Journal of Engineering and Applied Sciences 12 (8): 1987-1991, 2017
ISSN: 1816-949X

Detection and Cloning of Alpha Glucosidase Inhibitor Gene *Streptomyces* sp. IPBCC. B. 15.1539 and Potential as an Anti Hyperglycemic in Education Science

Deden Makbuloh and Yessy Velina
IAIN Raden Intan Lampung, Bandar Lampung, Indonesia

Abstract: Diabetes mellitus is a metabolic disorder characterized by the presence of hyperglycemia due to defective insulin secretion, defective insulin action or both. One therapeutic approach for treating diabetes is to decrease the post-prandial hyperglycemia. This is done by preventing the absorption of glucose through the inhibition of the carbohydrate-hydrolysing enzymes α-glucosidase and α-amylase in the digestive tract. Actinomycetes have been known as source of commercialized acarbose, an α-glucosidase inhibitor. Acarbose is keto analog moieties of the C7N aminocyclitols. Sedoheptulose 7-phosphate is converted into 2-5 epi-valiolone via the activity of sedoheptulose 7-phosphate cyclase at the first step of the biosynthesis of C7N aminocyclitol. This research was aimed to detect and cloning sedoheptulose 7-phosphate cyclase gene. Detection gene was done by using Polymerase Chain Reaction (PCR) with with design primers C7N aminocyclitol. The primer used was designed on the basis of the comparison of the sequence of known sedoheptulose 7-phosphate cyclase (ACBC) from that of *Actinoplanes* sp. SE50/100 which was then cloned by T-Vector pMD20. The result show that there was similiarity of nucleotide series sedoheptulose 7-phosphate cyclase of *Streptomyces* sp. IPBCC. B. 15.1539 with GenBank database analysis through blast program. The result indicated that the designed primer was able to amplify the sedoheptulose 7-phosphate cyclase acarbose although the accomplishment in amplifying the gene is still up to 300 bp and have 100% sequence similiarity with DNA fragment of sedoheptulose 7-phosphate cyclase *Actinoplanes* sp. complete acarbose (ACB) gene cluster, strain SE50/110, accses number Y18523.4 reported in GenBank.

Key words: *Diabetes mellitus*, *Streptomyces* sp. IPBCC. B. 15.1539, inhibitor α-glucosidase sedoheptulose 7-phosphate cyclase, nucleotide, aminocyclitols

INTRODUCTION

Global Diabetes Mellitus (DM) prevalence data shows serious increases. The total number of diabetics worldwide is projected to rise from 171 million in 2000 to 366 million in 2030 (Wild *et al*., 2004) and become the growing global problem of overweight, obesity and physical inactivity. Both modern and traditional antidiabetic therapies have been used to treat people with type 2 DM which is the most common form of DM. Type 2 DM comprises 90% of people with diabetes around the world (WHO, 2014). One of the modern antidiabetic drug treatment mechanisms is based on α-glucosidase inhibitor activity which inhibits the absorption of glucose from the intestine to the blood.

Acarbose is a pseudo-oligosaccharides which acts as a competitor for α-glucosidase, non-digestible and non-toxic. *Tinospora crispa* has been studied for its potential antidiabetic treatment mechanism. Water extract of *T. crispa* significantly lowered blood glucose levels and increased plasma insulin levels in diabetic rats (Noor and Ashcroft, 1998). This effect may be due to The α-glucosidase inhibitor of acarbose is used in the therapy of type 2 DM (non-insulin-dependent) (Wehmeier and Piepersberg, 2004; Morshed *et al*., 2011). On the other hand, various medicinal plants have been traditionally used to treat diabetics. Recent scientific evidence supports the use of the medicinal plants in DM therapy, e.g., *Terminalia arjuna*; *Tinospora crispa*, *T. cordifolia*, *Lagerstroemia speciosa*; *Andrographis paniculata*, *Phaleria macrocarpa*, *Curcuma aeruginosa*, *C. xanthoriza*, *Centela asiatica*, *Xoncus arvensis*, *Caesalpinia sappan*, *Alloe vera*, *Parcia speciosa*, *Gynura procumbens*, *Physalis peruviana*, *Hibiscus sabdariffa*, *Berberis aristata* (Morshed *et al*., 2011; Bnouham *et al*., 2006).

Tinospora crispa has been studied for its potential antidiabetic treatment mechanism. Water extract of *T. crispa* significantly lowered blood glucose levels and increased plasma insulin levels in diabetic rats (Noor and

Corresponding Author: Deden Makbuloh, IAIN Raden Intan Lampung, Bandar Lampung, Indonesia

Ashcroft, 1998). This effect may be due to the modulation of Ca^{2+} concentration in pancreatic beta cells (Sriyapai *et al*., 2009). Other data showed that *T. crispa* treatment reduced plasma glucose levels as much as 7.45% for 40 days in rats induced by streptozotozin (Grover *et al*., 2003).

Actinomycetes are known to produce bioactive compounds with various biological function including α-glucosidase inhibition. Published data on Actinomycetes which function as an α-glucosidase inhibitor have been mainly on non-endophytic Actinomycetes. Supporting evidence is available for *Actinoplanes* sp. SE50/110 (Stratmann *et al*., 1999; Zhang *et al*., 2003) *Actinoplanes* sp. CKD485-16 (Choi and Shin, 2003). *Micromonospora* sp. VITSDK3 (EU55138) and *Actinoplanes* sp. A56 (Hyun *et al*., 2005; Lamba *et al*., 2011). Now days, acarbose which was originally isolated from *Actinoplanes* sp. from Africa has been successfully commercialized as an antidiabetic drug *Streptomyces glaucescens*.

Based on the research that has been done by Pujiyanto *et al*. (2012) has obtained 1 isolate Actinomycetes IPBCC. B. 15.1539 endophytic brotowali were isolated from the roots brotowali that isolates can reduce blood glucose but so far there have not done a study on the detection of gene-producing inhibitor of alpha-glucosidase in this case is Sedo heptulosa 7 phosphate cyclase as well as the ability to lower blood glucose levels. The results of this study can provide information to the field of science education particularly information relating to health especially in the study of metabolism in hormone regulation (Heiner, 2002).

MATERIALS AND METHODS

Growth and production of metabolites: *Streptomyces* sp. IPBCC. B.15.1539 (1% volume) was grown in a bioreactor filled with ISP 2 medium for 10 days and assayed for its α-glucosidase inhibition. The optimum time for the production of α-glucosidase inhibitor was determined based on α-glucosidase inhibitory activity produced by crude extract the *in vitro* α.

Detection of gene sedo heptulosa 7 phosphate cyclase: Detection of gene Sedo heptulosa 7 phosphate cyclase recation performed by Polymerase Chain (PCR). Primers used are the result of design at ACBC gene in *Actinoplanes* sp. SE50/110 (11, 12). PCR amplification (Takara PCR Thermal Cycler, Japan) was conducted in 50 mL reaction mixture containing 10 pmol of each primer by 5 mL, 200 ng mold genomic DNA, 2.5 mM deoxynucleotide Triposphate (dNTP) as much as 4 mL, 10 x Ex taq Buffer as much as 5 mL and 5 units/mL Takara Ex TaqTM much as 1 mL (Takara Japan) and ddH_2O up to volume 25 mL.

Oligonucleotide primer sedo heptulosa 7 phosphate cyclase: Forward primer: 5'-ACCTACGAGGTGCGCT TCCGGGACGACGT-3'andReverse: 5'-GGCGGCCTGCA GCTCGGCGGCCGTCACGT-3' is used to detect gene Sedo heptulosa 7 phosphate cyclase. Target gene PCR product using this primer is 1068 bp. PCR cycles were performed consisting of 94°C initial denaturation for 2 min, followed by 25 cycles of denaturation 94°C for 15 sec, annealing 55°C for 15 sec, elongation of 72°C for 45 sec and a final elongation for 5 min.

DNA purification and cloning DNA with t-vector pmd 20: Gel containing the target DNA and then purified from gel using GeneClean II® kit (Qbiogene, Japan). DNA was quantified using a NanoDrop ND-2000 spectrophotometer (Thermo scientific, Japan). DNA fragments are then subsequently cloned. cloning is done with T-vector pMD20 by ligation reaction using 2 X Ligation Mix (Wako Nippon Gene).

Transformation: Transformation was conducted by heat shock. A total of 5 mL ligation reaction was added to the cell suspension of *E. coli* DH5α that had been competent. This mixture is placed for 3 min dies, then do heat shock treatment (heat shock) at a temperature of 42°C for 45 sec. After the tube containing the reaction mixture ligation and *E. coli* DH5α competent rapidly incubated on ice for 3 min. After incubation on ice, the addition of 200 mL of SOC media treatment liquid on the tube and incubated at 37°C for 45 min. Then the mixture was spread on LB medium containing ampicillin 100 mg/mL, Isopropyl beta-D-Thiogalactopyranoside (IPTG) 100 mL and 100 mL X-Gal in two bowls. Then incubated for 24 h at a temperature of 37°C. After 24 h was observed color of the colony grows. White colonies containing inserts.

PCR colonies: Colony PCR performed to select a recombinant plasmid containing the insert of white colonies E.coli DH5α. Colonies that were taken were white colonies with a sterile toothpick tip then moved to cup replica LB containing ampicillin. Furthermore, the tip of a toothpick in the input and shake vigorously for 10 mL ddH_2O as template DNA for PCR. Positive colony containing the correct inserts subsequent isolation of plasmid. Then measured the concentration of plasmid DNA inserts in the NanoDrop.

Cutting with restriction enzyme: This technique is used as a step to verify whether the DNA insert in plasmid DNA is the desired target. This plasmid was cut with two kinds of enzyme composition includes enzymes XBA I, BAM HI-HF. Each combination is done on a micro tube with a reaction volume of 20 mL.

DNA sequencing: Plasmids have been positively contained DNA fragment sequenced using applied biosystems big dye terminator cycle sequencing kits (v3.1) using M13 primer RV as a forward primer and M13 primer 14 as reverse primer.

RESULTS AND DISCUSSION

Alpha glucosidase inhibitory activity: The data showed that alpha glucosidase inhibitory activity of extracts and the average weight of biomass increased at 5-10 days of the production time and decreased in 15-20 days (The crude extract containing α-glucosidase inhibitor showed 98.5 of alpha glucosidase inhibitory activity after 10 production with 15.6 mg produced biomass the crude (Fig. 1).

Amplification gene of sedo heptulosa 7 phosphate cyclase: Primers used to amplify the gene Sedo Heptulosa 7 Phosphate cyclase managed to amplify the specific gene by 300 bp. Furthermore, the colony PCR to confirm the size of the DNA inserts in several colonies were taken. This can be seen in Fig. 2.

Then the recombinant plasmid verified using restriction enzymes Bam Hf-HI, Bam HI+HF-XBA I. Verification showed one band measuring approximately 3000 bp for the restriction enzyme Bam HII and the HF-2 tape sized 2700 and 300 bp for the restriction enzyme HF-Bam HI and XBA I. This suggests that the insert DNA fragments have been cloned (Fig. 3).

Analysis of gene fragments sedo heptulosa 7 phosphate cyclase with a database in gen bank: Nucleotide sequence aligment analysis of gene Sedo heptulosa 7 phosphate cyclase with a database in Gen Bank conducted with BLAST program. The result of nucleotide sequence similiarity of gene fragments sedo heptulose 7 phosphate cyclase with Gen Bank data center shown in Table 1.

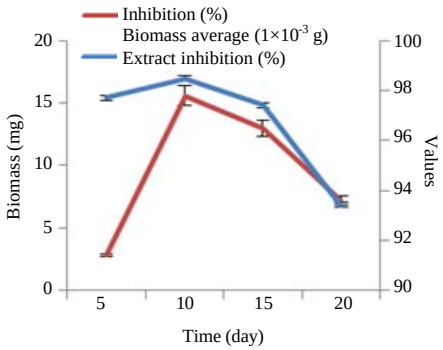

Fig. 1: The α-glucosidase inhibitory activity and biomass production of *Streptomyces* sp. IPBCC. B. 15.1539 grown at ISP2-medium for 5-20 days at room temperature

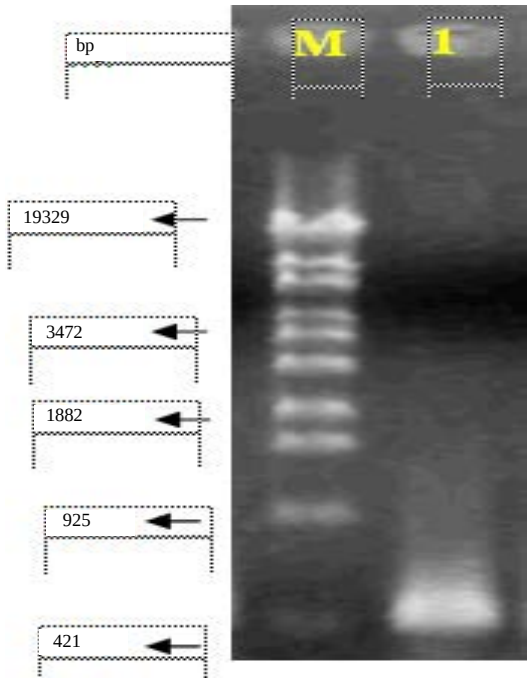

Fig. 2: The colony PCR: DNA Amplification Inserts Sedoheptulosa 7 phosphate cyclase gene with a size of 300 bp in lane 1. M = Marker 1 kb (invitrogen)

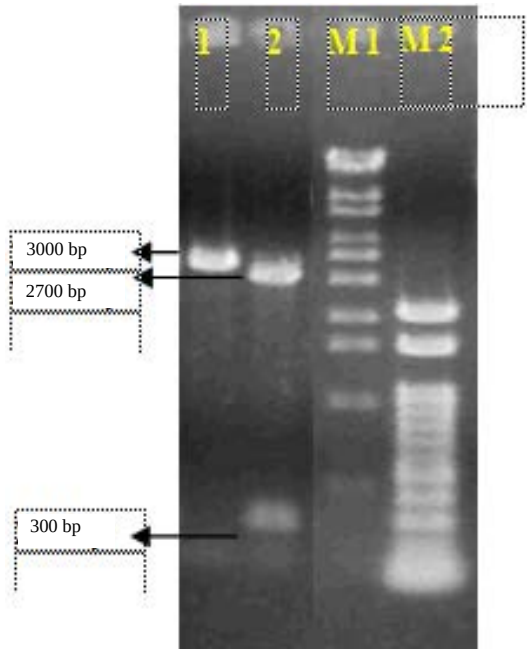

Fig. 3: Verification DNA insert with the rate v1. Recombinant plasmid Hf-Bam-HI, 2. Plasmid rekombinan-Bam HI+HF-XBA I., M1 = Marker 1 kb, M2 = Marker 100 bp

Table 1: Results of nucleotide sequence similarity sedoheptulosa 7 phosphate cyclase gene acarbose on blast program

Clon	Nucleotide sequences most similar	Identity (%)	Access number
Streptomycess IPBCC. B. 15.1539	*Actinoplanes* sp. SE 50/110, complete genom	100	CP003170.1
	Actinoplanes sp. SE50/110 complete acarbose (acb) gene cluster, strain SE50/110	100	Y18523.4

CONCLUSION

Sedoheptulosa 7 phosphate cyclase is an enzyme that catalyzes the cyclization sedoheptulosa 7 phosphate into 2-5 epi-valiolone C7N-aminocyclitol in the biosynthesis of natural products produced by microbes. The results of cyclization of 2-5 epi-valiolone is C7N-aminocyclitol precursor formation in the form validamycin and acarbose useful in the fields of health and agriculture.

Validamycine is an antifungal antibiotic that has trehalase inhibitory activity and is used to control sheath blight of rice plant diseases caused by Rhizoctonia solani. Acarbose alpha-glucosidase inhibitors used in the treatment of type 2 diabetes acarbose works as a competitive inhibitor of the enzyme alpha-glucosidase that break the glycosidic bonds in catalyzing the release of glucose which causes inhibition of glucose absorption, thereby lowering glucose levels after a meal. Acarbose is a complex pseudooligosakarida isolated from Actinoplanes strain SE 50/110 where acarbose able to perform inhibitory activity against sucrase, maltase, dextrinase and glucoamylase.

Piepersberg prove that intermediate 2-5 epi-valiolone catalyzed by sedoheptulose 7 phosphate cyclase (ACBC) in the biosynthesis of acarbose in *Actinoplanes* sp. SE 50/110. Thus, detection of gene acarbose which is class-aminocyclitol C7N designed using Primer PCR based on the nucleotide arrangement known from sedoheptulosa 7 phosphate cyclase (ACBC) in *Actinoplanes* sp. SE 50/110 (Wehmeier and Piepersberg, 2004).

The results of the PCR amplification has been designed to detect gene Sedo heptulosa 7 phosphate cyclase in *Streptomyces*s IPBCC. B. 15.1539 produce specific band and the size of the partial DNA fragments of about 300 bp. Several other types of *Actinomycetes* produce gene sedo reported heptulosa 7 phosphate cyclase has a number of partial gene fragment size such as *Streptomyces* abikoensis strain ATCC 21066, Saccharothrix espanaensis strain ATCC 51144 and *Streptomyces* sp. NAIST13/40.

Alignment through BlastN results showed that the Sedo heptulosa 7 phosphate cyclase shows similarity 100% identity with ACBC gene in *Actinoplanes* sp. SE50/110 complete acarbose (ACB) gene cluster, strain SE50/110. This suggests that genes involved in the biosynthesis of acarbose detected in *Streptomyces*s IPBCC. B. 15.1539.

REFERENCES

Bnouham, M., A. Ziyyat, H. Mekhfi, A. Tahri and A. Legssyer, 2006. Medicinal plants with potential antidiabetic activity-A review of ten years of herbal medicine research 1990-2000. Int. J. Diabetes Metab., 14: 1-25.

Choi, B.T. and C.S. Shin, 2003. Reduced formation of byproduct component c in acarbose fermentation by actinoplanessp: CKD485-16. Biotechnol. Prog., 19: 1677-1682.

Grover, J.K., V. Vats, S.S. Rathi, R. Dawar, 2003. Tradition Indian antidiabetic plants attenuate progressive renal damage in streptozotozin induced diabetic mice. J. Ethnopharmocol, 81: 233-240.

Heiner, L., 2002. Acarbose an update of its therapeutic use in diabetes treatment. Clin. Drug Invest, 22: 141-156.

Hyun, C.G., S.Y. Kim, J.H. Hur, M.J. Seo, J.W. Suh and S.O. Kim, 2005. Molecular detection of alpha-glucosidase inhibitor-producing actinomycetes. J. Microbiol., 43: 313-318.

Lamba, H.S., C.S. Bhargava, M. Thakur, S. Bhargava, 2011. Alpha glucosidase and aldolase reductase inhibitory activity in vitro and anti diabetic activity in vivo of Tribulus terrestris L (Dunal). Int. J. Pharm. Pharm. Sci., 3: 270-271.

Morshed, M.A., A. Haque, B. Rokeya and L. Ali, 2011. Anti-hyperglycemic and lipid lowering effect of Terminalia arjuna Bark extract on Streptozotocin induced type-2 diabetic model rats. Int. J. Pharm. Pharm. Sci., 3: 449-453.

Noor, H. and S.J. Ashcroft, 1998. Pharmacological characterisation of the antihyperglycaemic properties of Tinospora crispa extract. J. Ethnopharmacol., 62: 7-13.

Pujiyanto, S., Y. Lestari, A. Suwanto, S. Budiarti and L.K. Darusman, 2012. Alpha-glucosidase inhibitor activity and characterization of endophytic actinomycetes isolated from some Indonesian diabetic medicinal plants. Int. J. Pharm. Pharmaceut. Sci., 4: 327-333.

Sriyapai, C., U.R. Dhumma, S. Sangwatanaroj, N. Kongkathip and S. Krittiyanunt, 2009. Hypoglycemic effect of tinospora crispa dry powder in outpatients with metabolic syndrome at king Chulalongkorn memorial hospital. J. Health Res., 23: 125-133.

Stratmann, A., T. Mahmud, S. Lee, J. Distler, H.G. Floss and W. Piepersberg, 1999. The Acbc protein from actinoplanes species is a c7-cyclitol synthase related to 3-dehydroquinate synthases and is involved in the biosynthesis of the alpha -glucosidase inhibitor acarbose. J. Biol. Chem., 274: 10889-10896.

WHO, 2014. 10 Facts ab out Diabetes. World Health Organization, Geneva, Switzerland.

Wehmeier, U.F. and W. Piepersberg, 2004. Biotechnology and molecular biology of the a-glucosidase inhibitor acarbose. A ppl. Microbiol. Biotechnol., 63: 613-625.

Wild, S., G. Roglic, A. Green, R. Sicree and H. King, 2004. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. Diabetes Care, 27: 1047-1053.

Zhang, C.S., M. Podeschwa, O. Block, H.J. Altenbach and W. Piepersberg *et al*., 2003. Identification of a 1epi valienol 7 kinase activity in the producer of acarbose, Actinoplanes sp.: SE5 0-110. FEBS. Lett., 540: 53-57.