INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

IMPROVING ANTIBIOTIC ACTIVITIES FROM ENDOPHYTIC ISOLATE BES-5 GRAM POSITIVE BACTERIA

Anthoni Agustien [1]*, Nasril Nasir [1], Feskaharny Alamsjah [1], Imelda [2] and Akmal Djamaan [3]
[1]Deparment of Biology, University of Andalas, Padang, Indonesia
[2]Deparment of Chemistry, University of Andalas, Padang, Indonesia
[3]Faculty of Pharmacy, University of Andalas, Padang, Indonesia
*Corresponding Author Email: anthoni_agustien@sci.unand.ac.id

Article Received on: 04/12/18 Approved for publication: 02/02/19

DOI: 10.7897/2230-8407.1004119

ABSTRACT

Antibiotics are metabolites of microorganisms that can kill or inhibit the growth of other microorganisms. This study aims to increase the inhibition of antibiotics produced from endophytic BES-5 positive Gram bacteria. The increasing of antibiotic activity is carried out by optimizing the production of antibiotics from bacteria. Antibiotic activity was determined by the Kirby method with *E. coli* as test bacteria. The results showed to obtain antibiotics with very strong category characteristics, with optimum conditions for incubation temperature of 33^0 C; pH of production media 7.5; agitation of 150 rpm; 5% inoculum; 3% soaking corn water as inducer, 5% inoculum, 0.5% glucose as carbon source. The optimum condition besides increasing the inhibition also accelerates the production of antibiotics.

Keywords: improving, endophytic, antibiotics, BES-5 isolates

INTRODUCTION

Endophytes are microorganisms including bacteria, fungi and actinomycetes that live on intra and inter cells in plant tissues for their entire life or part of their life cycle without endangering their hosts[1]. Each high-level plant can contain several endophytic microbes that are capable of producing secondary metabolites that are thought to be the result of coevolution or transfer of genetics from host plants into endophytic microbes[2]. The ability of endophytic microbes to produce secondary metabolites according to their host plants is a very large and reliable opportunity to produce secondary metabolites from endophytic microbes isolated from their host plants[3].

endophytic bacteria BES-5 was isolated from *Piper betle* in the HPPB forest area have the ability to produce secondary metabolites in the form of antibiotics that are narrow spectrum and include moderate strong category antibiotics[4]. Increasing the ability of bacteria to produce antibiotics with higher inhibition of microorganisms can be done by optimizing the fermentation process so that optimum conditions for antibiotic production include bacterial growth curve profile and antibiotic activity, type and concentration from inducers to antibiotic activities, effects of temperature, pH and agitation on the production media on the antibiotic activity of the concentration effect of the inoculum on antibiotic activity, the effect of type and concentration of carbon and nitrogen sources and trace element to antibiotic activity[3]. This study aims to increase antibiotic activity of BES-5 endophytic bacteria.

MATERIALS AND METHODS

Preparation and cultivation of isolate BES-5 endophytic bacterial

The source of antibiotics in this study were the three endophytic isolates of BES-5 which were antibiotic producers. Bacterial cultivation is carried out as has been done routinely in the microbiology laboratory. A single colony of bacteria was inoculated in a streak plate on a Petri dish containing medium NA pH 7.0. Then incubated at room temperature for 24 hours. Single colonies were inoculated in a test tube containing NA media as a sloping culture which was used as a stock for optimization work.

Determination of antibiotic activity

Testing of antibiotic activity from each endophytic bacterial isolate was carried out by paper disc method. Disc paper is made by attaching 3 layers of Whatman no. 42, then punched with paper holes so that the disc is obtained 6 mm in diameter, sterilized by autoclaving. Fifteen ml NA medium was poured on a Petri dish. Then each medium in the petri dish was applied to *E. coli* paper discs were immersed in a crude antibiotic solution from each isolate, then aseptically the paper discs were transferred to a sterile and awaited container to dry. Aseptic disc paper is placed on NA medium. Then incubated at room temperature for 48 hours. The diameter of the resistance formed around the paper disc is measured with the help of the caliper. Microbes are indicative of producing antibiotics if inhibitory zones are formed around the disc paper. Endophytic microbes are recorded which can produce antibiotics along with the diameter of the inhibition zone.

The effect of inducer on the production medium on antibiotic inhibitory

Determination of the effect of type and inducer concentration on the production medium on antibiotic activity was carried out by making variations in the inducer concentration starting at 1.0;2.0; 3.0; 4.0 and 5.0% in the marinade and corn extract as control is without inducer.

Effect of incubation temperature on antibiotic inhibitory

Determination of the effect of incubation temperature on the production medium to obtain antibiotics with large inhibitory power is carried out by making temperature variations: 31; 33; 35; 37 and 39^0 C.

Effect of pH of the production medium on antibiotic inhibitory

Determination of the influence of the pH of the production medium to obtain antibiotics with a large inhibitory power is carried out by varying the pH of the production medium: 5.0; 5.5; 6.0; 6.5; 7.0; 7.5 and 8.0.

Effect of agitation on antibiotic inhibitory

Determination of the effect of agitation on the production medium to obtain antibiotics with large inhibitory power is carried out by making variations in agitation: 100, 125, 150,175 and 200 rpm.

Effect of inoculum on antibiotic inhibitory

Determination of the effect of the concentration of inoculum to obtain antibiotics with a large inhibition was carried out by making a variation inoculum dose: 1.0; 2.5; 5.0; 7.5 and 10%.

Effect of carbon sources on the production medium

Determination of the effect of carbon sources on the production medium to obtain antibiotics with large inhibitory power is carried out by adding different carbon sources such as starch, glucose, maltose, lactose and sucrose with varying concentrations of each: 0,5; 1.0; 2.0; 3.0; and 4.0%.

RESULTS AND DISCUSSION

Inducer effect on antibiotic activity

Endophytic BES-5 bacterial isolate, in producing antibiotics requires an inducer, where as an inducer for antibiotic biosynthesis in bacterial cells can be used either corn water or corn extract. In contrast shows that if the production medium does not contain both types of inducers, bacteria cannot synthesize secondary metabolites in the form of antibiotics, this is indicated by the absence of antibiotic activity against *E. coli* and *S. aureus* test bacteria. Three percent of corn soaking water is the best for inducing biosynthesis of antibiotics in bacterial cells (Fig.1).

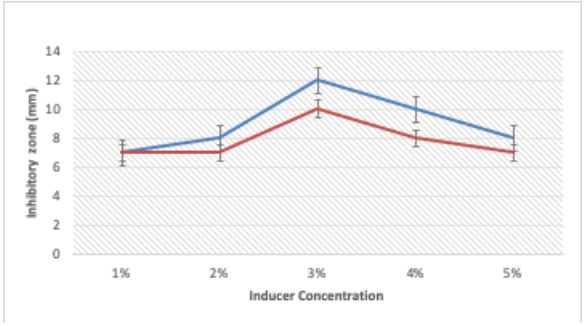

Fig. 1. Inducer effect on antibiotic activity; soaking corn water, corn extract

Inducers with levels higher than 3%, cause a decrease in antibiotic activity, this is probably due to the higher moisture content of corn in the production media, will increase the level of carbon content contained in corn, so it will result in repression of metabolites that cause low antibiotic biosynthesis. The antibiotic production medium of bacterial isolates can be used as a producer of antibiotics by making slight modifications to the composition by adding corn soaking water, so that corn soaking water can be used as an inducer for antibacterial biosynthesis[5].

Incubation temperature effect on antibiotic activity

Endophytic betel-leaf bacterial BES-5 bacterial cells have the ability to produce antibiotics with a temperature range of 31 to 37^0 C, while at 39^0 C, although bacterial isolates can survive but do not have the ability to produce antibiotics. The optimum temperature condition of endophytic BES-5 bacteria isolates in producing antibiotics is at 33^0 C (Fig 2).

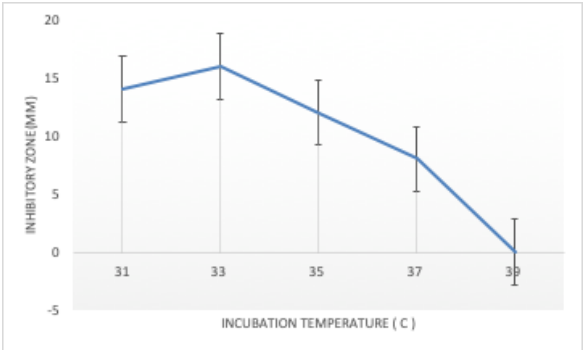

Fig.2. Profile of incubation temperature effect on antibiotic activity

This means that at 33^0 C, it is the ideal temperature for maximum antibiotic biosynthesis of bacterial isolates. Temperature is one of the most important factors in the life of microorganisms, because it greatly affects the catalytic enzyme in metabolic processes in cells. Very significant temperature fluctuations can have fatal effects on cell metabolic processes, thus indirectly affecting antibiotic biosynthesis in microbial cells. At relatively higher temperatures will increase metabolic activity that occurs in the stationary phase, and lower temperatures to metabolic slow down[6].

pH of production media effect on antibiotic activity

In the life of microbial the factor that greatly influences the growth is the pH of the environment in which the microbes are present. pH media for the growth and production of a primary or secondary metabolite, plays an important role, this is due to the process of anabolism and catabolism that occurs in cells of

microorganisms is very dependent on enzymes that play a role in enzymatic processes, because enzymes are arranged by amino acids, then the active center of the enzyme which is an amino acid is strongly influenced by pH. A very significant change in pH can cause conformational changes in enzyme molecules, which will have an impact on the catalytic power of enzymes in enzymatic processes. Endophytic BES-5 bacterial isolates produce antibiotics in a wide range between pH 5.0 to pH 8.0, where the media of antibiotic production with pH 7.5 is the optimum pH for BES-5 bacterial isolates to produce antibiotics with maximum activity (Fig 3.).

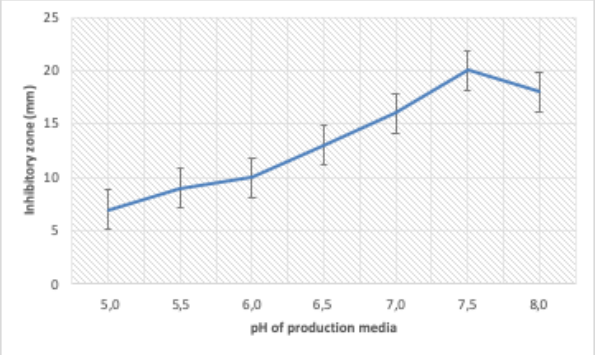

Fig.3. Profile of pH production media effect on antibiotic activity

Determination of optimal initial pH will affect cell growth and antibiotic activity (pH 7.0). Cultivation at a constant pH, highest value of activity at pH 7.5. PH values at higher levels will inhibit biomass formation and antibiotic activity. Although lower pH values such as pH 6.5 cell level are limited, biomass and emotion activity are relatively higher at the initial stage when compared to higher pH values[7].

Agitation effect on antibiotic activity

The need for oxygen is one of the factors that influence the life of microorganisms. Obligate aerobic bacteria are very dependent on the availability of oxygen in their environment, will be different from bacteria that do not need oxygen at all, but there are also bacteria that can survive at low oxygen levels and most bacteria can live in their existing conditions and not presence of oxygen (facultative anaerobes). Agitation in the fermentation process which is one of the factors that influence the survival of microorganisms, where the speed of agitation influences the occurrence of air diffusion into the media of production and also the homogeneity of a production medium. Agitation in the range of 100 to 200 rpm can be used for the production of antibiotics from endophytic BES-5 bacterial isolates, where at 150 rpm agitation the bacterial agitation conditions produce antibiotics maximally (Fig. 4).

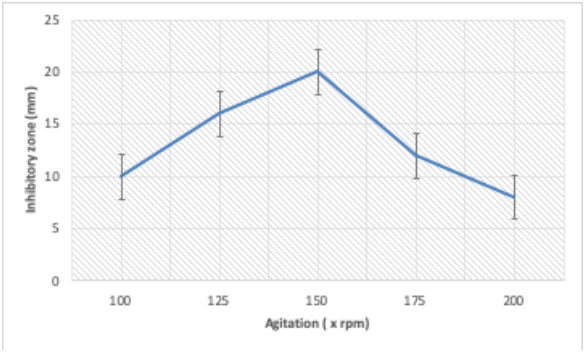

Fig. 4. Profile of agitation effect on antibiotic activity

There was a decrease in antibiotic activity if agitation was used at 175 and 200 rpm. This is because the shaking speed at 175 and 200 rpm will cause foam on the surface of the media so that it can cause bacterial cells to attach to the foam. Whereas if under the optimum conditions of agitation (100 and 125 rpm) it will cause slow shaking so that it will reduce the homogeneity of the production media and also cause a lack of diffusion of oxygen into the production medium Knowing the oxygen mass transfer coefficient (KLa) will be known to users of agitation and aeration. An increase in KLa causes biomass and antibiotic activity to increase. The activity unit and dry cell weight became 232 U ml $^{-1}$ and 19.58 g $^{-1}$, respectively, productivity in cells and antibiotics rose by more than 30% the increase in KL increased from 115.9 hours $^{-1}$ to 185.7 /jam[8].

Inoculum effect on antibiotic activity

The inoculum is a biotic factor that plays a very important role, this is because a microorganism to produce its metabolites is determined by the size of the microorganism, meaning that the microorganism can only produce its metabolites if the number of cells has reached its optimum condition. The percentage of bacterial cells contained in the production medium determines antibiotic biosynthesis. If the percentage is too low, the adaptation phase will be long, if the percentage is too high it will cause competition for nutrition. Inoculum of BES-5 bacterial isolates with a percentage of 1.0 to 10% can produce antibiotics, where the conditions are optimum with a percentage of 5% inoculum (Fig.5).

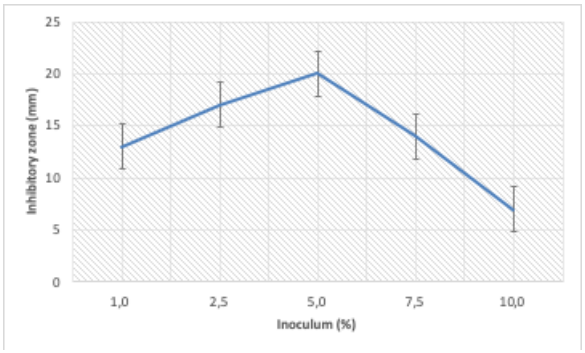

Fig. 5. Profile of inoculum effect on antibiotic activity

Carbon sources effect on antibiotic activity

Carbon sources, which are part of the composition of antibiotic production, play a very important role in microorganisms in carbohydrate metabolism, where carbohydrates are generally used as a source of primary energy income, where the energy formed will support the occurrence of metabolite biosynthesis in microorganism cells. Endophytic BES-5 bacterial isolate can use carbon sources from monosaccharides, disaccharides and polysaccharides (Fig. 6).

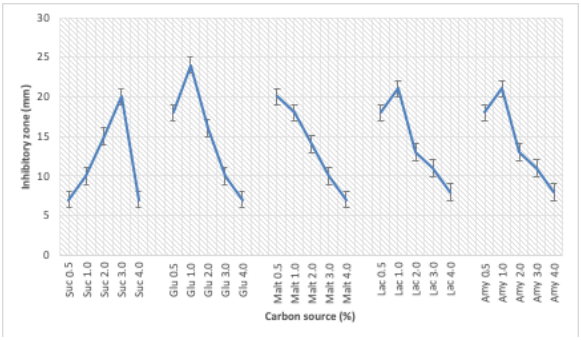

Fig. 6. Profile of carbon source effect on antibiotic activity

This means that these bacteria can produce various extracellular enzymes to hydrolyze disaccharide carbohydrates such as sucrose, maltose and lactose or polysaccharides such as starch. 1% glucose is the best source of carbon for BES-5 bacterial isolates to produce antibiotics with the maximum power. Glucose above 1%, it appears that antibiotic activity decreases, this may be due to the repression of metabolites, which will affect antibiotic biosynthesis. The phenomenon of the use of carbon sources is regulated by the repression of carbon catabolites in Gram positive bacteria, this regulatory mechanism was studied from *Bacillus subtilis.* Carbon Catabolite Repression (CCR) is caused by a complex of two proteins that act at the cis-acting locus, upstream of the catabolite gene[9]. Carbon source regulation is one of the conservative mechanisms to protect against protein biosynthesis, cell synthesis, and operate when more than one substrate that can be used is present in the environment[10].

CONCLUSION

The optimum condition in producing antibiotics with maximum activity from BES-5 bacterial isolates with maximum activity is by using a 3% soaking corn water as inducer; incubation temperature 33^0 C; media with a pH of 7.5; agitation of 150 rpm, inoculum 5%, glucose 1% as a source of carbohydrate.

ACKNOWLEDGEMENT

The author would like to say special thanks to The Rector of Andalas University for his support this research under Acceleration of Professor Research Grant Project, Andalas University 2018, with Contract Number: 028/UN.16.17/PP.PGB/LPPM/2018.

REFERENCES

1. Arnold, A.E. Understanding the diversity of foliar endophytic fungi: progress, challenges and frontiers. Fungal Biology Review. 2007; 21, 51-66.
2. Tan, R.X and W.X. Zou. Endophytes: a rich sourceoffunctionalmetabolites.*Nat.Prod.Rep.* 2001; 18, 448-459.
3. Strobel, G.A. and B.Daisy.Bioprospecting forMicrobial Endophytes and Their Natural Products.Microbiol. and Mol. BiologyRev. 2003;67(4), 491-502.
4. Agustien, A., P. Santoso, N.P., Sari, F. Annisa, N. Nasir, Y. Rilda, and A. Djamaan. Screening of Endophyte *Piper betle* Bacteria from the Forests of HPPB University Andalas as Antibiotics Producer. International Journal of Current Microbiology and Applied Sciences. 2017; 6(12), 3970-3975.
5. Djamaan, A., A. Agustien, S.I. Zam, M. Jannah, R.S. Lalfari, Y. Aldi, A.P. Dewi, and R.P. Suci. Selection of medium for biopesticides fermentation process by *Bacillus subtilis* AAF2 UAAC 20701. International Research Journal of Pharmacy. 2018; 9(8), 21-26.
6. A. Bassam, N. Anuar, and A. A. H. Kadhum. The Effects of Temperature, pH and Carbon Sources on Antimicrobial Peptide An5-1 Production Using *Paenibacillus alvei*An5. Asian Jr. of Microbiol. Biotech. Env. Sc. 2013; 15(1), 195-201.
7. Wang, Y.H., X. Fang, Y. Cheng, and X. Zhang. Manipulation of pH shift to enhance the growth and antibiotic activity of Xenorhabdus nematophila. Journal of Biomed and Biotech. 2011.
8. Wang, Y.H., and X. Zhang. Influence of agitation and aeration on growth and antibiotic production by *Xenorhabdus nematophila*. World Journal of Microbiology and Microbiology. 2007;23(2), 221–227.
9. Hueck, C.J. and W. Hillen. Catabolite repression in *Bacillus subtilis*: a global regulatory mechanism for the Gram-positive bacteria? Mol Microbiol. 1995; 15, 395–401.
10. Sanchez, S. and A. L., Demain. Metabolic regulation and overproduction of primary metabolites. Microb Biotechnol. 2008; 1(4), 283–319.

Cite this article as:

Anthoni Agustien *et al*. Improving antibiotic activities from endophytic isolate BES-5 gram positive bacteria. Int. Res. J. Pharm. 2019;10(4):32-35 http://dx.doi.org/10.7897/2230-8407.1004119

Source of support: Professor Research Grant Project, Andalas University 2018, Conflict of interest: None Declared

Disclaimer: IRJP is solely owned by Moksha Publishing House - A non-profit publishing house, dedicated to publish quality research, while every effort has been taken to verify the accuracy of the content published in our Journal. IRJP cannot accept any responsibility or liability for the site content and articles published. The views expressed in articles by our contributing authors are not necessarily those of IRJP editor or editorial board members.