Isolation and Characterization of Endophytic Actinomycetes on Onion Root (*Allium Ascalonicum* L.) Palu Cultivar

Ayu Lestari(✉), Abd. Rahmat Sapa, Meryany Ananda, and I. Nengah Suwastika

Department of Biology Faculty of Sciences, Tadulako University, Jl.Soekarno Hatta KM 9, Palu 94118, Indonesia
ayulestari4234@gmail.com

Abstract. Endophytic microbes are microbes that during their entire life cycle are in plant tissues. Actinomycetes is one of the endophytic microbes that produce secondary metabolites and can be found in the roots of onions. This study aimed to isolate, characterize and determine the growth rate of endophytic actinomycetes from *Allium ascalonicum* L. Palu cultivar. The isolation was done by spread plate method using SCA (*Starch casein agar*) medium, then purified and characterized their properties macroscopically (morphology of colony) and microscopically (morphology of cell) before it is determined their growth rate. Actinomycetes were isolated from the roots of onion namely, JO1, JO2, and JO3 which showed different in characters of each isolate. The result also showed that Actinomycetes growth rate was quicker on onion root extract than it was in SCB *(Starch casein broth)* medium. Based on that data, it can be concluded that endophytic Actinomycetes can be grown in onion root extract medium.

Keywords: Actinomycetes · Onion · growth rate · growth medium

1 Introduction

Microorganisms known as endophytic bacteria spend their whole life cycle inside plant tissues. Microbes can enter plant tissues in a number of ways, including through root pores, leaf stomata, and tissue wounds [1]. Actinomycetes is one form of endophytic bacterium. Microbes called actinomycetes produce enormous quantities of bioactive substances. Actinomycete active chemicals are typically utilized as antibacterial, anticancer, and antitumor agents [2].

One host plant for endophytic actinomycetes that can make secondary metabolites is shallots. One of Central Sulawesi's top exports is the Palu Valley variety of shallots, which is also the raw material for the fried onion processing sector and has established a "local brand" in Palu [3]. Depending on the type of bacteria and the surrounding environment, the rate of bacterial development varies dramatically. Every stage of a bacterium's growth occurs at a separate time [4].

Actinomycetes grow far more slowly than the majority of bacteria. Actinomycetes colonies can produce spores that resemble powder, are stuck to the surface of the media,

N. I. Inda et al. (Eds.): ISST 2022, APR 7, pp. 228–234, 2023.
https://doi.org/10.2991/978-94-6463-228-6_26

and take a long time to grow (2 weeks) [5]. Even though selective media, such as Starch Casein Agar (SCA) media, have been employed, actinomycetes still take a long time to develop, therefore the best medium for their growth has not yet been discovered.

2 Materials and Methods

2.1 Materials

Onion root samples (*Allium ascalonicum L*), were obtained from local plantations in the Sigi area that do not use chemical herbicides and pesticides. Supporting materials in the lab are: Aquades, Alcohol 70%, Sodium Hypochlorite (Naocl) 5.25%, starch/starch, Casein, KNO_3, $MgSO_4.7H_2O$, K_2HPO_4, NaCl, $CaCO_3$, $FeSO_4.7H_2O$, Agar, UHT Milk, Aluminum foil, Label paper, Spritus, Rubber, Tissue and Plastic wrap.

2.2 Methods

A sampling of the shallot root A. ascalonicum L was obtained in Soulowe Village and Jono Oge Village, Kec. Sigi Biromaru Kab. Sigi, Central Sulawesi Province. SCA (Starch Casein Agar) and onion root extract medium were employed in this investigation. Spread-plate isolation that was cultured for 5–7 days at room temperature. The developed endophytic actinomycetes are then gradually and individually purified. The acquired Actinomycetes endophytic pure isolates were then put on SCA media. By using Gram staining to examine colony morphology and cell shape, Actinomycetes morphology was described. Actinomycetes' growth rate was measured, and observations were also made regarding their optical density (OD) value and number of cells.

3 Results and Discussion

There were three isolates of actinomycetes with the codes JO1, JO2, and JO3 that were taken from the roots of shallots of the Palu variety. The newly formed colonies of actinomycetes had a typical shape that was circular with elevated and convex elevations, flat edges, and smooth, rough, or wrinkled surfaces (Fig. 1).

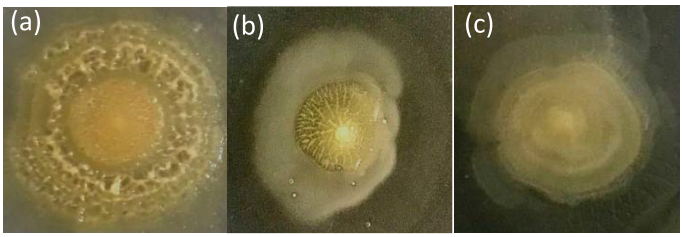

Fig. 1. Colonies grew on SCA media after three days of incubation. The pigmentation of (a) JO1, (b) JO2, and JO3 (c) was yellow. (Color figure online)

Table 1. Observations of macroscopic characters of Actinomycetes

No.	Isolate	Shape	Pigmentation	Elevation	Edge	Aerial mycelium	Substrate mycelium
1.	JO1	*Filamentous*	Yellow	*Flat*	*Curled*	Yellow	Yellow
2.	JO2	*Circular*	Yellow	*Raised*	*Entire*	Yellow	Yellow
3.	JO3	*Circular*	Yellow	*Flat*	*Curled*	Yellow	Yellow

Colony morphology analysis revealed that the three isolates had various traits or belonged to several species. Colonies with the JO1 isolate have a filamentous morphology, a yellow tint, a flat elevation, and curled edges. The JO2 isolates had a circular form, a yellow colony, and an elevated edge. Colony JO3 shape: round, color: yellow, elevation: flat, edges: curled. According to research [6], white, round shapes, whole edges, high elevations, and yellow pigmentation were the most prevalent colony colors. Table 1 displays the findings of macroscopic observations.

Gram staining was used to observe cell morphology. The purpose of gram staining is to identify the kind, shape, and size of vegetative cells. Gram staining is a microscopic morphological identification method to ascertain cell shape and gram type, according to Sari [7]. The outcomes indicated that all three of the isolated organisms were gram-positive. This is in line with the findings of a study by [8], who stained Actinomycetes and discovered that the isolates were Gram positive. The cell walls of Gram-positive bacteria contain a thick layer of peptidoglycan, which allows them to form a rigid structure. As a result, when a purple-iodine crystal complex enters the cells of Gram-positive bacteria, it cannot be washed off by alcohol due to the presence of alcohol, according to Pratiwi [9] explanation of the structure of bacterial cells. The cell wall's peptidoglycan layer is thick and present. The three isolates exhibited spherical cells according to their cell shape (coccus) Fig. 2. While JO2 and JO3 had 1.8 m in size, the vegetative cell size of JO1 isolates was the same at 1.7 m. According to [10] Table 2 shows the properties of Actinomycetes, which have vegetative cells with a diameter of 0.5 to 2.0 m.

Results in the form of a growth curve that depicts a faster growth of growth rate of actinomycetes, namely onion root extract media in comparison to SCB media, can be used to determine the rate of growth of actinomycetes. According to the curve, the SCA medium exhibits lower growth than the onion root extract media. The availability of nutrients, water activity, oxygen, and substances that impede bacterial development are among the variables that affect microbial growth, according to Fardiaz [11]. According

Table 2. Observations of microscopic characters of Actinomycetes

No.	Isolate	Cell Shape	Gram	Cell size pxl (μm)
1	JO1	Coccus	Positive	1,7 μm
2	JO2	Coccus	Positive	1,8 μm
3	JO3	Coccus	Positive	1,8 μm

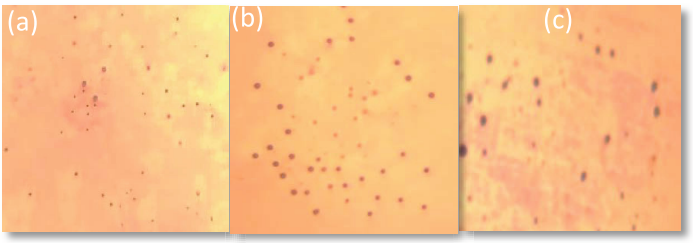

Fig. 2. The result of cell shape and gram staining. (a) isolate JO1, (b) isolate JO2 coccus dan (c) Isolat JO3 were coccus and gram positive bacteria.

to [12], the nutrients in shallots include calcium, iron, magnesium, phosphorus, potassium, sodium/sodium, zinc, and selenium. They also contain carbs, sugar, and protein. Onion bulbs include secondary metabolites like polyphenols, sulfur, flavonoids, tannins, saponins, essential oils, kaempferol, flavon glycosides, furoglucin, dihidroaliin, cycloaliin, methylaliin, and quercetin [13].

The growth curve (Fig. 3) The adaptation phase of the JO1 isolate on SCB media and onion root extract media exhibited a comparatively gradual onset, as evidenced by the curve, on the first day. Because the bacteria are growing on the same medium as they were in the prior phase, adaption happens swiftly. Microorganisms introduced into the medium, according to [14], first go through an adaptation phase to adjust to the ambient parameters. The quantity of cells injected, the ideal physiological and morphological circumstances, and the necessary growth media all have a significant role in determining how long the adaptation period lasts. It might not be necessary to allow for adaption time if the growth media and surroundings are the same as those used previously.

On SCB media and onion root extract media, the growth curve also reveals that on day 3, it entered a logarithmic phase, which is characterized by a considerable increase

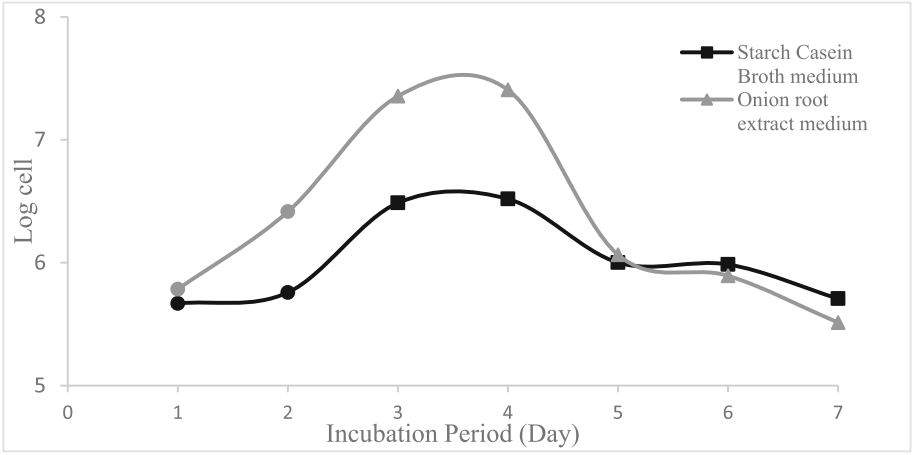

Fig. 3. Growth curve of JO1 isolate on onion root extract and SCB media.

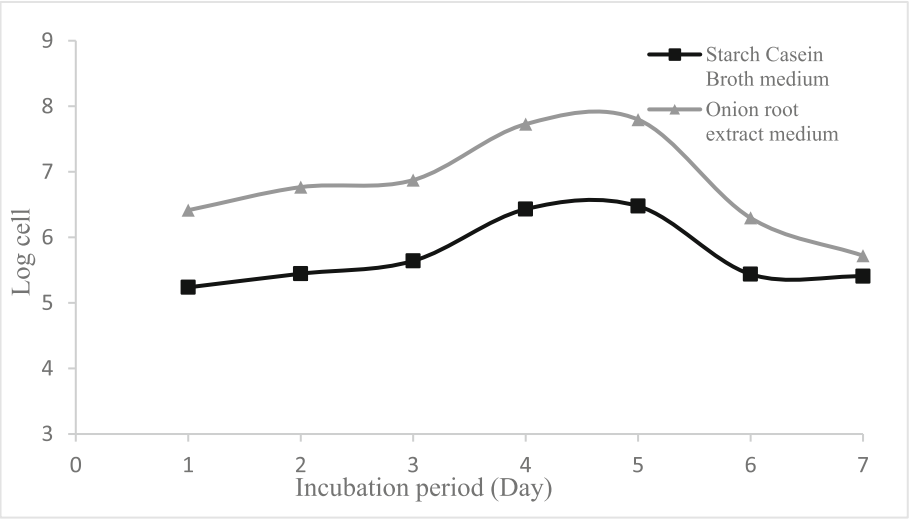

Fig. 4. Growth curve of JO2 isolate on onion root extract and SCB media.

in the number of cells. On SCB media, the stationary phase had a growth phase that was largely constant, and the onion root extract media reached its peak on days 4–5. Because the number of growing and dying cells is equal during this phase, the number of cell populations stays. This is consistent with the claim made by [15] that the stationary phase causes a reduction in cell size because the cell continues to divide despite food depletion, maintaining a relatively constant rate of growth. On days 5–7, the death phase began to reduce. This is because there aren't enough growth factors, like vitamins and minerals [16]. The absence of some vital nutrients in the medium, the buildup of autotoxins in the medium, or a combination of the two may also result in the stoppage of growth.

Growth curve (Fig. 4) The growth phase for JO2 isolates was the same on SCB media and media with onion root extract. Day 1 saw an adaptation period for both the SCB and onion root extract medium. Then, on days 2–3, it goes through an exponential phase during which the curve steepens. It entered a stagnant phase on days 4–5, then on days 6–7, it dwindled gradually toward the death phase.

Growth curve (Fig. 5) The growth phase of the isolate differed between SCB media and media containing onion root extract. The type of bacteria and the surrounding environment have a significant impact on the growth rate of bacteria. Each phase of a bacterium's growth takes place at a separate period [4]. The SCB medium and onion root extract media were used on day 1. Went through a period of adaptation. Then, on the SCB media, an exponential phase occurred on the second day, whereas on the onion root extract media, it took place on the fourth to the sixth day. On SCB media for days 3–4, and on onion root extract media for days 5–6, it then entered the stationary phase. On the SCB medium, the curve continued to decline as it entered the death phase on days 5–7, whereas it did so on day 7 in onion root extract.

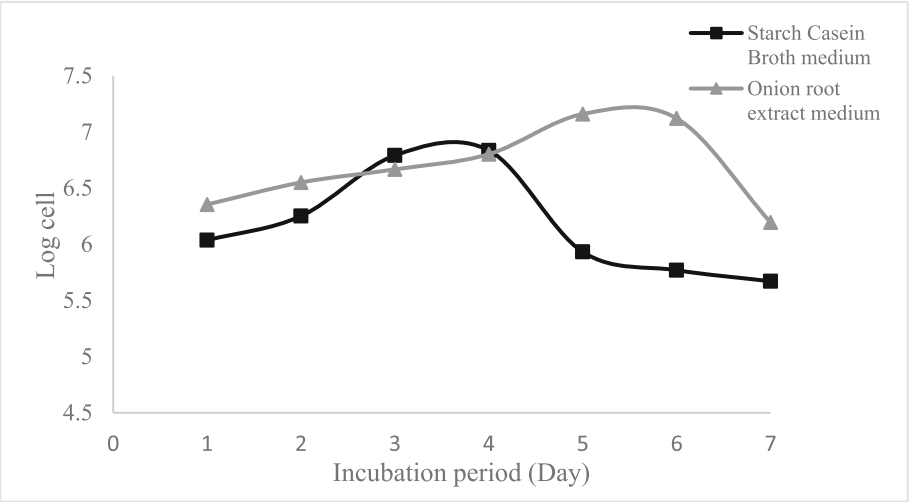

Fig. 5. Growth curve of JO3 isolate on onion root extract and SCB media.

4 Conclusion

There were three isolates of actinomycetes with the codes JO1, JO2, and JO3 that were found in the roots of shallots of the Palu variety. The three isolates displayed distinctive traits in their cell and colony morphologies, indicating differences in species. Actinomycetes were cultivated on various SCB media and onion root extract media, and variable growth rates were observed. Onion root extract media provides a higher productive medium for the growth of endophytic Actinomycetes.

Acknowledgement. Thank you to Mr. Suroso, an onion farmer in Jono Oge village, who provide a sample. Thank you to Ms. Sami Bukang, S.P., Rian Aristiawan, Dewi Purnamasari, Nurul Rahma, and Titik Nur Vitasari for helping while in the laboratory works.

References

1. Sukmadi, R. B.: Aktivitas fitohormon Indole-3-Acetic Acid (IAA) dari beberapa isolat bakteri rizosfer dan endofit, Jurnal Sains dan Teknologi Indonesia, 14(3), 221–227 (2012).
2. Pujiati, P.: Isolasi actinomycetes dari tanah kebun sebagai bahan petunjuk praktikum mikrobiologi, Jurnal Biologi Dan Pembelajarannya, 1(2), 42–46 (2014).
3. Limbongan, J., Maskar.: Potensi Pengembangan dan Ketersediaan Teknologi Bawang Merah Palu di Sulawesi Tengah, Jurnal Litbang Pertanian, 22(3), 103–108 (2003).
4. Mahjani, M., Putri, D. H.: Growth Curve Of Endophyte Bacteria Andalas Plant (Morus macroura Miq.) BJT A-6 ISOLATE, Biologi, 5(1), 29–32 (2020).
5. Krieg, N.R., Holt.: Bergey's Manual of Determinative Bacteriology, p. 605–675, William and Wilkins, London (1994).

6. Ekowati, C. N., Achmad, A.: Pengaruh Kompos Kulit Buah Kopi (Coffea robusta Lind.) dan Kacang Pinto (Arachis pintoi Krapov dan Gregory) terhadap Keanekaragaman Actinomycetes, Sains MIPA, 13(3), 177–182.(2008).
7. Sari, D. W.: Perencanaan Rehabilitasi Hutan Dan Lahan Kritis Pada Kawasan Daerah Aliran Sungai (Das) Sumber Brantas Di Kecamatan Bumiaji, Tesis, Fakultas Teknologi Hasil Pertanian Pasca Sarjana Universitas Brawijaya. 2011.
8. Arifuzzaman, M., Khatun, M. R., Rahman: Isolation and screening of actinomycetes from Sundarbans soil for antibacterial activity, Journal of Biotechnology, 9(29), 4615-4619. (2010)
9. Pratiwi, S.T.: Mikrobiologi Farmasi, Jakarta, Penerbit Erlangga (2008).
10. Ambarwati, S.: Keanekaragaman Streptomyces yang Berasosiasi dengan Rizosfer Jagung (Zea Mays), Journal of Microbiology Research, 6(1) (2012).
11. Fardiaz, S.: Mikrobiologi Pangan I. Gramedia Pustaka Utama, Jakarta (1992)
12. Aryanta, I. W. R.: Bawang merah dan manfaatnya bagi kesehatan, Widya Kesehatan, 1(1), 29–35 (2019).
13. Arora, E., Sharma, V., Khurana, A., Manchanda, A., Sahani, D., Abraham, S., Kundu, D., Gupta, H., Chiru, L., Sharma, N., Garg, N., Jomy, S.: Phytochemical analysis and evaluation of antioxidant potential of ethanol extract of Allium cepa and ultra-high homoeopathic dilutions available in the market: A comparative study, Journal of Research in Homoeopathy, 11(2), 88 (2017).
14. Middlebeek, E.J., Jenkins, R.O., Drijver-de Haas, J.S.: In Vitro Cultivation of Micro-organisms, Growth in batch culture. In Vitro Cultivation of Micro-organisms. Biotechnology by Open Learning (1992).
15. Mangunwidjaja, D., Suryani, A.: Teknologi Bioproses, Penerbit Swadaya, Jakarta (1994).
16. Gaman, P.M., Sherrington, K.B.: Pengantar Ilmu Pangan Nutrisi dan Mikrobiologi, Gadjah Mada University Press. Yogyakarta (1994).