ORIGINAL ARTICLE

Enhancement of Anti-MRSA Potential Produced by an Endophytic Fungus *Ceratobasidium Ramicola* IBRLCM127 via Submerged Fermentation System

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ABSTRACT

Introduction: Exploring endophytic fungi isolated from medicinal herbs could be a turning point in the research of secondary metabolites biosynthesis, as these endophytic fungi are capable of synthesizing the similar compounds as their host plant. The advantages of manipulating endophytic fungi for bioactive compound production are the reduction of dependency rate on slow-growing and rare plants, cost-effective, continuous process, environmentally friendly and high yield in a short period. Thus, the current study envisages investigating the influence of culture conditions against the anti-MRSA potential production of the endophytic fungal isolate, Ceratobasidium ramicola IBRLCM127 isolated from the local medicinal plant Curcuma mangga Valeton & Zijp. **Methods:** The endophytic fungal isolate was used to produce fungal metabolites through submerged fermentation. The physical parameter improvement was investigated using the 'one-factor-at-a-time' technique. The fungal fermentative broth was subjected to an anti-MRSA assay using Lorian method, whereas the growth of a fungus was determined based on the cell growth weight. **Results:** The highest anti-MRSA potential of 42.50±0.1 U/ml and 5.49±0.1 g/L of mycelial growth was observed after improving the basal medium containing yeast extract sucrose broth incorporated with water extract from the host plant, 6 days old of inoculum age, 2 agar plugs of mycelia, incubation temperature of 25 0C and 12 days of cultivation 12 days of cultivation shaken at 120 rpm in the absence of light. **Conclusion:** The improved culture conditions shorten the incubation period and yield a significant enhancement of anti-MRSA potential and fungal growth with 13.27% and 10.91%, respectively.
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Keywords: Fungal endophyte; Culture conditions; Ceratobasidium ramicola; Anti-MRSA; Fungal growth

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INTRODUCTION

Endophytic fungi can be found ubiquitously colonizing higher plant tissues without causing any adverse effect and they are regarded as untapped sources of novel bioactive metabolites with chemically diverse structure and biologically active potential. They have formed symbiotically close biological association with their host plant after living together for a very long time, thus they are capable of secreting similar bioactive compounds as their host plants that possess antibacterial, antiviral, antifungal, antitumor and anti-inflammatory activities (1-3). Extraction of these beneficial metabolites plays an essential role to obtain a complete profiling of metabolites and it can be done through fungal fermentation by using the most common method, submerged fermentation (SMF) (4). SMF refers to a simple fermentation technique that adopted liquid nutrient media by inoculating the desired microbes into the flask or bioreactor system. This method is very famous due its simplicity method that offer a better adjustment of fermentation parameters such as temperature, pH, growth media types, incubation period, dissolved oxygen, etc. (5,6). These parameters can be easily manipulated to obtain a various secondary metabolite with pharmaceutical and industrial importance (7).

Substantial work has been done involving the role of fungal endophyte extracts to combat the infection of Methicillin-resistant Staphylococcus aureus, one of

the prime causes of hospital-acquired infection. We also have reported the potential of Ceratobasidium ramicola IBRLCM127, a potent endophytic fungal isolate isolated from *C. mangga* Valeton & Zijp rhizome which possess remarkable anti-MRSA potential (8,9). This fungal ethyl acetate extract has demonstrated significance effects against the cell of MRSA and capable to kill them (bactericidal effect). Therefore, the enhancement of culture conditions by optimizing the physical parameters involved in fungal fermentation such as initial pH of media used, inoculum age, period of incubation, temperature, agitation speed and size of inoculum were conducted to increase the anti-MRSA potential and fungal growth of *C. ramicola* IBRLCM127. The current study is very significance as this is the first report of *C. ramicola* IBRLCM127 isolated from the rhizome of *C. mangga* Valeton & Zijp and data of this fungal endophyte has yet to be documented.

MATERIALS AND METHODS

Culture of endophytic fungi maintenance

Endophytic fungal isolate, *C. ramicola* IBRLCM127 used in this study was previously recovered from *C. mangga* rhizome and grown on potato dextrose agar (PDA) incorporated with powdered host materials (2 g/L) followed by incubation process under designated conditions (30 0C, 6 days) prior to storing under 4 ^{0}C for further use. The isolate was subjected to subculturing routinely once a month using fresh PDA to preserve its purity.

Culture media preparation

The host plant powder was prepared by grinding the dried plant parts of *C. mangga* into a fine powder form. A total of 5 g/L of this plant powder was incorporated into the culture media consisting of (g/L) yeast extract, 20; sucrose, 40 and magnesium sulphate, 0.5. On the other hand, host plant extract was prepared by boiling 5 g of host plant powder in 1000 mL of distilled water for 30 minutes, then filtering the mixture using the Whatman No 1 filter paper. This filtrate was used for culture media preparation.

Bacterial inoculum

Methicillin-resistant Staphylococcus aureus ATCC 33591 culture was cultured on nutrient agar (NA) and incubated at 37 oC overnight. Bacterial inoculum was prepared by transferring four to five single colonies from the culture to five mL of saline (0.85%, w/v). The turbidity was standardized to be similar with 0.5 McFarland standard.

Anti-MRSA potential profiling

The profile for anti-MRSA potential and growth of fungi before the improvement of culture conditions were performed to determine the period of cultivation time (in days) with the highest activity. For this purpose, the involved physical parameters were fixed before the improvement since the information gained in this section was used to compare the result obtained after the improvement process. The fixed physical parameters (pre-culture age, 6-days old; culture medium, supplemented with host plant extract; presence of light, no; temperature, 30 0C; inoculum size, 1 mycelial plug; agitation speed, 120 rpm) also served as a control in the current investigation. First, a six days old endophytic fungal isolate plug was transferred into 250 mL Erlenmeyer flask consisting of 100 mL of yeast extract sucrose broth (YESB) incorporated with host plant extract. The pH of culture medium was adjusted the to 6.0. (10). The flask then was incubated according to the predetermined conditions (120 rpm, 30 ^{0}C, dark condition). The anti-MRSA potential and fungal growth were measured at interval of two days for consecutively 30 days. The steps for this investigation were performed independently three times (10).

Culture conditions enhancement for anti-MRSA potential and fungal growth

Enhancement of physical parameters for culture conditions in submerged fermentation was conducted to improve the anti-MRSA potential production and growth of fungus by manipulating inoculum age. Inoculum age was prepared by inoculating mycelial fragment on PDA incorporated with powdered host substances (2 g/L), followed by incubating for 3, 6, 9, 12 and 15 days at 30 ^{0}C.

Another parameter for culture conditions improvement in the current study including supplementation of host plant powder to the culture medium, form of host plant (powdered or extracted form), presence of light, inoculum age, temperature of incubation (20, 25, 30, 35, 40 and 45o C), agitation speed (0, 50, 100, 120 and 150 rpm) and number of mycelial plug (1, 2, 3, 4 and 5). The experiments were performed thrice in separate occasion (10).

Extraction of fermentative broth

The fermented broth of fungi was extracted by adopting the technique proposed by Tong (11). Fermented broth and fungal biomass were separated out by filtration using the sterile Whatman filter paper (No. 1). The filtrate then was stored in sterile universal bottle for anti-MRSA assay determination.

Assay for anti-MRSA potential

Anti-MRSA potential of fungal fermentative broth filtrate was measured quantitatively using method proposed by Lorian (12) with some modifications. A fermentative broth filtrate (2.0 mL) was introduced into a sterile universal bottle consisting of nutrient broth (NB) (7.9 mL) followed by the addition of 24-hour old bacterial suspension (0.1 mL) to make up a total volume of 10.0 mL. Blank was prepared by

adding fermentative broth filtrate (2.0 mL) into universal bottle consisting of NB (8.0 mL) while control used in the current investigation consisting of 24-hour old bacterial suspension (0.1 mL) mixed with NB (9.9 mL) in universal bottle. The samples were incubated for 18 hours at 370 C prior to measuring OD using a wavelength of 560 nm. This step of investigation was done thrice in separate occasion. The anti-MRSA potential of extract referred to the one unit (U) of activity that reduces or inhibits the bacterial growth (13). The calculation is shown below:

$$\text{Anti-MRSA potential (Unit/mL)} = \frac{(OD_{con} - OD_{sample})}{OD_{con} \times VOL\ (ml)}$$

(Where ODcon and ODsample are the turbidity of control and sample measured spectrophotometrically at 560 nm respectively, whereas VOL is the total volume of NB used)

Fungal growth determination

Method proposed by Darah (13) was adopted to determine the fungal growth and dry weight of fungal biomass was recorded as g/L.

Statistical analysis

The significance difference of data (mean value ± standard deviation) was analysed using ANOVA and Duncan's Test with PASW Statistic 18. A confidence level of 5% (α = 0.05) was opted for data validity.

RESULTS

Time course of fungal growth and antibacterial activity before physical parameters enhancement

The profiling of time course prior to enhancement of culture conditions (YESB incorporated with powdered host plant, one mycelial agar plug, pH 6.0, agitation speed of 120 rpm and incubation temperature of 37 0C) was observed for consecutively 30 days. The result for time course profiling is shown in Fig. 6. The results revealed the anti-MRSA potential was detected starting day 4 and increase gradually until its maximal production on day 16 of incubation (37.52±0.5 U/mL). A sudden drop of anti-MRSA potential was recorded on day 18 (25.39±0.6 U/mL) and decreased steadily afterwards. On the other hand, the fungal growth showed a fluctuate pattern with the growth was detected starting day 4 and achieved maximum fungal growth on day 18 (5.65±0.2 g/L). Ironically, on day 20, there was a sharp decrease in fungal growth and the growth keep decreasing gradually afterwards until day 30. The significance of the investigation was then validated using Duncan's test, $p < 0.05$. The result revealed that day 16 significantly possessed the highest antibacterial activity and was retained for the next investigation.

Enhancement of culture conditions for optimal production of anti-MRSA potential

Effect of inoculum age

Five different inoculum age (3, 6, …… and 15 days old) for the tested fungal endophyte were used in this study and the result is depicted in Fig. 1. The six days old yielded the highest value of anti-MRSA potential (33.83±2.1 U/mL) and growth of fungi (4.17±0.09 g/L). On the other hand, the least anti-MRSA potential and fungal growth was 15 days old with the values of 1.54±0.3 U/mL and 2.11±0.2 g/L, respectively. The production of anti-MRSA potential and fungal growth for 3, 9 and 12 inoculum age were 8.79±1.0 U/mL; 2.27±0.2 g/L, 13.55±1.6 U/mL; 2.84±0.1 g/L, 3.36±0.4 U/mL; 2.11±0.2 g/L, respectively. Duncan test, $p<0.05$ was used to test the significance of the study. The statistical analysis showed that inoculum age of six days old was significantly enhanced the best anti-MRSA potential, thus it was adopted as an inoculum age for the next step of experiment.

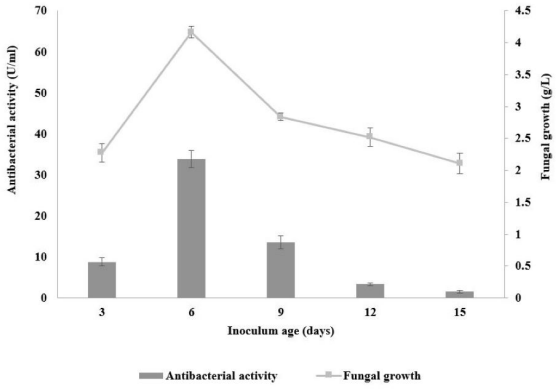

Figure 1 : Effect of inoculum age on antibacterial activity and fungal growth of C. ramicola IBRLCM127. The values of standard deviation for triplicate analyses are indicated by error bars.

Effect of host material supply and presence of light

The effects of host material supply in the culture media and presence of light during incubation period against anti-MRSA potential were investigated in the current study. The results obtained are depicted in Fig. 2. The best anti-MRSA potential of fungal endophyte *C. ramicola* IBRLCM127 was dark condition supplemented with extract form of the host plant (41.90±0.8 U/mL) whereas the least anti-MRSA potential was light condition without host plant supply (14.83±0.8 U/mL). The finding revealed that the incorporation of host extract in the media was significantly improved the anti-MRSA potential (37.11±1.1 U/mL) as compared to the powdered host materials (28.84±0.7 U/mL). Meanwhile, the addition of host materials into the media either in a powdered or extracted form (32.97±0.9 U/mL) was significantly increased the anti-MRSA potential as compared to the media without any inclusion of plant substances (19.84±1.1 U/mL). Current findings reveal that supplementation of the host materials in the culture

media can enhance the anti-MRSA potential of the fungal isolate. On the other hand, the media incubated in the dark yielded the best anti-MRSA potential as compared to the one that incubated under the light (23.90±0.9 U/mL). Ironically, the production of antibacterial potential was not growth-dependant. Duncan's test ($p<0.05$) was opted to test the data significance. The result disclosed that the supplementation of host extract in the dark possessed significantly best anti-MRSA potential, thus it was chosen as the optimized culture condition that would be applied for the next investigation.

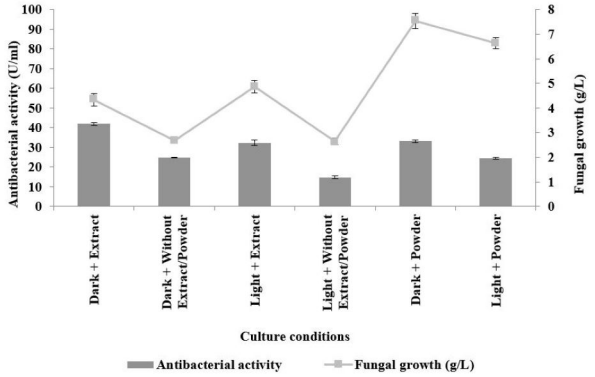

Figure 2 : Effect of host plant supply and light intensity on antibacterial activity and fungal growth of C. ramicola IBRLCM127. The values of standard deviation for triplicate analyses are indicated by error bars.

Effect of incubation temperature

The effects of various incubation temperature were investigated in the current study and the results are shown in Fig. 3. The increase of incubation temperature from 20 to 25 ^{0}C enhanced the anti-MRSA potential production of *C. ramicola* IBRLCM127 from 45.56±1.0 U/mL to 47.67±0.5 U/mL. Further rising in incubation temperature (30 ^{0}C and 35 ^{0}C) caused a sudden declining in anti-MRSA potential production. Besides, further increment of incubation temperature beyond 40 ^{0}C obstructed the antibacterial activity and fungal growth. Additionally, the incubation temperature below or above 25 ^{0}C (optimal incubation temperature) yielded lower anti-MRSA potential. Since the incubation temperature of 25 ^{0}C yielded the best anti-MRSA potential (Duncan's test, $p<0.05$), it was then selected for the next investigation.

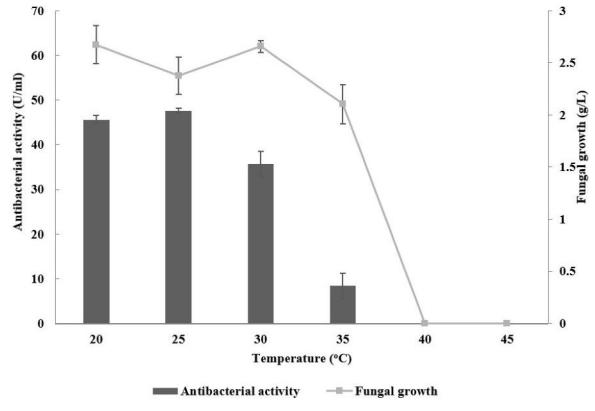

Figure 3 : Effect of temperature towards antibacterial activity and fungal growth. The values of standard deviation for triplicate analyses are indicated by error bars.

Effect of inoculum size

The result for the effects of inoculum size (number of fungal agar plugs) on the anti-MRSA potential is presented in Fig. 4. The best anti-MRSA potential was recorded by adopted two fungal agar plugs of the six days old with anti-MRSA potential and fungal growth of 47.83 ±1.2 U/mL and 2.66 ± 0.1 g/L, respectively (Duncan's test, $p<0.05$). Any further increment in the inoculum size (3, 4 and 5) led to the steady decrement of anti-MRSA potential as well as the growth of fungi. Therefore, the inoculum size of two agar plugs that gave the highest anti-MRSA potential was chosen for the subsequent experiment.

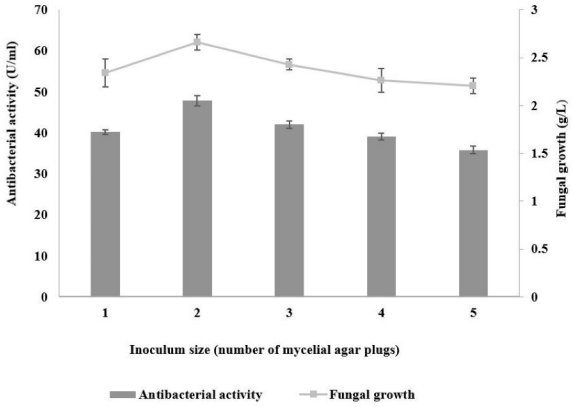

Figure 4 : Effect of fungal inoculum size towards antibacterial activity and fungal growth. The values of standard deviation for triplicate analyses are indicated by error bars.

Effect of agitation speed

The effects of agitation speed on the anti-MRSA potential and growth of fungi were also investigated in the current study as it was one of the essential conditions in submerged fermentation. There were five agitation speeds tested and the result obtained is concluded in Fig. 5. The result obtained disclosed that the increasing of agitation speed from 0 to 120 rpm also increased the ani-MRSA potential until it reached the maximal production of 41.46±1.4 U/mL and the growth of fungi of 3.126667± 0.1 g/L (Duncan's test, $p<0.05$). Any further increased of agitation speed (150 rpm) caused a sudden declining in anti-MRSA potential (11.31±0.6 U/mL). Since the agitation speed of 120 rpm gave the highest anti-MRSA potential of *C. ramicola* IBRLCM127, it was accepted as optimized agitation speed and would be used for the next step of investigation.

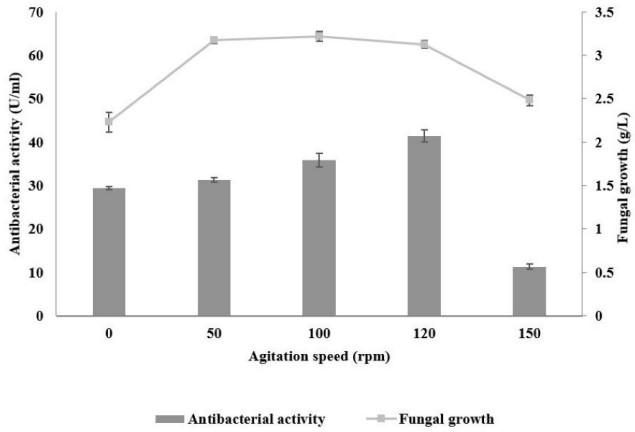

Figure 5 : Effect of agitation speed on fungal growth and antimicrobial activity. The values of standard deviation for triplicate analyses are indicated by error bars.

Time course of fungal growth and antibacterial activity after physical parameter improvement

The enhanced physical parameters from the previous investigations (6 days old of inoculum age, culture media incorporated with host extract, dark environment, temperature of 20 ^{0}C, size of inoculum of two mycelial agar plugs and agitation speed of 120 rpm were incorporated in this single time-course study and samples were taken every two days for 30 days consecutively for anti-MRSA potential and fungal growth determination. The results obtained in this study is shown in Fig. 6. Overall, the anti-MRSA potential and fungal growth increased steadily with incubation period. However, there was no anti-MRSA potential detected on day two of the incubation period possibly due to the no production of antibacterial metabolite since the fungi was still at early growth stage with abundance of nutrient elements available in the culture medium. The anti-MRSA potential was detected starting at day four and increased as the incubation period increased until it achieved maximal anti-MRSA potential at day 12 with the value of 42.50±1.1 U/mL and 4.95±0.1 g/L of the growth of fungi.

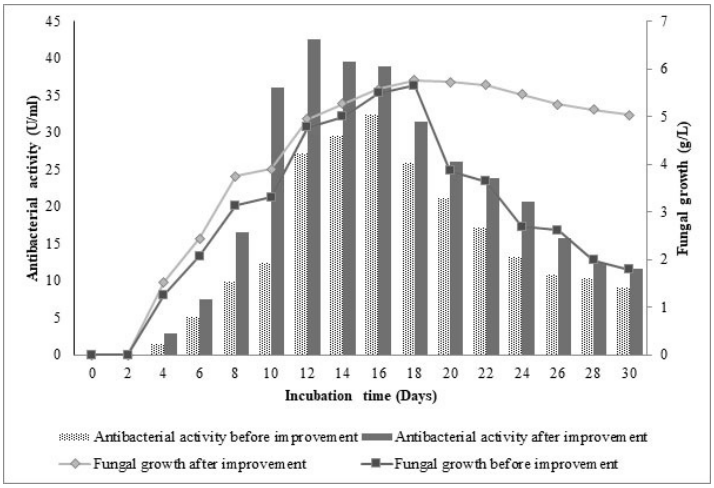

Figure 6 : Time course of fungal growth and antibacterial activity before and after physical parameter enhancement. The values of standard deviation for triplicate analyses are indicated by error error bars.

After day 12, the anti-MRSA potential started to decrease gradually until it reached the lowest activity at day 30 (11.66±0.4 U/mL) whilst the fungal growth kept increasing until day 18 and decreased gradually afterwards. The findings from this investigation disclosed that the enhancement of physical parameters of culture conditions imposed a significant effect on the anti-MRSA potential production by the endophytic fungus, *C. ramicola* IBRLCM127.

A brief comparison of culture condition before and after enhancement of physical parameters was summarized in Table I. The findings from this investigation showed that the anti-MRSA potential of *C. ramicola* IBRLCM127 increased to 11.72% under improved conditions, contradicting with the fungal growth as the growth was reduced to 9.84%. The reduction in fungal growth occurred because endophytic fungus isolate *C. ramicola* IBRLCM127 was growth independent. Throughout this investigation, it was observed that the temperature and inoculum

Table I : The summary of antibacterial activity and fungal growth of *C. ramicola* IBRLCM127 before and after enhancing the physical parameters of culture conditions via submerged fermentation system.

Parameters	Profiling before improvement	Profiling after improvement
Inoculum age (days)	6	6
Host plant supply	Host plant extract	Host plant extract
Light intensity	Dark condition	Dark condition
Temperature (^{0}C)	30	25
Inoculum size (no. of mycelial agar plugs)	1	2
Agitation speed (rpm)	120	120
Optimum anti-MRSA potential	Day 16	Day 12
Anti-MRSA potential (U/ml)	37.52	42.50
Increment of anti-MRSA potential (%)	-	11.72
Fungal growth (g/L)	5.49	4.95
Decrement of fungal growth (%)	-	9.84

size played a significant role in improving the synthesizing of anti-MRSA compound. Interestingly, the enhancement culture medium parameters could not only enhance the anti-MRSA potential but also shorten the incubation time from 16 to 12 days only. Incubation time also serves as one of the essential parameters in fermentation process to obtain the desired fungal metabolites.

DISCUSSION

Fungal secondary metabolite is low molecular mass product that is not essential for culture growth, but very beneficial for human wellness such as for the production of antibiotics, anticancer agent, cholesterol-lowering drugs and others. Fermentative production of fungal metabolites is cost effective, continuous process, environmentally friendly and high yield in a short period. The secretion of antimicrobial compounds by fungal endophytes is greatly influenced by nutrients and cultural conditions (14). Therefore, the nutrients, cultural conditions and interaction between fungi-host plants play a vital role in the onset and synthesizing of these compounds by endophytic fungi (15). A proper cultivation medium and efficient fermentation system can secure the high yield of fungal metabolites (16). These metabolites can be synthesized at a large scale by manipulating the physical parameters of cultural condition such as temperature, pH of culture media and incubation time as the biosynthesis of fungal compounds is influenced by the environment, nutrient availability as well as the stage of fungal development (17).

The pre-culture seed age used in fungal cultivation is essential to optimum fungal growth and antimicrobial metabolite production. The present finding revealed that young seeds yield the highest growth of fungus and antibacterial activity compared to old seeds. This occurrence was likely due to the young culture being ready to enter the active growth phase with abundant nutrients still available in the media. Meanwhile, the old stage of seed culture was already entering the inactive phase, and it was challenging for them to reproduce and form new mycelia in a scarcity of nutrients in a medium. A similar pattern of finding was also reported by a previous study on the production of dextranase enzyme by new fungal endophyte recovered from Red Sea Sponge that disclosed the dextranase secretion increased with the increasing of inoculum age and a further increase in inoculum age caused the yield to decrease, of which led them o conclude that the microbial death phase might cause this occurrence (18).

Endophytes refer to endophytic microorganisms that reside either the whole or part of their lifecycle inhabiting higher plant tissues asymptomatically and endophytic fungi are the most frequently isolated (19). During the long time of co-evolution between fungal endophyte and their host, these fungi have been gradually adapted themselves to the unique microenvironment in the host plant via genetic variation (20). In symbiotic relationship between fungi and their host, the host provides protection, nutrition and propagation opportunities to endophytes (21,22). In return, endophytic fungi benefitting the host by producing beneficial metabolites to increase the growth, development and biomass (23,24). Endophytes also increase plant resistance towards pathogens, insects, herbivore and other abiotic stress (25,26). The current finding disclosed that the amendment of the host materials, especially in the form of extract into the culture media was significantly enhancing the antibacterial potential of the isolate, coinciding with the previous study (27) that showed the antibacterial potential of endophytic fungi *Fusarium sp.* DF2 was improved by adding host plant extract into the culture media. Additionally, the production of the fungal metabolite is alleviated when grown in conditions that resemble the internal host plant condition likes the addition of extract of crude plant metabolite or plant defensive compounds into the culture media (28).

Light intensity is another physical parameter of cultural conditions significantly affecting fungal growth and antimicrobial potential. Previous study reported the dark condition is favored by endophytic fungal isolates compared to the light condition since the shared feature of all endophytic fungi is the preference for the dark condition as they live in internal host tissues (29). Additionally, light also serves as one of the pivotal factors in the production of secondary metabolites by endophytic fungi as the fungal master regulator called LaeA/VeA is primarily controlled by light (30,31). The inhibitory effects of light on fungal metabolite biosynthesis are mainly due to the absence of LaeA nuclear protein that serves as a main regulator for metabolite synthesis, thus leading to the inactivation of bioactive metabolite production (32).

The temperature used for fungal cultivation is a determining factor affecting fungal growth and antimicrobial metabolite synthesis. A previous study reported that incubation temperature plays an essential role in the overall growth of any organisms, and at the same time, it can influence the organisms' physiology that will subsequently affect the synthesis of plentiful bioactive metabolites (33). Temperature also has an immense effect on the growth curve of any microbe, which comprises a series of phases, viz., lag, log, stationary and decline, where retaliation occurs during the fermentation kinetics (34). The current study revealed 25 ^{0}C yielded the highest antibacterial activity of *C. ramicola* IBRLCM127, in agreement with previous study (27) which reported endophytic fungus *Fusarium sp.* DF2 required a temperature of 25 ^{0}C for optimum growth and antimicrobial metabolite

production. However, a further increase in incubation temperature exceeding 40 ^{0}C could kill the endophytic fungi as they are very sensitive to elevated temperatures. Therefore, the current study showed a temperature exceeding 40 ^{0}C failed to support any growth of endophytic fungus as well as the production of secondary metabolites. A previous study emphasized that growth and secondary metabolite production by fungus ceased at temperatures 40 ^{0}C to 50 ^{0}C as the high temperature killed the fungal cells (35).

Fungal inoculum size also was believed to significantly affect its growth and metabolite production. The inoculum size can be determined using two methods, either spore count or agar plug. The agar plug method was adopted for endophytic fungi that did not produce spores. In the current study, two fungal agar plugs were found to be the optimal inoculum size for the highest antibacterial potential production of *C. ramicola* IBRLCM127, similar to previous study (11) that reported the usage of two fungal agar plugs in the cultivation of endophytic fungi isolated from Orthosiphon stamineus can significantly enhance their antibacterial potential. The more the inoculum size used, the higher the fungal growth but not necessarily more production of fungal metabolites because during the stationary phase, the fungal growth was declining, but the production of metabolite was enhanced (36). Besides, the larger size of mycelial plug caused the low yield and poor quality of the secondary metabolite obtained from the fermentation broth (37). Any increase in optimal inoculum size did not stimulate biomass growth and productivity but led to a significant reduction in antibiotic activity (38). There is a specific limitation for the minimum inoculum size, where any inoculum size below this range will fail to grow, while an optimal stage of inoculum size can successfully promote fungal growth and its secondary metabolite production (39).

Optimal agitation rate is an important factor for the maximum potential of culture in submerged fermentation as it can increase the dissolved oxygen content and dispersion of macromolecules in a culture medium at a correct level (40). Dissolved oxygen is a critical oxygen source for microorganisms in submerged fermentation as they grow immersed in the culture medium without direct contact with the oxygen gas phase (41). However, the content of organic and inorganic elements in the culture medium leads to the reduction of dissolved oxygen in the flask due to the oxidation process. Thus, proper aeration is necessary to meet oxygen demand during fermentation (41). The current finding disclosed that an agitation rate of 120 rpm produced the highest antibacterial activity and further increase from this point caused the activity to decrease. This finding coincided with a previous study that also reported the highest bioactivity of Pseudoalteromonas rubra BF1A IBRL at an agitation speed of 120 rpm (42). However, too high agitation speed could damage culture microorganisms, resulting in cell concentration and decreasing productivity (43). In contrast, low agitation speed did not yield a good product due to the over-aggregation of cells that led to the large pallet formation.

Determining optimal growth and production conditions was necessary to outline the efficient strategies for the production compound of interest with bioactivity by endophytic fungi at the commercial level. Endophytic fungi serve as a potential natural product that possess innately large-scale structural diversity as compared to synthetic compound. They could be used as lead structures to search for new antimicrobial agents representing vast chemical diversities (44). Hence, the research in natural products is very promising if the correct exploitation of novel source organisms coupled with advanced bioassays and efficient structure elucidation has been employed. This will further contribute to producing many novel antimicrobial agents for pharmaceutical use. The exciting fact about endophytic bioactive metabolites is the reduction of cell toxicity to human cells, as these metabolites do not harm or kill the eukaryotic host plant system (45). This finding proved that the symbiotic nature of the fungi-host plant relationship yielded bioactive metabolites from endophytic fungi with reduced cell toxicity, and it is imperative to the medical world as potential drug candidates that do not adversely affect human cells can be developed to cure many diseases (46). Thus, these studies disclosed the potential of endophytic fungi as a versatile arsenal of antimicrobial compounds.

CONCLUSION

The present findings suggested that the fermentative broth of endophytic fungi isolate, *C. ramicola* IBRLCM127 obtained from submerged fermentation demonstrated a prominent anti-MRSA potential. The optimal culture conditions significantly enhanced the production of anti-MRSA potential of the isolate by 11.72%. This was the first study to evaluate the anti-MRSA potential of the endophytic fungus, *C. ramicola* IBRLCM127, isolated from a local medicinal plant, *C. mangga*, by manipulating the physical parameters of the culture condition. This study also compensates for the absence of research on the endophytic fungus *C. ramicola* and provides new insight for antibiotic development from this untapped source.

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