Antimicrobial activity of Streptomyces griseoviridis K10 against ESBL Escherichia coli, MRSA, and other paathogenic microorganisms

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Antimicrobial activity of *Streptomyces griseoviridis* K10 against ESBL *Escherichia coli,* MRSA, and other pathogenic microorganisms

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Abstract. The emergence of resistant pathogenic bacteria is serious threat to global public health.This problem can be address through discovering new antibiotics from nature. *Streptomyces* are known as the source of more than fifty percent commercially available antibiotics, but it is predicted that only less than 5% were identified. *Streptomyces griseoviridis* K10 were fermented in ISP-4 medium pH 7.2 for four days in 32 °C incubated shaker with 150 rpm agitation. The antimicrobial activity of *Streptomyces griseoviridis* K10 were analyzed using diffusion method. The antibiotic production curves were made to determine the optimal antibiotic production time. The result showed that these bacteria had activity against extended-spectrum beta-lactamases (ESBL) *Escherichia coli*, Methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *S. aureus* ATCC 25923, and *Candida albicans*, with inhibitory zone of 17.25 ± 0.43 mm; 13.95 ± 0.60 mm; 20.96 ± 0.41 mm; 20.64 ± 0.92 mm; 21.73 ± 0.53 mm; and 16.90 ± 1.27 mm, respectively. The antibiotic production was optimum in the second and third days.

1. Introduction

Antibiotics resistance is a serious threat to global public health [1,2].Although there is considerable progress in the fields of chemical synthesis and engineered biosynthesis of antimicrobial compounds, most antibiotics are too complex to synthesize [3]. One of the strategies to address such crisis is by discovering and developing new antibiotics from nature.

Microorganisms have been an enormous source of biodiversity and chemical diversity. They have capability to produce highly complex molecules from common nutrients in the fermentation process. They become the main source drugs and often used in the industrial-scale production of drugs[4]. Terrestrial derived microorganisms have been the most important resource for discovery of new drugs, especially actinomycetes. Streptomycetes, one of actinomycetes member, is well known producer of vast majority antibiotics, but only 1-3% have been discovered [5]. Thus, Streptomyces is still a potential source of natural product with anti-infective activity.

Staphylococcus aureus and *Escherichia coli* are two of the most common pathogenic bacteria that caused infection [6, 18, 19]. These bacteria have been identified to gain resistance against various antibiotics and have spread around the world, even in the countries that known to have low prevalency such as Norway, Denmark, and Finland [7,8]. Thus, there are urgency to find new antibiotics which had activity against these resistance bacteria.

Streptomyces griseoviridis K10 which had been isolated from kale plantation area in Sidoarjo were analyzed for its antimicrobial activity against several microorganisms such as extended-spectrum beta-lactamases (ESBL) *Escherichia coli*, Methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *S. aureus* ATCC 25923, and *Candida albicans*. These study also evaluated the growth curve of the bacteria.

2. Materials and Methods

2.1. Materials

Bacteria that had been used in these study were *Streptomyces griseoviridis* K10, ESBL *E.coli*, MRSA, *Pseudomonas aeruginosa* ATCC 27853, *E. coli* ATCC 25922, *S. aureus* ATCC 25923, and *Candida albicans*.

Materials that had been used in these study were International Streptomyces Project 4(ISP-4) media(Himedia), Nutrient agar (NA) media (Himedia), Sabouraud Dextrose Agar (SDA) (Himedia) and agar bacteriological (Himedia). All other materials were analytical grade.

2.2. Characterization of S. griseoviridis K10

The isolate were cultivated on ISP-4 medium and characterized by following the direction in *Bergey's Manual of Systematic Bacteriology* [9].The bacteria characteristics such as the color of aerial mycelium, substrate mycelium and pigmentation of the isolate were observed [10].The ability of the isolate in the utilization of various carbon sources were evaluated [11]. The isolate also observed for its abilityto grow in different pH, salinity, and temperatures.

2.3. S. griseoviridis K10 *fermentation*

Single isolate of *S. griseoviridis* K10 were put into 25 mL ISP-4 broth medium and incubated in thermoshaker (Gerhardt) with controlled condition, 32±2°C and agitation of 150 rpm, for two days. 10^{-6}-10^{-7}cfu bacteria from the pre-culture inoculated into 150 mL ISP-4 broth medium and growth in the same fermentation condition [12]. Each day, every 24 hours, the culture were sampled and analyzed for its antimicrobial activity.

2.4. Determination of S. griseoviridis K10 *growth curve*

The growth curve of S. *griseoviridis* K10 were determined using dry weight cell method based on Moreira [13]. Briefly, 5 mL of cultured were sampled every 24 hours, centrifuged in 10.000 rpm, and the pellet were dried in oven in controlled condition at 105 °C until the weight of the cell constant. The determination of dry weight cell were done in five replication.

2.5. Antimicrobial screening

The pathogenic microorganism were growth in NA media for bacteria and SDA for fungi. Bacteria, such as ESBL *E.coli*, MRSA, *Pseudomonas aeruginosa* ATCC 27853, *E. coli* ATCC 25922, and *S. aureus* ATCC 25923 were grown in NA slant media and incubated in thermally controlled incubator (Memmert) in 32±2 °Cfor 18 hours. The pathogenic fungi, *Candida albicans,* were grown in SDA media under the same condition. The pathogenic microorganisms then suspensed in 10 mL NaCl 0.9% sterile solution. The transmittance of the suspension were measured using spectrophotometer (UV-Vis Spectrophotometer, Agilent 8453) in 580 nm and diluted until the transmittance reached 25% [14].

12 mL of NA media were used as base layer and 8 mL NA media were inoculated with 8 μL pathogenic microorganism suspension for antimicrobial activity test.

The antimicrobial assay conducted using well diffusion method. 100 µL of 3 days old *S. griseoviridis* K10 culture supernatant was put into the well of test media containing pathogenic microorganism that had been made using cork borer. The media then incubated in thermally controlled incubator (Memmert) in 32±2 °C for 18 hours. The antimicrobial assay done in five replicate.

2.6. Antimicrobial production curve

The antimicrobial production curve were determined using supernatant of *S. griseoviridis* K10 culture, which were grown in ISP-4 broth medium according to section 2.3 and sampled every 24 hours for four days. The antimicrobial assay were done using ESBL *E.coli* and MRSA as test organisms and the method was described previously in section 2.5.

3. Result and Discussion

The isolate, *S. griseoviridis* K10 (Figure 1), had yellowish pink aerial miselium, light orange yellow substrate miselium, and had melanoid pigment. It can utilize various carbon sources such as amylum, arabinose, dextrose, fructose, galactose, glucose, lactose, lactulose, sarose, and sucrose. The isolate also had ability to grow in temperature between 25 °C to 37 °C, pH range 5 to 9, and 2% salinity of the media. If the salinity was 5% or higher, the isolate were unable to grow.

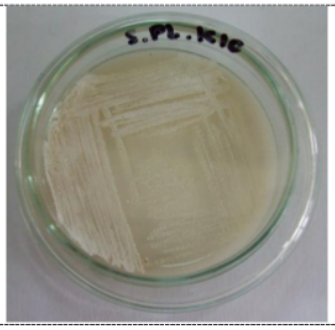

Figure 1. *S. griseoviridis* K10

These study showed that *S. griseoviridis* K10 had broad antimicrobials activities against ESBL *E. coli*, MRSA, *Pseudomonas aeruginosa* ATCC 27853, *E. coli* ATCC 25922, *S. aureus* ATCC 25923, and *Candida albicans* (Table 1).

Table 1. Antimicrobial activities of *S. griseoviridis* K10 against pathogenic microorganisms

Test microorganism	Inhibition zone (mm)
ESBL *Escherichia coli*	17.25 ± 0.43
MRSA	13.95 ± 0.60
Pseudomonas aeruginosa ATCC 27853	20.96 ± 0.41
Escherichia coli ATCC 25922	20.64 ± 0.92
S. aureus ATCC 25923	21.73 ± 0.53
Candida albicans	16.90 ± 1.27

These finding correlate with the previous study that reported that *S. griseoviridis* had activity against gram positive bacteria, such as *S. aureus, Micrococcus luteus*, *Bacillus pumilus*, and *Bacillus subtilis,* and gram negative bacteria, such as *E. coli* and *P. aeruginosa* [15].It was also reported that these species produce griseoviridin and viridogrisein that exert bactericidal activity which also effective against many multi-drug resistant microorganisms [16], such as vancomycin-resistant *Enterococcus faecium* [17]. Thus, it proved the potency of these isolate to produce antimicrobial substances with board spectrum activity.

The growth curve and antimicrobial production curve of *S. griseoviridis* K10 were showed in Figure 2. The *S. griseoviridis* K10 had the optimum activity against MRSA, which reach 14.34 ± 0.25 mm, in the second day of fermentation when the growth in the late log phase and ESBL *E. coli*, which reach 17.25 ± 0.43 mm, in the third days of fermentation when the growth in about the stationary

phase. The determination of the bacterial growth phase need to study futher with narrower sampling time, to predict the growth phase more accurately.

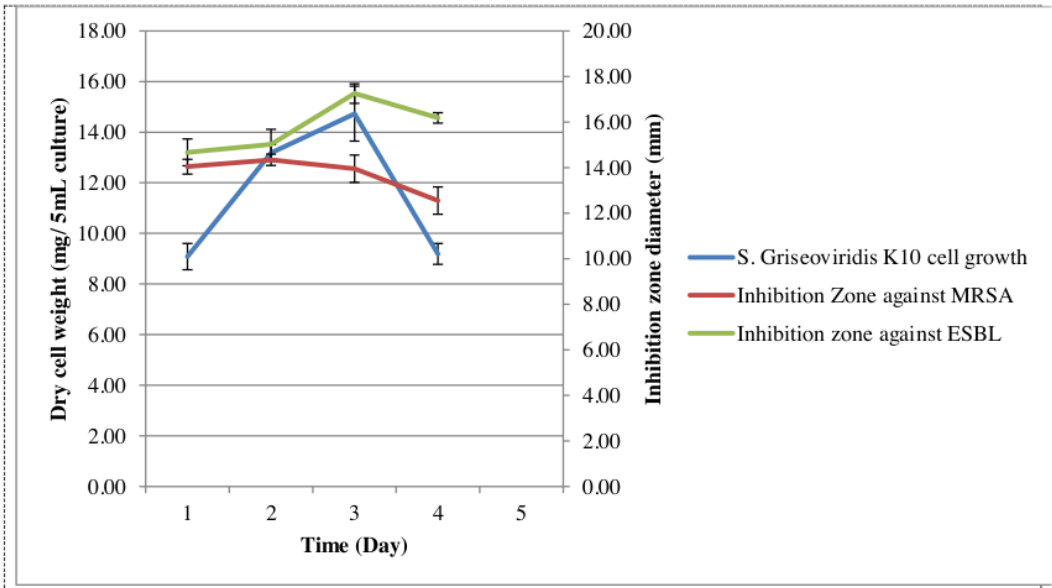

Figure 2. The growth curve and antimicrobial production curve of *Streptomyces griseoviridis* K10

The graph showed that *S. griseoviridis* had two different optimum antibiotic production day, for two different resistance bacteria, it is probably because it can produce several different chemical compound with antimicrobial activity.

4. Conclusion

S. griseoviridis K10 had broad range antimicrobial activity against both pathogenic fungi and bacteria. It also had activity against resistance bacteria such as MRSA and ESBL *E. coli*. Thus, they are potential to be studied further.

5. Acknowledgement

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