VOLUME 2 • NOMOR 1 • JUNI 2015 ISSN 2442 - 2606

JURNAL
BIOTEKNOLOGI & BIOSAINS INDONESIA

Homepage Jurnal: http://ejurnal.bppt.go.id/index.php/JBBI

PENICILLIN PRODUCTION BY MUTANT OF *Penicillium chrysogenum*

Produksi Penisilin oleh Mutan *Penicillium chrysogenum*

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ABSTRAK

Penisilin adalah antibiotika yang pertama kali ditemukan dan digunakan untuk pengobatan infeksi bakteri. Sejak ditemukan penisilin sebagai antibiotika oleh Alexander Fleming pada tahun 1928, banyak usaha dilakukan untuk meningkatkan produktivitas Penicillium chrysogenum*. Pemuliaan galur untuk meningkatkan produksi penisilin dapat menggunakan mutasi acak secara fisika dan kimia. Pada penelitian ini, radiasi sinar ultraviolet digunakan untuk mendapatkan mutan* P. chrysogenum*. Produksi penisilin ditentukan menggunakan HPLC dan produktivitas mutan dibandingkan dengan induk* P. chrysogenum*. Mutan M12 menghasilkan penisilin 1,23 kali lebih banyak dibandingkan dengan induk* P. chrysogenum*.*

Kata kunci: *Penisilin,* Penicillium chrysogenum*, ultraviolet, mutan, radiasi*

ABSTRACT

Penicillin is the first antibiotic discovered and used for treatment of bacterial infections. Since the discovery of penicillin as antibiotic by Alexander Fleming in 1928, much effort has been invested to improve productivity of *Penicillium chrysogenum*. Strain improvement to increase the penicillin production can be carried out by physical and chemical random mutation. In this research, ultraviolet irradiation was used to obtain *P. chrysogenum* mutant. Penicillin production was determined by using HPLC and productivity of *P. chrysogenum* mutants was compared to the wild type. Mutant M12 produced 1.23 fold higher penicillin than the wild type did.

Keywords: Penicillin, *Penicillium chrysogenum*, ultraviolet, mutant, radiation

INTRODUCTION

Penicillin is the first antibiotic discovered and used for treatment of bacterial infections. The structure of β-lactam penicillin consists of a bicyclic nucleus formed by a β-lactam ring and a thiazolidine ring containing a sulfur atom and an acyl side chain bound to the amino group present at C-6 (Liras dan Martin 2006). Although some bacteria are resistant to penicillin, this antibiotic is still widely used today. Penicillin is used in treatment of many gram-positive bacterial infections, such as *Staphylococcus pyogenes* (strep throat) and *Streptococcus pneumoniae* (respiratory tract infection, otitis media) (Christensen et al. 2000; Rayamajhi et al. 2000). Bacterial cell wall synthesis is inhibited by penicillin. Penicillin binds to the enzyme transpeptidase that link the peptidoglycan molecules in bacterial cell wall.

Penicillin industrially is produced by the filamentous fungus *Penicillium chrysogenum* and commercial production of penicillin began in 1941 (Newbert et al. 1997). The production of penicillin by the submerged fed-batch fermentation in stainless tank reactors of 30,000-100,000 galon capacity is important for several decades (Elander 2003) and optimization of penicillin production is very important for the penicillin companies (Thykaer dan Nielsen 2003). Since the discovery of the production of antibiotics by Alexander Fleming in 1929, much effort has been invested in selection and synthesis of strains with improved productivity (Harris et al. 2009). Classical strain improvement with random mutation and screening has been used to obtain overproducing strains. The UV irradiation is the one of techniques for strain improvement and its technique is very effective for mutation because the bases DNA absorb the UV irradiation. The effect of the absorbed UV irradiation is the formation of thymine dimers and cross links in the same strand (Parekh et al. 2000). Mutants are unstable, mutagenic events are random and do not necessarily affect only the genes involved in antibiotic synthesis. Re-isolation of mutants, since prolonged storage of high producing strains is needed to know stability of mutants.

The increase in penicillin fermentation productivity and high recovery yield (>90%) has led to significant cost reduction fispite increasing labor, energy, and raw materials costs. In 1953, the bulk cost for penicillin production was ~$300/kg. In 1980, the bulk price for penicillin was ~$35/kg. In the late 1990s, bulk penicillin cost ranged from $10 to 20/kg and bulk marketed costs for 6-APA have been estimated to range from $ 35 to 40/kg (Elander 2000).

The aim of this research was obtained *P. chrysogenum* mutant that produces higher amount of penicillin than wild type.

MATERIALS AND METHODS

Microorganism

Strain of *P. chrysogenum* in this study was obtained from Biotech Center Culture Collection, Agency for the Assessment and Application of Technology.

Mutation by UV irradiation

Czapek Dox Agar was used to select *P. chrysogenum* mutants. A conidial from 10 days old culture of *P. chrysogenum* was suspended in striled water and ajusted to about 10^3 conidia/mL. Conidial suspension was irradiated by short wave UV irradiation of 254 nm and the time of irradiated variated from 5-30 minutes with interval 5 minutes. 50 µL conidial suspension of the growth of *P. chrysogenum* mutants were inoculated, spreaded into Czapek Dox Agar plates, and the inoculated plates were incubated for 10 days at 28°C.

Isolation and selection of mutants

After 10 days, mutant colonies were separated by inoculation each mutant colony on Czapek Dox Agar plates. The inoculated plates were incubated for 10 days at 28°C.

Production of penicillin

One of mutants colony was inoculated in seed medium (6.1% corn steep liquor, 2.0 % sucrose, 0.5% $CaCO_3$) and incubated in shaker for 36 hours at 28°C. Ten percent

suspension of inoculum was inoculated in fermentation medium (4.2% corn step liquor, 13% lactose, 0.40% KH_2PO_4, $(NH_4)SO_4 \cdot 7H_2O$ 0.45%, 0.15% Na_2SO_4, 0.1% $CaCO_3$ pH 5.9) and incubated in shaker for 10 days at 28°C.

Analysis of penicillin

Broth of fermentation was centrifugated at 15000 g, and supernatant was filtered through filter 0.45 µm. 10 µL sample was injected to HPLC mechine from Waters with C18 column (Symmetry), mobile phase: 5 mM KH_2PO_4 and 6 mM H_3PO_4 : Acetonitrile (60 : 40), flow rate 1.0 mL/min, and ultraviolet detector λmax: 210 nm.

RESULTS AND DISCUSSION

The survivals of *P. chrysogenum* mutants were selected and tested for penicillin production. Penicillin yield of Mutants varied from 1635 to 4594 ppm. Mutant M12 produced 1.23 fold (4594 ppm) compared to wild type (3305 ppm) (Table 1.). The hyperproduction of mutant may be caused mutation of one or more biosynthesis of penicillin gene or the disrupsion of the *lys2* gene (gene for lysine biosynthesis). The mutation of biosynthesis of penicillin gene may increase enzymes activity for penicillin production or disruption of the *lys2* gene increase aminoadipic acid (precursor for penicillin biosynthesis).

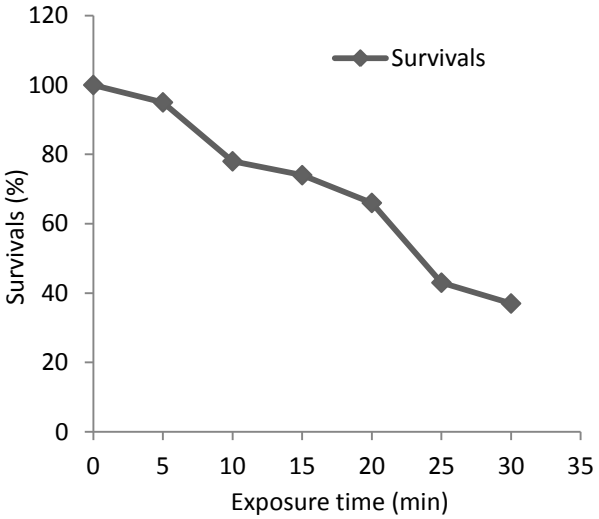

Figure 1. Effect of UV exposure time on *P. chrysogenum* and mutants from 50% and above survivals were selected and used for production of penicillin

In *P. chrysogenum* the biosynthesis of penicillin and lysine have several steps in common (Figure 2). It should be possible to increase penicillin production by the disrupsion of *lys2* gene (Casqueiro et al. 1999). The poor result of penicillin of mutants may be caused by mutation in penicillin biosynthesis genes. The mutation of biosynthesis of penicillin gene may cause decrease enzymes activity for penicillin production.

Table 1. Penicillin production of mutants

P. chrysogenum	Concentration of Penicillin (ppm)
Wild type	**3711**
M1	2534
M2	3193
M3	2674
M4	2068
M5	2482
M6	2638
M7	3193
M8	2859
M9	2650
M10	1635
M11	2087
M12	**4594**
M13	3067
M14	2548
M15	4404
M16	4216
M17	3663
M18	4138
M19	4272

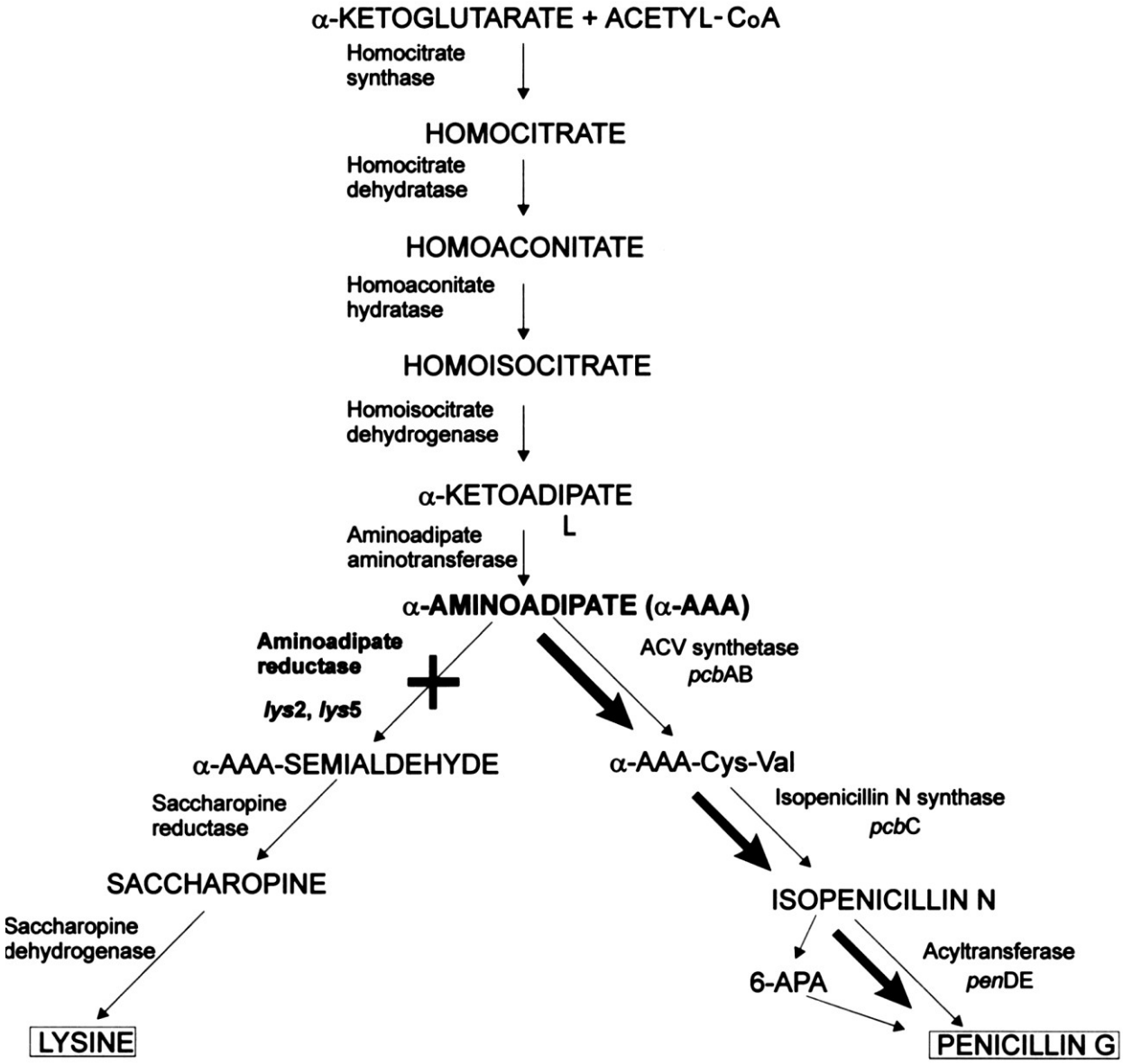

Figure 2. Biosynthesis pathway of penicillin and lysine in *P. chrysogenum* (Casqueiro et al. 1999)

CONCLUSION

The penicillin yield of mutants varied from 1635 to 4594 ppm and mutant M12 from 30 minutes-irradiated produced 1.23 fold compared to wild type.

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