

MUTAGENESIS DEVELOPMENT OF *ACTINOPLANES* SP. KCTC 9161 BY N-METHYL-N'-NITRO-N-NITROSOGUANIDINE (NTG) AND SCREENING FOR ACARBOSE PRODUCTION

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SUMMARY

Acarbose has been widely used in the therapy of type II diabetes (non-insulin dependent) because it controls blood sugar contents of patients after meals. Acarbose, a pseudo-oligosaccharide, acts as a competitive α -glucosidase inhibitor. Acarbose is produced by the strains of *Bacillus*, *Streptomyces* and *Actinoplanes* sp. The aim of this study was to develop mutagenesis for an *Actinoplanes* sp. strain and screening for acarbose production. The spores of *Actinoplanes* sp. KCTC 9161 strain were subjected to be mutated by N-methyl-N'-nitro-N-nitrosoguanidine (NTG) for screening and finding mutant strains that were capable of production of higher acarbose (an inhibitor of α -glucosidase) higher than wild type strain. Firstly, the original NTG solution was prepared in phosphate buffer 0.05 M, pH 6.9 and the safety concentration of NTG was determined at 5 mg/ml. Then, the spores were incubated with different NTG amounts and duration. The living colonies were transferred to fermentation medium. The results obtained showed that 15 mutant strains were produced higher acarbose than wild type when used thin layer chromatography method for analysis and comparing with standard acarbose (Sigma). Three cell lines among total tested 15 mutant lines of *Actinoplanes* sp. KCTC 9161 produced acarbose at a higher level or indicated a higher inhibitory activity toward α -glucosidase than the original strain. Enzymatic inhibitory activity of α -glucosidase of three mutant strains (*Actinoplanes* sp. KCTC-L4, L11, L14) was increased 1.3 fold higher than wild type and *Actinoplanes* sp. KCTC spores were very sensitive to NTG toxic, 98% spores could not survive at the treatment condition of 50 μ g NTG for 30 minutes. In addition, an applicable protocol for mutating *Actinoplanes* sp. using NTG was suggested for further research.

Keywords: Acarbose, *Actinoplanes* sp. KCTC 9161, N-methyl-N'-nitro-N-nitrosoguanidine, α -glucosidase, mutant

INTRODUCTION

Acarbose is an α -glucosidase inhibitor. It was used as a novel therapeutic agent for treating hyperglycemia in non-insulin-dependent diabetes mellitus (NIDDM) (Inoue *et al.*, 1997). The action of acarbose depends on its structure: a compound of acarviosyl and maltose. In which, acarviosyl is core, which is involved in valienamine and 4-amino-4,6-dideoxyglucose by N-glycosidic bond. Therefore, acarbose seems to be like a pseudo-oligosaccharide. It acts as the causing agent in a competitive way with oligosaccharides, delays the digestion of starch and is used to reduce postprandial plasma glucose and insulin levels in diabetic patients (Baron *et al.*, 1987). Acarbose has high biological activity,

inhibitor of intestinal α -glucosidase with $IC_{50}=11$ nM (Caspary, Graf 1979). This reported demonstrated that the affinity of acarbose with intestinal sucrase was much higher (15,000 folds) if compared to sucrose. The acarbose binding resulted in a very stable enzyme/substrate complex. Acarbose is only minimally absorbed from the gastrointestinal tract (<2%), and its action is extended throughout the small intestine; it will be metabolized by colonic bacterial enzymes (Lebovitz, 1998).

Acarbose was the first to be identified in the fermentation broth of *Actinoplanes* sp. SE50 isolated in the form of coffee soil in Kenya last 1960s (Schmidet *et al.*, 1997). The production of acarbose depends on various sources, i.e., maltose (Choi, Shin,

2003) extracts of corn (Wei *et al.*, 2010), monosodium glutamate (Wang *et al.*, 2011). *Actinoplanes* sp. SE50/110 (*Actinoplanes* sp. KCTC 9161) produced 1 g/l of acarbose and was widely used for researches on the acarbose synthesis gene (Schwientek, 2012; Klein *et al.*, 2013). *Acb*-gene cluster in *Actinoplanes* sp. SE50/110 is the acarbose production cluster of 41,323 bp (Accession number Y18523 in GenBank) (Hemker *et al.*, 2001; Schwientek *et al.*, 2012).

N-Methyl-N'-nitro-N-nitrosoguanidine (NTG) has been widely used to induce mutations in bacteria. Because of highly effective chemical, it has been suggested to be the most potent mutagen yet discovered (Moore, 1968). NTG causes the chemical mutagenic process named alkylation. This methylation modifies the O4 position of thymine, or the O6 position of guanine, which becomes stably maintained when replicated. The mutations caused by this are transitions.

So far, the research about mutagenic process on *Actinoplanes* sp. in the world is very little. In Viet Nam, we had some mutagenic *Actinoplanes* sp. VTCC A1779 by NTG. Six of the 60 mutant strains had α -glucosidase inhibitor higher three folds than wild strain (Quyen Dinh Thi *et al.*, 2012). Based on this success, in this experiment we used NTG on *Actinoplanes* sp. KCTC 9161 strain to screen the mutant strains for higher acarbose production. The methods for assessment of acarbose production ability is thin layer chromatography (TLC) and determination of α -glucosidase inhibitor activity (Yamaki, Mori, 2006; Mori *et al.*, 2011).

MATERIALS AND METHODS

Materials and chemicals

Actinoplanes sp. KCTC 9161 purchased from the Korea collection for type culture. Acarbose standard and NTG were from Sigma (USA); *p*-nitrophenyl- α -D-glucopyranoside, α -glucosidase, peptone (Biobasic INC, Canada); n-butanol, ethanol, methanol, ethyl acetate, acid formic, CaCl₂, CaCO₃, KCl, FeSO₄.7H₂O, K₂HPO₄, KH₂PO₄.3H₂O, MgSO₄ were purchased from AR Co, China.

Methods

Activation of cultured Actinoplanes sp. KCTC 9161

Actinoplanes sp. KCTC 9161 from the tubes was

maintained on agar slant containing (g/L): glucose 20; peptone 5; KCl 0.5; K₂HPO₄ 1.0; MgSO₄.7 H₂O 0.5; and agar, 2.0; pH 7 (Wei *et al.*, 2010)

Inoculum of Actinoplanes sp. KCTC 9161

Actinoplanes sp. KCTC 9161 was culture sinking after the activation of propagation medium, at 28°C, 200 rpm, 72 hours in an incubator shaker. The inoculum medium (CPC) was composed (g/l): sucrose 30; peptone 2; KCl 0.5; MgSO₄.7H₂O 0.5; FeSO₄.7H₂O 0.1; KH₂PO₄.3H₂O 1; casein hydrolysate type 1.

Fermentation of Actinoplanes sp. KCTC 9161

The MT1 medium (Nguyen *et al.*, 2013) (fermentation medium) was added 5% of seed varieties of *Actinoplanes* sp. KCTC 9161, then the bacteria was cultured at 28°C, 200 rpm in 168 hours shaking. The MT1 medium (g/L): maltose 50; glucose 30; core powder 15; CaCl₂ 2.0; CaCO₃ 2.5; monosodium glutamate 1.0; KH₂PO₄.3H₂O 1.0. After 168 hours, broth solution was collected and the produced acarbose in culture were checked by TLC with acarbose standard and the activity of enzyme α -glucosidase inhibitor was measured.

Thin layer chromatography

The fermentation broth was centrifuged at 12,000 rpm, 4°C for 10 minutes to remove bacterial cells. Proteins in the culture was precipitated and the supernatant was cleaned by ethanol (1 sample: 4 ethanol v/v) in 30 minutes. The supernatant was separated by centrifugation at 12,000 rpm, 4°C for 10 minutes, and then dropped on TLC silica gel. Thin-layer chromatography was performed on a Merck silica gel 60 F254, 0.25 mm thick with a solvent system 94% A and 6% B (A contain ethyl acetate: methanol = 1:1 and B contain H₂O: acid formic = 5:2), then by the color burst slightly acid (10% H₂SO₄ in ethanol) at 121°C in 15 minutes.

Determination of α -glucosidase inhibition activity

α -Glucosidase cuts the α -1,4 glycosides in polysaccharide. It also digests the α -1,4 bonds in *p*-nitrophenyl- α -D-glucopyranoside. If those bonds in the compounds are digested, *p*-nitrophenyl - a yellow substance, will be released and absorb light at the wavelength of 405 nm.

After centrifugation at 12,500 rpm, 4°C for 15 min, 10 μ l supernatant of broth sample was mixed

with 40 µl buffer of 0.1 M phosphate pH 6.9 and 100 µl of α-glucosidase 1 µg/ml solution in experienced wells. In the controlled wells, the 10 µl of broth sample was replaced with 10 µl of buffer. This mixture was incubated at 25°C for 10 minutes. Then, 50 µl of 5 mM *p*-nitrophenyl-α-D-glucopyranoside was added and incubated for 5 minutes. OD measures were performed before and after incubations.

Ability of inhibition of α-glucosidase (%) was defined as.

$$\% \text{ inhibition} = \frac{\Delta A_c - \Delta A_s}{\Delta A_c} \times 100,$$

Where, ΔA_c is the changing of OD values of controlled sample before and after incubation

ΔA_s is the changing of OD values of experienced sample before and after incubation

Mutation technique

The mutagen solution was freshly prepared by dissolving NTG in phosphate buffer (pH 7). The final concentration of the mutagen solution was 5 mg/ml and was kept in dark.

Actinoplanes sp. KCTC 9161 spores were selected from CPC medium (agar or liquid). We collected spores by 0.2% Tween 20 solution in CPC agar medium, slightly shake in 30 minutes; with CPC liquid medium, we centrifuged the broth in 5 minutes, 8,000 rpm and dissolved again by water.

Spores were incubated with NTG (50, 100, 150 and 200 µg for one milliliter of spores). Each NTG concentration was studied in 30, 60 and 90 minutes at 25°C. After incubation, the stopping NTG action was carried out by washing with phosphate buffer, centrifugation in 5 minutes, 8,000 rpm. The spore was collected again then repeated 3 times for mutation. Finally, the spores were cultured on CPC agar medium at 25°C. After 120 hours, the numbers of the colonies raised on agar plates were recorded to compute the survival rates. These mutant strains will be used to ferment and determine the ability of acarbose production.

RESULTS AND DISCUSSION

The characteristics of *Actinoplanes* sp. KCTC 9161

Actinoplanes sp. KCTC 9161 is gram-positive, has fibrous structure, the fibers tightly linked together to form a separate orange-colony, bulging in the middle colonies that developed to sporangia contain spores. Spores can be mobile. The fibrous is just a cell, there is no horizontal septum, simple nuclear, no nuclear membrane. *Actinomyces* reproduces by spores, prefers moist, appropriate with pH 6-8 in culture. In agar medium, the colony of *Actinoplanes* sp. KCTC 9161 strain had round shape, smooth surface, slow development (5-7 days), orange colonies 1-2 mm in diameter (Fig. 1A). In liquid medium, they grew well, forming circular pellet particles, insoluble when shaken up, orange color (Fig. 1B).

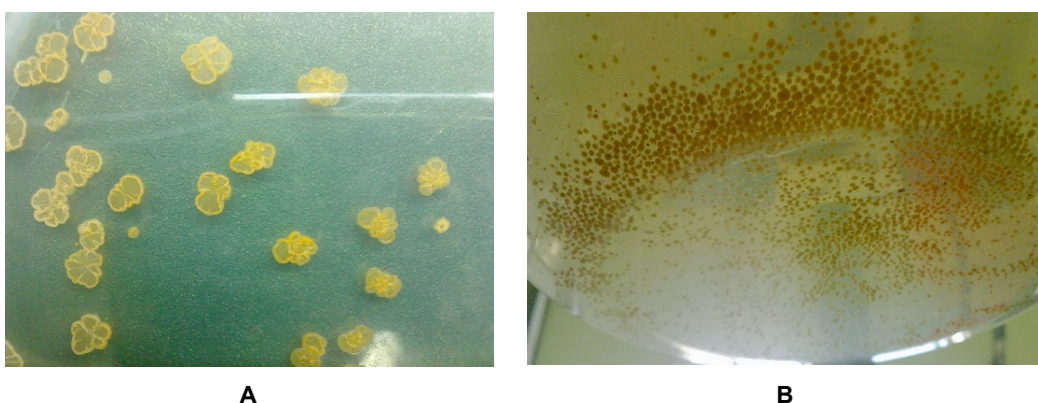


Figure 1. The colonies of *Actinoplanes* sp. KCTC 9161 strain (A) in agar medium; (B) in liquid medium

The acarbose production ability of this strain was expressed on MT1 medium. After 7 days (168 hrs), the fermentation broth was cleaned and acarbose in culture was checked by TLC and α -glucosidase inhibitor ability. The TLC result (Fig. 2) exhibited a deep-clearly band, same position in horizontal lines with acarbose standard and α -glucosidase inhibition activity was 50%.

The concentration of NTG original solution

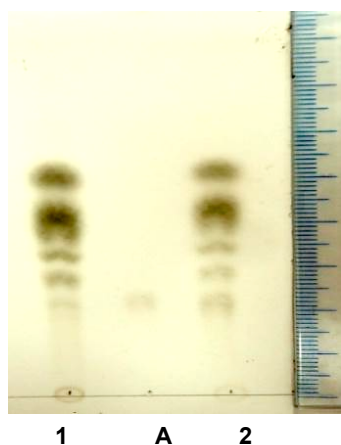


Figure 2. TLC analysis of the acarbose samples from fermentation broth of *Actinoplanes* sp. KCTC 9161.1,2: fermentation broth; A: acarbose standard (Sigma).

The concentration of original NTG is very important with mutation experiment. If the NTG original solution is not clean, the experiment will fail by infection. In theory, NTG is toxic for bacteria, but in the low concentration of NTG, the organism can be survival. Firstly, 50 μ l of the 100 μ g/ml NTG was used to spread on CPC agar medium for mutation test. After 5 days, a lot of bacteria appeared on agar plate. Other NTG solution was prepared (5 mg/ml), after 5 days, the agar plate is clean without the attention of bacteria data not show. The NTG concentration of 5 mg/ml was used for mutation experiment after.

The NTG mutation experiment with liquid spore

Base on the characteristics of *Actinoplanes* sp KCTC 9161, the problem about weak growth of this strain can be resolved by spores taking from liquid medium. We can collect high amount of spores which is in the growing process.

Actinoplanes sp. KCTC 9161 was cultured on CPC liquid medium, 200 rpm, 28°C, 120 hours. The spore was collected by centrifugation, removed surfactant, added water to same volume before. Four milliliters of spore in four Eppendorf tubes were treated with 50, 100, 150 and 200 μ g NTG, respectively. After 30, 60 and 90 minutes, 300 ml of spore were taken out, washed 3 times with phosphate buffer and cultured on CPC agar plate, 100 ml per one plate. The results were shown in Tab. 1.

Table 1. The survival colony in NTG mutation liquid spore

NTG (μ g)	Control	30 min		60 min		90 min	
		Number of living colonies	Survival ratio (%)	Number of living colonies	Survival ratio (%)	Number of living colonies	Survival ratio (%)
50	3927 \pm 100.1	620 \pm 12.3	15.8	820 \pm 28.1	20.9	1072 \pm 84.5	27.3
100	3927 \pm 100.1	295 \pm 17.8	7.5	514 \pm 45.1	13.08	1042 \pm 114.4	26.5
150	3927 \pm 100.1	235 \pm 32.1	6.0	371 \pm 24.2	9.4	838 \pm 29.7	21.3
200	3927 \pm 100.1	1098 \pm 67.9	27.9	450 \pm 14.6	11.4	847 \pm 48.2	21.5

In theory, the survival ability of spore after treatment with NTG depends on the concentration of NTG and the duration of incubation time. With low NTG concentration or short time treatment, the number of survival colony on agar plate will be higher than in plate that was treated with

higher concentration of NTG or longer time incubation. The effect of NTG on survival ability of spores was seen clearly when compared the number of colony on control plate with treatment plates. It seemed true with plates in different NTG concentrations. The survival ability of

spores was decreased when the concentrations of NTG increased. On overall view, the number of colonies on plates that treated with 50 µg NTG were highest, higher than plates treated with 100 µg NTG, the next was 150 µg and in the end was 200 µg NTG. The numbers of colonies were increased with longer treatment time. In 90 minutes of treatment, the number was highest, then in 60 minutes; and those in 30 minutes were smallest.

The number of living colonies was too much for acarbose production faculty. Thus non-control concentration of spores before experiment was the reason for this problem. In other hand, the higher NTG concentration can be used but it will be very dangerous for the researcher. Studying NTG mutation on agar plate was chosen as our solution in the next result.

The problem we have when do mutagenic with spore from liquid medium is the killing effect of NTG is not right with more time treatment. The number of living spore in 90 min treatment with NTG in each concentration are higher than in 60 min and least in 30 min. The result should be reverse, highest number in 30 min, then in 60 min and last in 90 min as table 1 with 50 µg NTG treatment. The reason caused by the pelleting shape spore of KCTC 9161 strain in liquid medium. The pellet of *Actinoplanes* sp. KCTC 9161 strain consisted of many spores and the fibrous can link strongly together, making the pellet too hard to be broken when shaking. In experiment process, after shaking to take 300 µl spore the pellet is rapidly go down to the bottom. Because the fibrous of pellet are stabilized, they can act as a shield which prevents the toxic of NTG on the spore in deep of a pellet. It means with long time treatment, the amount of pellets we take out is increased and the number of spore will be increased. The solution for

the defect of this method is masticated to break pellet shape, and use filter to removing the pellet as possible.

The mutation experiment with agar spore

Actinoplanes sp. KCTC 9161 strain was cultured on CPC agar medium. After 120 hours, two agar plates were used to collecting spore by 10 ml Tween 20 0,2% and divided 1 ml spore for 1 Eppendorf tube. The steps to do experiment were the same with experiment on liquid pore.

In this experiment, the result was better than result in the experiment with liquid spore. The number of living colonies on control plate was about 1000. Thus concentration of living spores was 10⁴/ml. The survival ratio of NTG treated -KCTC 9161 spore for each concentration of NTG was indicated in Tab. 2. It's clearly that KCTC 9161 spore is very sensitive with toxic effect of NTG. In just only 30 min of treatment with 50 µg NTG, more than 99% spores were killed. It's is possible to make the comparison with other bacteria; with a 30 min treatment time only 39% of *Escherichia coli* organisms were killed by 300 µg NTG/ml (Adelberg *et al.*, 1969), while 80 % of treated organisms of *Schizosaccharomyces pombe* were killed by exposure to 2 mg NTG/ml, again for 30 min (Megnet, 1965). Therefore, KCTC 9161 strain has 200 times more sensitive than *E. coli* and above 400 times more sensitive than *S. pombe* to the killing effect of NTG. Sixteen colonies includes: 4 colonies (named L1, L2, L3, L4) from treatment of NTG 50, 30 min, 4 colonies (named L5, L6, L7, L8) from treatment of NTG 50, 60 min; 4 colonies (named L9, L10, L11, L12) from treatment of NTG 50, 90 min; 1 colony (named L13) from treatment of NTG 150, 30 min, 2 colonies (named L14, L15) from treatment of NTG 200, 30 min and 1 colony from control were cultured to test acarbose production.

Table 2. The number of living colonies and survival ratio in mutation experiment with agar spore

Time(min) NTG (µg)	Number of living colonies			Survival ratio (%)
	30	60	90	
50	63 (3+17+43)	44 (12+10+22)	41 (30+10+1)	0.0148 (0.0063+0.0044+0.0041)
100	0	0	0	0.0000
150	1	0	0	0.0001
200	2	0	0	0.0002

The acarbose production ability of 15 mutation strains

Sixteen strains (15 mutants and 1 normal strain) were cultured on CPC liquid medium to increasing number of spores. After 120 hours, they were transfer to fermentation medium (MT1) at 28°C, 200 rpm, 168 hrs. The fermentation broth was centrifuged at 12,000 rpm for 10 min. The surfactant solution was used to check the acarbose production and inhibitor α -glucosidase.

In TLC plate, all cultures from 16 strains made dark bars in the same position with acarbose standard in horizontal (Fig. 3). The inhibitor activity of α -glucosidase showed in Fig.4. Base on this result, the strain L4, L11 and L14 had the highest α -glucosidase inhibitory activity (1.3 folds higher) than control.

The improvement of the high acarbose yielded by the mutant KCTC 9161 strains may be of 3 reasons. The first, Lee *et al.* (2008) have studied 3 gene clusters of *TreX-Y-Z* in *Actinoplanes* sp. SN233/29. *TreY* was encrypted for an isomerase that can convert acarbose (Ac-1,4-Glc-1,4-Glc) to component C (Ac-1,4-Glc-1,1-Glc). The prevention component C production is useful in increasing acarbose level in fermentation broth by mutation

TreY gene (Lee *et al.*, 2008). The second, the acarbose production was of the process by 25 genes, encoding for some enzymes and important factors. If the intensity expression or the ability of one gene is increased by mutation, probably the acarbose level would be higher. The last, maltose has important influences on the acarbose production in *Actinoplanes* sp. A56 (Wei *et al.*, 2010). The amount of acarbose was increased from 837 to 1043 mg/l with maltose from 30 g/L to 61.25 g/L. Here is only putative reasons, that should be confirmed in future by experiments.

The acarbose production capacity of 15 mutants strains were shown in Fig. 3. Overall, all of strain increased the production of acarbose, presented by the dark of the acarbose band and α -glucosidase inhibition (Fig.4). However, the highest inhibitor of acarbose activity (L4, L11, L14) was 1.3 folds higher than control, less than the data resulted from the study of QDT (2012)- 3 times higher than control on *Actinoplanes* sp. VTCC-A1779. It was impossible to make exactly comparison because some colonies were not tested. We supposed that NTG had effect on improving acarbose production of *Actinoplanes* sp. KCTC 9161. Based on our experiment, we suggested a protocol to do NTG mutant with *Actinoplanes* sp. KCTC 9161.

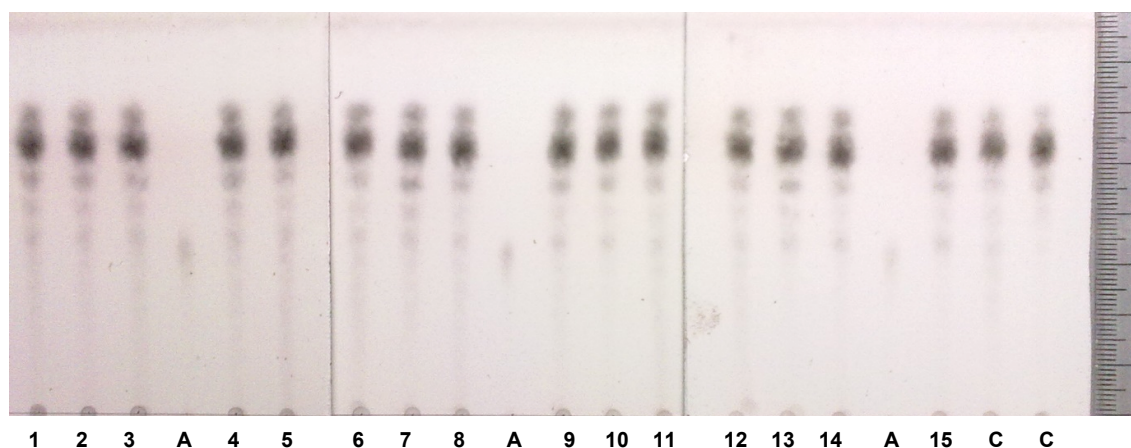


Figure 3. Acarbose production from cultures developed from 16 strains by thin layer chromatography. 1-15: Strain L1-L15; A: acarbose standard (Rf = 0.41); C: control

We hope this experiment can help some researchers in next study: The *Actinoplanes* sp. KCTC 9161 strain is cultured on CPC liquid medium at 28°C, 200 rpm, 120 hrs. Transfer 100 μ l spore in the CPC agar plate, keep in 120 hrs, 28°C. After that,

collect the colonies by stick, put in Tween 20 0.2% solution, vortex in 30 min to have final concentration of spore about 10000 for 1 ml. One milliliter spore is incubate with 50-100 μ g NTG (concentration of NTG original 5 mg/ml phosphate buffer pH 6.9, 0.5

M) in 30, 60 and 90 min. Stop the action of NTG by washing with phosphate buffer (pH 6.9, 0.5 M), centrifuge at 8000 rpm for 5 min; repeat 3 times. Finally, 100 µl spores are cultured on CPC agar. After 5 days, the living ratio of spore is less than 2% with control.

Each survival colony is transferred to CPC liquid medium to increasing number of spore, 120 h, 28°C, 200 rpm. 5% (v/v) of seed varieties added to fermentation medium, continue in 168 hrs, 28°C, 200

rpm. Then, the fermentation broth is centrifuged at 12,000 rpm, 10 min, 4°C. The surfactant is cleaned by methanol (1 sample: 4 ethanol (v/v)) in 30 min. After that, centrifuged at 12,000 rpm, 4°C for 10 min and use surfactant to point on TLC plate. Solvent system is 94% A and 6% B (A contain ethyl acetate: methanol = 1:1 and B contain H₂O : acid formic = 5:2 v/v), then dye by slightly acid (10% H₂SO₄ in ethanol) at 121°C in 15 minutes. Use surfactant of fermentation broth after centrifuged for α-glucosidase inhibitor ability.

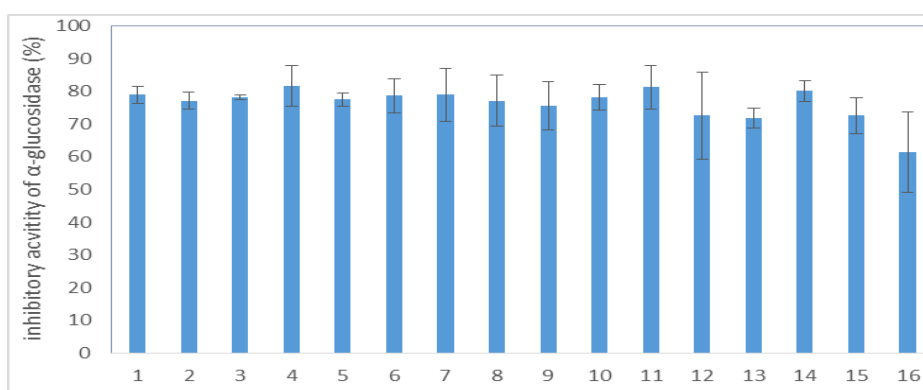


Figure 4. The α-glucosidase inhibitor ability of the cultures from 16 strains 1-15: L1-L15; 16: Control

CONCLUSION

In this experiment, the novel findings were that three mutant strains (*Actinoplanes* sp. KCTC 9161 – L4, L11, L14) have α-glucosidase inhibitory higher 1.3 times than wild type. It is necessary to build up a good protocol for the next study and to continue to conduct experiments to find which the best mutant strain is.

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GÂY ĐỘT BIẾN CHỦNG *ACTINOPLANES* SP. KCTC 9161 BẰNG N-METHYL-N'-NITRO-N-NITROSOGUANIDINE (NTG) VÀ SÀNG LỌC DÒNG CÓ KHẢ NĂNG SINH ACARBOSE CAO

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TÓM TẮT

Acarbose đã được sử dụng rộng rãi trong điều trị bệnh đái tháo đường type 2 không phụ thuộc-insulin. Acarbose là hợp chất hữu cơ giả đường (pseudo-oligosaccharide), hoạt động như một chất ức chế cạnh tranh α -glucosidase. Acarbose có thể kiểm soát hàm lượng đường trong máu của bệnh nhân sau khi ăn và được sinh tổng hợp từ các chủng vi sinh vật như *Bacillus*, *Streptomyces* và *Actinoplanes*... Mục đích của nghiên cứu này là gây đột biến chủng xạ khuẩn *Actinoplanes* sp. và sàng lọc các dòng có khả năng sinh tổng hợp acarbose cao. Bào tử của chủng *Actinoplanes* sp. KCTC 9161 được gây đột biến bằng NTG (nitrosoguanidine) để tìm ra các dòng đột biến có khả năng sinh tổng hợp acarbose cao hơn chủng gốc. Nồng độ NTG gốc được chuẩn bị là 5 mg/ml trong đệm phosphate pH 6.9. Bào tử được ủ với NTG nồng độ 50, 100, 150 và 200 μ g trong 30, 60 và 90 phút và được nuôi trên môi trường thạch CPC. Sau 120 giờ, khuẩn lạc sống sót được đếm và chuyển sang môi trường lên men để kiểm tra khả năng sinh tổng hợp acarbose và khả năng ức chế α -glucosidase. Kết quả cho thấy, có 3 dòng đột biến (*Actinoplanes* sp. KCTC 9161- L4, L11, L14) ức chế hoạt tính của α -glucosidase cao hơn 1,3 lần so với chủng gốc ban đầu và bào tử KCTC 9161 rất nhạy cảm với độc tính của NTG, 98% bào tử chết khi xử lý với 50 μ g NTG trong 90 phút. Bên cạnh đó, một quy trình hoàn thiện để làm đột biến NTG với *Actinoplanes* sp. được đề nghị để hỗ trợ những nghiên cứu tiếp theo.

Từ khóa: Acarbose, α -glucosidase inhibitor, *Actinoplanes* sp. KCTC 9161, NTG, fermentation