DESAIN DAN *BIOASSAY* INHIBITOR PROTEIN Bgl2 PENYUSUN BIOFILM PADA *Candida albicans*

Safinah, Merry
Pembimbing : **Dr. Afaf Baktir, MS**
CANDIDA ALBICANS; KANAMYCIN
KKC KK TK 02 / 11 Saf d
Copyright© 2011 by Airlangga University Library Surabaya

Abstract

IN SILICO DESIGN AND BIOASSAY OF Bgl 2 LIGAN ACTING AS INHIBITOR OF Candida albicans BIOFILM

Candida albicans is one of the most commonly isolated fungal pathogens in human body and also most commonly associated with biofilm formation. Biofilm protects microorganism from immune system of the host cells and antimicrobials mmunereponses.It is reported that *C. albicans* biofilms are highly resistance to eleven antifungal compounds such as *fluconazole, ketokonazole, amphotericin B, clotrimazole, miconazole, zaragozic acid B, terbinafine, cerulenin, chlorhexidine,* and *nystatin.*Approximately 80 to 90% of the cell wall of *C. albicans* consist of carbohydrate mostly containing polymers of glucose _-1,3 and _-1,6 linkages (_-glucans), 50 – 60%, mannoprotein 30 – 40%, and chitin 0.6 – 9%. Instead of being cell wall components, Bgl2 composed ectracellular biofilm matrix. Using modeling simulation the research blocking the interaction of Bgl – Bgl to formed biofilm. Based on method 2D-Structure matching, activity relationships represent an attempt to correlate structural or property descriptors of compounds with activities. Kanamicyn are the structure which similarity with _-D-glucose. Second method is Virtual Screening using the docking program such as *AutoDock Vina* and *AutoDock*4 and we obtained the docked energy for kanamicyn are -7,71 kcal/mol, and the inhibition constant are 2,23 µM. Kanamicyn has identified the binding interaction at Glu192 and Glu292 which known as the catalytic residues of Bgl2. The effect of 5µM kanamicyn inhibit 61,69% protoplast regeneration and the electro scanning microscopy showed that *C. albicans* growth was predominantly composed of fragile blastospore.

Key Word : C. albicans; Biofilm, Virtual Screening, Kanamycin

SUMMARY

IN SILICO DESIGN AND BIOASSAY OF Bgl 2 LIGAN ACTING AS INHIBITOR OF Candida albicans BIOFILM

This research divided into two part, first is *in silico* study by using *docking* software to found compound which have the best value of binding energy (Gibbs pre energy), inhibition constant and fingerprinting interaction of compound to active site Bgl2 protein. Second is *in vitro* to examined bioactivities selected compound. Interaction

of proteins with ligands, other proteins or the surface is controlled by a complex arrangement intermolecular interaction. Similarly, the interaction between the two depends on two specific interactions, namely interactions of binding pocket, as well as interactions that occur in areas that are not binding pocket. Using Autodock4 kanamycin showed binding energy -7,71 kcal/mol, and using AutoDockVina sowed -7,7 kcal/mol and inhibition constant (Ki) 2,23 μM. Kanamycin showed interaction with Glu 192 and Glu 292, which both of the amino acid residue are the catalytic residue of Bgl2. *In vitro* assay showed that addition of 100 μL of 5μM kanamicyn into 10 mL YNB medium, which is the minimal growth medium for *C. albicans*, the regeneration of inoculated protoplast reduced 82,35% *C. albicans*, whereas on YPD medium reduced 23,65%. Assay for *C. albicans* morphology using *scanning electromagnetic*, showed that addition of 100 μL of 5μM kanamicyn into *C. albicans* protoplast which growth into biofilm formation in YNB medium look more fragile. Result of scanning *electromicroscopy* showed the effect of Kanamycin on *C. albicans* biofilm formation. On weight grain method the normal biofilm showed mass as big as 308 g, and when cells were treated with 100μl of 5μM kanamicyn at beginning time of the incubation, biofilm development was reduced showed with rough architectures predominantly composed of blastospore. Slope of mass biofilm formation are 61,69%. In conclusion kanamicyn can reduce the biofilm formation of *C. albicans*.

RINGKASAN

Penelitian ini terbagi dalam 2 tahapan, pertama secara *in silico* menggunakan program *docking* menguji aktifitas senyawa hasil penapisan ligan dam mendapatkan senyawa terpilih dengan aktifitas terbaik. Kedua secara *in vitro* untuk menguji bioaktifitas senyawa terpilih . Penapisan senyawa ligan didasarkan pada kesamaan struktur dengan castanospermin dan _–D-glukosa, dimana senyawa tersebut masing – masing berfungsi sebagai inhibitor dan substrat protein Bgl2 yang telah dilaporkan oleh Cutfield, *et al*, (1999). Kanamisina menunjukkan nilai energi bebas pengikatan terendah dan nilai konstanta inhibisi -6,1 kkal/mol, dengan nilai konstanta inhibisi (Ki) sebesar 2,23 μM memberikan interaksi ikatan hidrogen sebanyak pada residu Glu 192 dan Glu 292, dimana kedua residu ini merupakan residu katalitik pada protein Bgl2. Pengujian secara *in vitro* menunjukkan bahwa penambahan kanamisina sebanyak 5μM ke dalam 10 mL suspensi biakan cair media YNB protoplas *C.albicans*, dapat menurunkan jumlah pertumbuhan sel *C. albicans* sebanyak 82,35%. Sedangkan dalam media YPD, penurunan pertumbuhan protoplas *C. Albicans* hanya 23,65%. Pengujian terhadap analisis morfologi biofilm *C. albicans* dilakukan menggunakan SEM (*scanning electromagnetic*). Pengamatan dilakukan dengan membandingkan morfologi biofilm *C. albicans* yang ditumbuhkan dalam media biofilm (YNB) antara kontrol (tanpa penambahan kanamisina) dan penambahan kanamisina dengan kadar 5μM sebanyak 100μL pada (Gambar 5.5) di bawah ini. Gambar morfologi menggunakan SEM menunjukkan perbedaan yang signifikan antara biofilm kontrol dengan biofilm yang ditumbuhkan dalam media yang mengandung 5μM kanamisina. Dilakukan pula analisis biofilm menggunakan perhitungan berat kering. Hasil analisis berat kering dari pertumbuhan biofilm *C. albicans* yang ditumbuhkan selama 48 jam dengan peremajaan media setiap 24 jam sekali. Pertumbuhan biofilm *C. albicans* ini dilakukan di atas membran selulosa. Pertumbuhan biofilm *C. Albicans* tanpa penambahan kanamisina (kontrol)

menunjukkan massa 308 mg. Sedangkan untuk massa biofilm *C. albicans* dengan penambahan 5μM kanamisina sebanyak 100μL dalam media pertumbuhannya menunjukkan massa seberat 118 mg. Hasil ini menunjukkan pengaruh kanamisina dalam menghambat terbentuknya biofilm *C. albicans*. Penurunan massa biofilm akibat penambahan kanamisina sebesar 61,69%.