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Antibacterial Ability of Endophytic Bacteria Isolated from *Kemenyan* (*Styrax benzoin* L.)

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Abstract. *Kemenyan* is one of plant commodities in North Sumatera that has been used as medicinal plants to treat infection and illness. The aim of this study was to isolate endophytic bacteria from *kemenyan* (*Styrax benzoin*) and evaluate their antibacterial properties against pathogenic bacteria. Sixteen isolates were found from leaf, bark and root samples of *kemenyan.* Two isolates, RS01 and RS13 showed considerable antibacterial activities against *Staphyloccocus aureus, Escherichia coli* and *Streptococcus mutans*.

1. Introduction

Kemenyan is one of native commodity plants in North Sumatera and Malaysia that produce benzoin gum with high economical and medical value. In North Sumatera, two species of *Kemenyan* can be found namely *kemenyan Toba/ Styrax sumatrana* J.J.SM and *kemenyan durame/ Styrax benzoin* Dryand. [1]. Both species have been used in traditional remedies as topical medicine to treat various illness. Extensive studies have reported some of its biological properties. Benzoin gum produced from the stem, contained flavonoid and tannins possessing antibacterial properties [2,3].

Ethanolic extract from its leaves also possessed antibacterial properties against skin pathogens, *Propionibacterium acne* and *Staphylococcus epidermidis* [4]. Recent information of species distribution have showed that species is currently facing serious exploitation. The use of *kemenyan* is quite simple in many traditional practices. Continuous harvesting upon certain plant parts as traditional medicine may hinder plants to grow sustainably and may lead to species decline in the future. One of alternative ways in utilizing *kemenyan* as biological remedies without harvesting most of its parts, is by exploring and exploiting microbial endophytes living within. Microbial endophytes are community of microorganisms that inhabit internal environment of a healthy plant through various entry sites [5].

Endophytes are known to produce extracellular enzymes and metabolites to cope within host environment [6,7]. Endophytes are also known to synthesize similar compounds with their hosts that become key feature to utilize *kemenyan* in a more sustainable way [8]. Information regarding antibacterial properties from endophytic bacteria residing within *kemenyan* is still less reported. In this study, we reported that vegetative plant parts of *kemenyan* are known to harbor microbial endophytes, especially from bacteria group and antibacterial properties are also evaluated.

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2. Materials and Methods

2.1. Isolation of endophytic bacteria from kemenyan

Endophytic bacteria were isolated from leaf, bark and root of *kemenyan*, sampled from Parbuju village, North Tapanuli Regency, North Sumatera Province. Samples from healthy plant were collected randomly. Samples were immediately rinsed with water and stored in cold temperature prior laboratory test. In laboratory, plant parts were again rinsed with water and then cut into 1–3 cm pieces. Sample pieces of stems and roots were surface sterilized by soaking into solutions: 70% ethanol for 1 min, 5.25% NaOCl for 5 min, 70% ethanol for 30 secs. Sample pieces of leaves were surface sterilized using 96% ethanol and 5.25% NaOCl in the same manner. Sample pieces were rinsed with sterile distilled water prior plating on top of *Nutrient Agar* (NA) medium supplemented with Nystatin (0.01% b/v). Plates were incubated in ambient temperature for 7 days. Growing bacterial colonies from sample pieces were recovered and stored in stock medium.

2.2. Morphological and biochemical characterization of endophytic bacteria

Bacterial isolates were morphologically characterized by observing their colony shape, color, elevation and margin using standard observation. Gram staining was performed to observe cellular shape, arrangement and gram reactions. Biochemical tests was performed to observe physiological features such as: starch hydrolisis, gelatin hydrolisis, citrate utilization, sugar utilization, hydrogen sulfide production, motility and catalase test.

2.3. Antagonistic assay of endophytic bacteria

Antagonistic assay was performed against three pathogenic bacteria: *Staphylococcus aureus* ATCC 25923*, Escherichia coli* ATCC 25922 and *Streptococcus mutans* ATCC 251715 using disk diffusion assay [9]. Discs impregnated with 10 µL endophytic bacteria ($OD_{600}\approx10^8$ CFU/mL) were placed on top of pathogenic bacteria lawns grown on NA medium. Assay was done in duplicate. Clear zones around discs showed antibacterial activity and diameter of zones were measured.

3. Results and Discussion

3.1. Characterization of Endophytic Bacteria from Kemenyan

Sixteen bacterial isolates were found in this study: 5 isolates from leaf, 6 isolates from bark and 5 isolates from root. Each plant parts used as isolation source showed no significant differences in terms of number of bacterial isolates which may indicate that plant parts of *kemenyan* are evenly harbored by endophytic bacteria without any obvious limiting factor exposed by different parts. From morphological and biochemical characters, it may be concluded that all isolates were different each-others (Table 1).

Reports on finding endophytic bacteria within members of Styracaceae are still limited. Eight bacterial isolates are reported to harbor vegetative parts of *S. officinalis* from Iran [10]. Evidence showed that endophytic colonization occurred in leaf part of *S. camporum* from Brazil, indicating the assumption of metabolite co-production from endophytic bacteria in secretory glands of plant [11]. Interesting finding that most bacteria colonizing *kemenyan* were gram positive bacteria. Secondary metabolites from *kemenyan* which is mostly benzoin may affect the presence of certain group or even species of bacteria.

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Table 1. Morphological and biochemical characteristics of endophytic bacteria isolated from *Kemenyan*

	Colony Morphology					Cell Morphology		Biochemical Traits							
Isolate Code	Form	Margin	Elevation	Color	Gram Stain	Shape	Arrangement	Citrate	Gelatine	Motility	Amylase	Catalase	Glucose	Sucrose	Lactose
RS01	Circular	Undulate	Flat	Cream	-	Coccus	Mono	+	-	+	-	-	+	-	-
RS02	Irregular	Undulate	Flat	Cream	+	Coccus	Strepto	+	-	+	+	-	+	-	-
RS03	Filamentous	Filamentous	Flat	Cream	+	Bacil	Strepto	+	-	+	+	-	+	-	-
RS04	Irregular	Entire	Flat	Cream	-	Basil	Strepto	+	+	+	-	-	+	+	+
RS05	Irregular	Entire	Flat	Cream	+	Coccus	Strepto	+	-	+	+	-	+	+	+
RS06	Circular	Entire	Flat	Cream	+	Coccus	Strepto	+	-	+	+	-	-	-	-
RS07	Irregular	Undulate	Flat	Cream	+	Coccus	Strepto	+	+	+	+	-	-	-	-
RS08	Filamentous	Entire	Flat	Cream	+	Coccus	Mono	+	-	+	+	-	-	-	-
RS09	Irregular	Undulate	Flat	Cream	+	Coccus	Strepto	-	+	+	-	-	+	+	+
RS10	Irregular	Undulate	Flat	Cream	+	Coccus	Strepto	+	+	-	+	-	+	+	+
RS11	Irregular	Undulate	Flat	Cream	+	Coccus	Strepto	-	+	+	-	-	+	+	+
RS12	Circular	Entire	Flat	Cream	+	Coccus	Diplo	-	+	+	+	-	+	+	+
RS13	Filamentous	Filamentous	Flat	Cream	+	Basil	Diplo	-	-	+	-	-	+	-	-
RS14	Irregular	Undulate	Flat	Cream	+	Basil	Strepto	+	+	-	-	-	+	+	+
RS15	Circular	Entire	Flat	Cream	+	Coccus	Mono	+	-	-	+	-	+	+	+
RS16	Circular	Entire	Flat	Cream	+	Coccus	Mono	-	+	+	-	-	+	-	-

Most morphological characteristics of isolated bacteria were dominated by irregular form, undulate and entire margin, and cream-colored colonies. Most isolated endophytic bacteria (14 isolates) were categorized as gram positive in which 3 isolates of roots, 6 isolates of barks, 5 isolates of leaves while 2 isolates were gram negative, isolated from roots. The dominance of gram positive group found in this study is assumed to show the adaptive ability of the endophytic bacteria to withstand towards secondary metabolite produced from *S.benzoin*.

Sumatran benzoin tree is known to contain secondary metabolites, i.e. benzoic acid, cinnamic acid, *p*-coumaryl cinnamate, *p*-coumaryl benzoic and isovanillin [12-14]. The adaptive properties of gram positive endophytic bacteria varies greatly within host cells (plant tissues/organs), starting from the synthesize of pigments, spores and secondary metabolites. In addition, gram positive bacteria may secrete various compositions of secondary metabolites. In other study, 16 isolates of gram positive endophytic bacteria were successfully isolated from roots, stems and leaves of five medicinal plants namely *Vinca rosea* L., *Curcuma longa* L., *Eucalyptus globules* Dehnh, *Pongamia glabra* Vent, and *Musa Paradiasiaca* L. The study showed that isolated bacteria produced secondary metabolites such as enzymes, antibacterial, antifungal and antioxidative compounds [15].

3.2. Antagonistic Assay of Endophytic Bacteria from Kemenyan

Most bacterial isolates showed antagonisms against tested pathogens: *Staphylococcus aureus, Escherichia coli* and *Streptococcus mutans* with varying abilities (Table 2). Most bacterial isolates were able to inhibit three pathogenic bacteria as shown from inhibition zones measuring from 7.00 to 15.00 mm. Two selected isolates namely RS01 and RS13 were chosen as potential isolates and were subjected to the next step. Both isolates showed the best inhibition against *S.aureus* with diameter of inhibition zones reaching 15.00 and 13.00 mm for RS01 and RS13, respectively.

Table 2. Results of antagonistic assay against tested pathogens

Isolates	Source	Diameter of Inhibition Zones (mm)		
		S. mutans ATCC 251715	*S. aureus* ATCC 25923	*E. coli* ATCC 25922
RS01	Root	0.00	13.00	10.00
RS02	Root	7.00	9.00	8.50
RS03	Root	9.00	9.00	9.00
RS04	Root	8.00	7.40	8.00
RS05	Root	9.00	7.20	8.00
RS06	Bark	8.00	0.00	7.25
RS07	Bark	9.00	0.00	8.15
RS08	Bark	10.00	7.50	0.00
RS09	Bark	10.00	0.00	0.00
RS10	Bark	7.20	7.70	7.20
RS11	Bark	8.40	0.00	8.45
RS12	Leaf	7.00	8.75	6.30
RS13	Leaf	10.00	15.00	8.00
RS14	Leaf	7.25	0.00	7.25
RS15	Leaf	9.00	7.75	7.50
RS16	Leaf	8.00	0.00	0.00

Endophytic bacteria isolated from *Raru* (*Cotylebium melanoxylon*) and tea (*Camellia sinensis*) exhibit stronger inhibition towards *S. aureus* than other pathogenic bacteria tested [16,17]. The gram positive bacteria, *Staphylococcus aureus* is more sensitive against secondary metabolites produced by the endophytic bacteria. The isolate, RS13 displayed different antagonistic activities to the three pathogenic bacteria. Similar metabolites are assumed to be synthesized by endophytic bacteria residing within benzoin tree [18]. Antibacterial activity of benzoin tree compounds is already reported [19]. The metabolites are manifested as adaptive properties of endophytic bacteria to reside within

host micro-environment by secreting specific secondary metabolites and chemical trade-off among others.

The isolate, RS01 showed prominent antagonistic activities against *S. aureus* ATCC 25923 and *E. coli* ATCC 25922. The isolated endophytic bacterial strain was originated from root samples. Preliminary assumptions are that endophytic bacteria colonizing the roots of benzoin tree tissue synthesized higher variety of antibacterial secondary metabolites than the barks and leaves. Endophytic bacteria of benzoin tree may also provide protection against environmental stress and pathogenic bacteria to thrive within root tissues. The interaction between endophytic bacteria and plant rhizosphere is known to be initiated through specific chemical compounds secreted by both symbionts [20].

4. Conclusion

Sixteen isolates were found from leaf, bark and root samples of *Kemenyan.* Two isolates, RS01 and RS13 showed considerable antibacterial properties against *Staphyloccocus aureus, Escherichia coli* and *Streptococcus mutans*.

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