Extreme environments: environment that has very high temperatures (hot springs), very low temperatures (snow), high salinity (hypersaline ponds/lakes), very high pH or very acidic
- They have huge potential for various industrial application specifically for enzyme production.
- Waepella hot spring is one of the extreme habitats located in South Sulawesi
- Aim: to isolate and screen thermophilic bacteria from Waepella Hot Spring in Sinjai Regency, South Sulawesi, Indonesia for biotechnological application specifically for hydrolytic enzyme production.
- This research is expected to be the basis for the development of local extremophiles microorganisms for commercial applications.

Materials and methods

- Sampling Site
  - Waepella hot spring, Kampala Village, Sinjai Tengah District, Sinjai Regency, South Sulawesi (05o08'35.22"S, 120o12'07.67"E).
  - Water quality parameters were measured onsite including temperature, pH and salinity.
  - The water samples were collected using sterile glass bottles.

- Procedures
  - Isolation of thermophilic bacteria
    - Isolation of bacteria was carried out using agar plating technique (Andersen and Kawachi 2005).
    - Briefly, about 0,.2 mL of the water samples was plated on TSA medium and incubated at ambient room temperature (30±2°C).
    - The grown colonies emerged from on the plate were then re-streaked to obtain pure colonies.
  - Characterization of Isolates isolates
    - The obtained isolates were characterized by examination of colony colors, cell shapes, Gram staining, endospore test, catalase test and enzymatic activity (amylase, cellulose, protease, lipase and pectinase).

- Gram staining
  - For In Gram staining test, one a colony of bacterial is scratched smear was prepared on the surface of a sterile slide before adding 1 drop of crystal violet on the bacterial layer and allowed to stand for 1 minute.
  - After 1 minute, the slide is was rinsed with water until the dye fades. The slide is was dried over a bunsen burner before adding 1 drop of iodine solution to the surface of the slide and allowed to stand for 1 minute.
  - The slide is was rinsed with water followed by 70% alcohol rinsed until all the dye has had faded and then washed again with water.
  - The slide is was dried over a Bunsen burner before adding 1 drop of safranin and allowed to stand for 45 seconds.
  - The slide is was then washed with water and dried. Then slide was observed under microscope at (100x magnification)

- Catalase test
  - For catalase test, one colony of a loopful of bacterial isolate is was placed on a slide before adding 1- 2 drops of 3% H2O2 solution. If bubbles formed, it indicates catalase positive bacteria and if no bubble formed, it is catalase negative bacteria. The presence of bubbles means catalyze positive bacteria and the absence of bubbles means catalyze negative bacteria.
- Endospore test
  - For endospore staining, one colony of a bacterial smear was made bacterial isolate is scratched on the surface of a sterile slide then fixation is was carried out by adding 1-2 dopped of malachite green and heated for 2-3 minutes. If evaporation occursoccured, malachite green is was again dropped on the glass slide. After that, the glass slides are were rinsed with distilled water and dried before adding 1 drop of safranin is added to on the surface of the slide. The object glass is slide then allowed to stand for 1 minute. The slide is was washed and then observed using a microscope. Endospores positive will be green while endospore negarive/vegetative cells will be red

- Enzyme Activity activity Teststests
  - For enzyme activity tests, all the procedures are were the same except for the media used.
  - Proteolytic activity test was carried out on agar media using 1% skim milk (Nespolo et al. 2010).
  - Amylolytic activity test was carried out on agar media with 1% starch (Fossi et al. 2005). Lipolytic activity test was carried out on MRS agar media with the addition of 2 ml of olive oil (Svetlitshnyl et al., 1996).
  - Cellulolytic activity test was carried out on solid media containing 1% Carboxymethylcellulose (CMC).
  - Pectinolytic activity test was carried out on solid media containing 1% pectin and 0.1% Congo red indicator.
  - Briefly, 48 hours aged 20 µl bacterial culture aged 48 hours was pipetted onto a paper disc (5.5 mm diameter) then placed on the agar media and incubated at 30°°C for 3 days. The formation of a clear zone around the colony indicating indicates that the bacteria are were capable of hydrolyzing the media. The enzyme activity tests were carried out for 3 days. The clear zone diameter is was calculated by subtracting the diameter of the clear zone with the diameter of the paper diskdisc.

Results and discussion

Table 1. Water quality parameters at the Waepella Hot Spring Sinjai

Parameters	Stations		
	Station 1	Station 2	Station 3
Temperature (°C)	55	53	49
Salinity (ppt)	0	0	0
pH	7.43	7.61	7.28

Table 2. Characteristics of thermophilic bacteria isolated from ~~hot springs~~ Waepella Hot Spring Sinjai

No.	Isolate codes	Colony colour	Gram Staining	Cell shapes	Catalase	Endospore
1.	BHSS1	White	Positive	Rod	-	+
2.	BHSS2	White	Negative	Rod	-	-
3.	BHSS3	White	Negative	Cocci	+	+
4.	BHSS4	White	Positive	Cocci	+	-
5.	BHSS5	White	Negative	Cocci	+	-
6.	BHSS6	White	Negative	Cocci	+	-
7.	BHSS7	White	Negative	Rod	+	-
8.	BHSS8	Yellow	Negative	Cocci	+	-
9.	BHSS9	White	Negative	Cocci	+	-
10.	BHSS10	White	Positive	Cocci	+	+
11.	BHSS11	White	Negative	Rod	-	-
12.	BHSS12	White	Negative	Rod	+	-
13.	BHSS13	White	Negative	Cocci	+	-
14.	BHSS14	White	Negative	Cocci	+	+
15.	BHSS15	White	Negative	Cocci	+	-
16.	BHSS16	Brown	Negative	Cocci	+	-
17.	BHSS17	White	Negative	Rod	+	-
18.	BHSS18	Yellow	Negative	Cocci	+	-

Note: + = Presence, - = Absence

Table 3. Amylolytic activity of bacterial isolates

No.	Isolate Codes	Clear Zone Diameter (mm)		
		Day 1	Day 2	Day 3
1.	BHSS1	3.97±0.76	12.27±0.6	19.6±0.62
2.	BHSS2	11.1±0.15	12.23±0.38	18.57±0.60
3.	BHSS5	0	5.77±0.75	11.47±0.74
4.	BHSS7	0	8.3±0.7	15.63±0.76
5.	BHSS10	9.77±0.21	15.60±0.52	22.6±0.44
6.	BHSS12	0	4.2±0	5.73±0.15
7.	BHSS16	6.97±0.81	11.43±0.57	14.5±0.78
8.	BHSS18	4.53±0.32	7.77±0.31	11±0.36

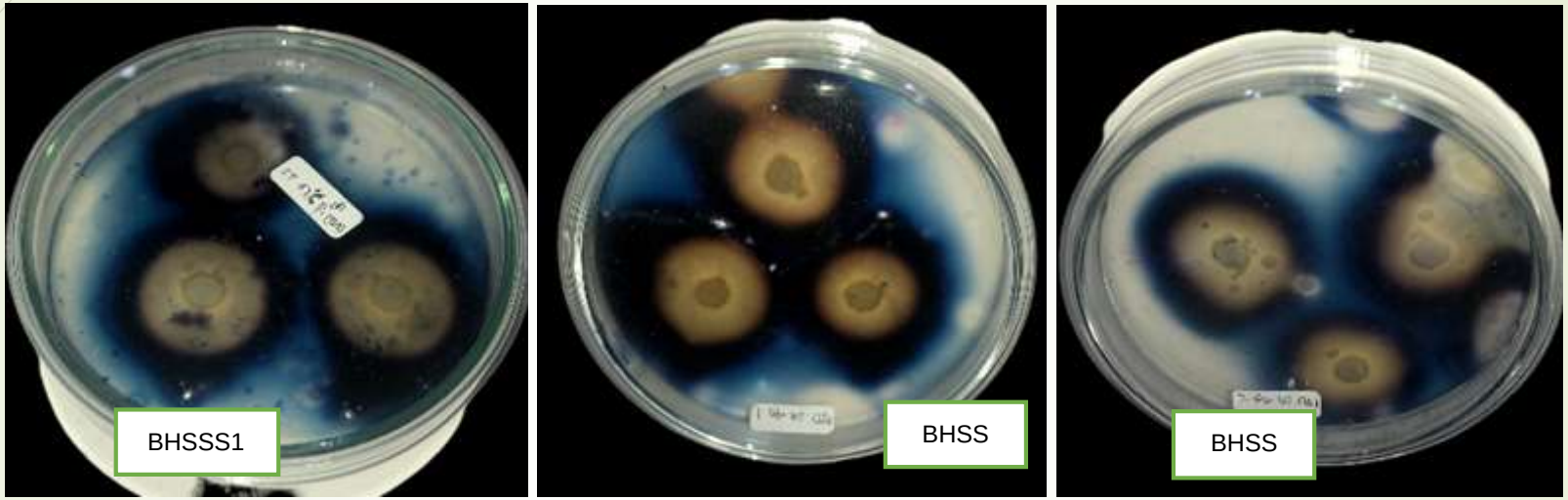

Figure 1. The formation of a clear zone around the paper disc containing bacteria on agar media with 1% starch

Table 4. Cellulolytic enzyme activity of bacterial isolates

No.	Isolate Codes	Clear Zone Diameter (mm)		
		Day 1	Day 2	Day 3
1.	BHSS1	2.23±0.32	3.63±0.47	3.93±0.67
2.	BHSS4	0	0	4.03±0.15
3.	BHSS5	0	0	3.13±0.15
4.	BHSS6	0	2.57±0.29	3.67±0.15
5.	BHSS7	4.5±0.3	7.73±1.01	11.6±0.4
6.	BHSS11	0	1.73±0.40	2.33±0.32
7.	BHSS12	0	0	2.8±0.2
8.	BHSS15	0	0	3.5±0.44
9.	BHSS17	1.6±0.2	2.13±0.23	2.4±0.26

Table 5. Pectinolytic enzyme activity of the isolates

No.	Isolate Codes	Clear Zone Diameter (mm)		
		Day 1	Day 2	Day 3
1.	BHSS2	7.53±0.40	8.1±0.67	10.53±0.49
2.	BHSS3	0	5.9±0.3	6.57±0.57
3.	BHSS4	2.37±0.67	6.37±0.35	8.73±0.31
4.	BHSS5	2.33±0.35	6.4±1.01	7.37±0.23
5.	BHSS6	0	3.37±0.11	3.5±0.26
6.	BHSS8	0	4.47±0.45	4.87±0.35
7.	BHSS9.1	5.03±0.15	5.67±0.25	6.4±0.5
8.	BHSS10	2.17±0.15	7.07±0.45	9.4±0.72
9.	BHSS11	0	4.97±0.40	5.23±0.59
10.	BHSS12	3.3±0.3	8.97±0.15	11.43±0.15
11.	BHSS13	3.7±0.46	6.9±0.79	8.7±0.56
12.	BHSS14	5.27±0.15	6.27±0.15	9.33±0.21
13.	BHSS15	2.67±0.23	7.43±0.15	9.73±0.15
14.	BHSS16	8.07±0.12	8.9±0.16	12±0.24
15.	BHSS17	2.47±0.15	3.3±0.2	3.43±0.15
16.	BHSS18	1.4±0.36	2.73±0.15	3.47±0.38

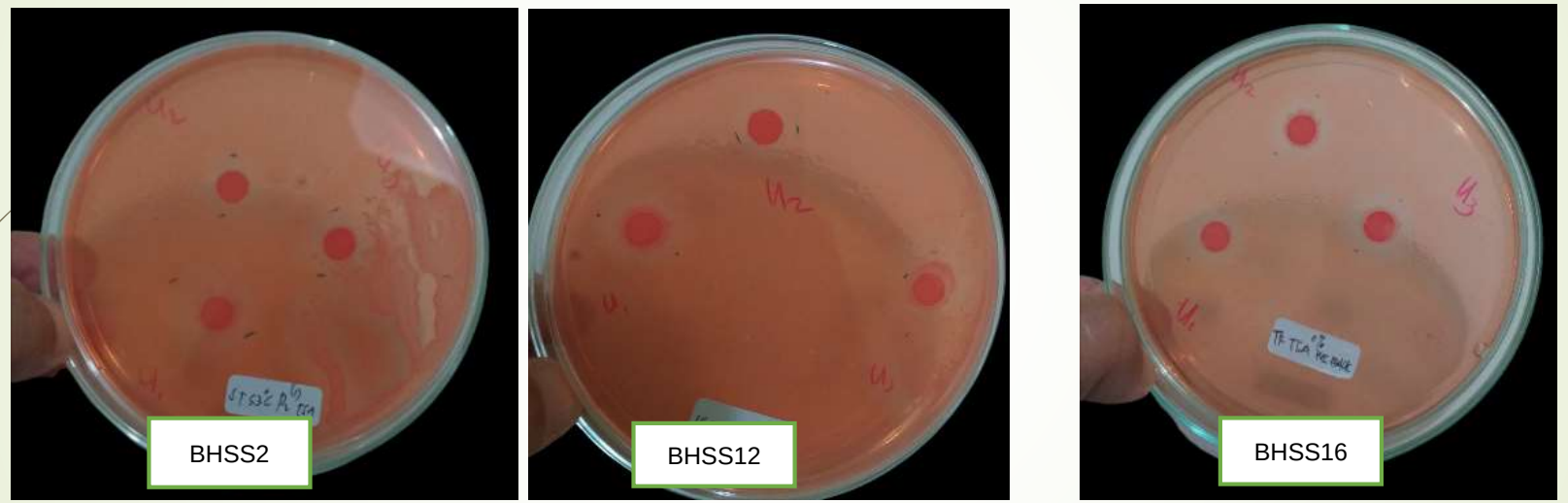

Figure 2. The formation of a clear zone around the paper disc containing bacteria on agar media with 1% pectin

Table 6. Proteolytic enzyme activity of bacterial isolates

No.	Isolate Codes	Clear Zone Diameter (mm)		
		Day 1	Day 2	Day 3
1.	BHSS13	0	5.57±0.72	6.67±0.60
2.	BHSS15	0	2.77±0.15	9.83±0.40
3.	BHSS17	0	5.43±0.25	6.53±0.35

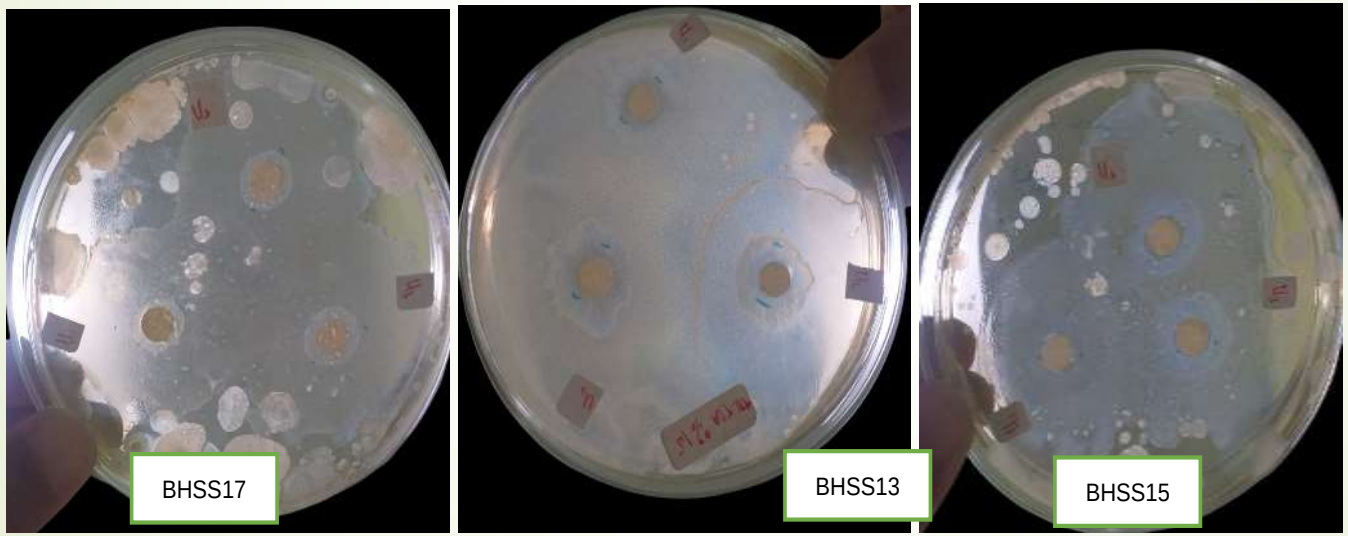

Figure 3. The formation of a clear zone around the paper disc containing bacteria on agar media with 1% skimmed milk

Conclusion

- The bacteria isolated from the Waepella hot springs have the ability to produce various important hydrolysis enzymes that have very wide industrial applications.
- It is clear that this study is very important as a basis for the development of the thermophilic bacteria as a producer of hydrolytic enzymes for industrial application.

Thank You