# Mantar Biyofilm İnfeksiyonlarında Tedavi

Dr. Ömrüm Uzun

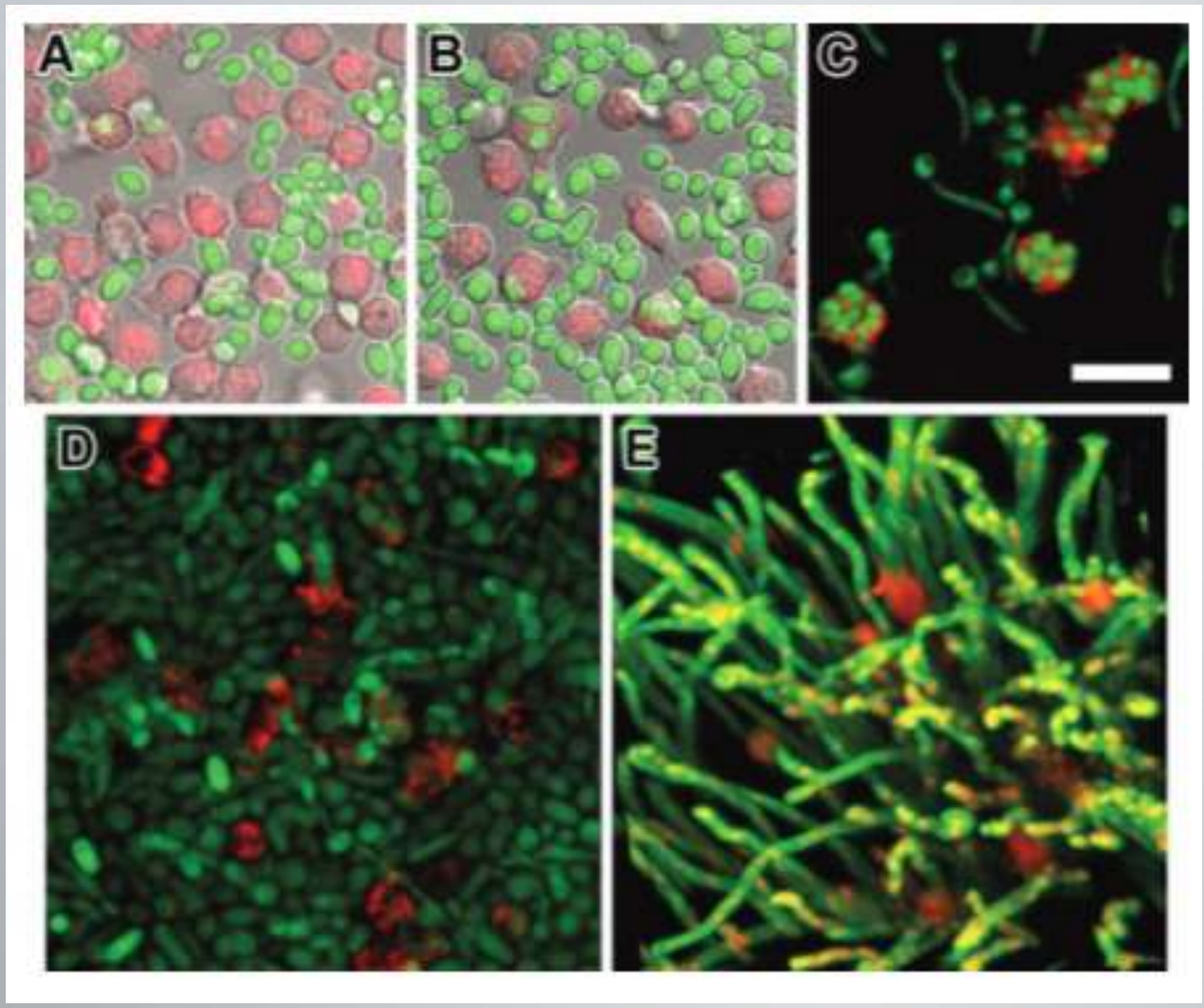
# Fagosit-Biyofilm Etkileşimi

*Katragkou A, et al. JID 2010;201:1941.*

**Table 1. Comparative Effects of Human Phagocytes (Polymorphonuclear Leukocytes [PMNs] and Monocytes) on Damage to *Candida albicans* Biofilms and Planktonic Cells, as Determined by XTT Assay**

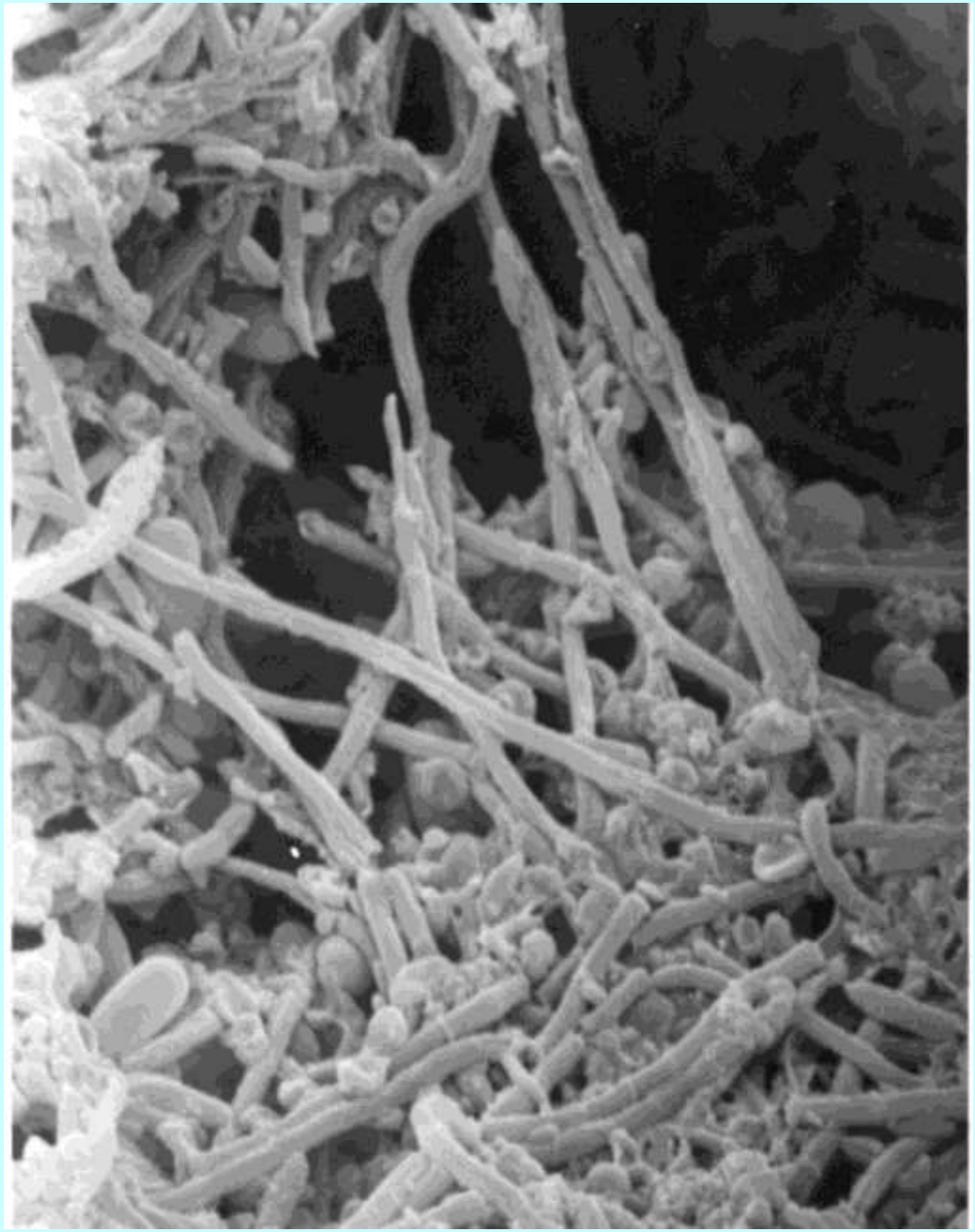
| Treatment time, E:T ratio | PMNs | | Monocytes | |
|---|---|---|---|---|
| | Biofilm | Planktonic | Biofilm | Planktonic |
| 2 h | | | | |
| 1:1 | 20.9 ± 3.9 | 40.4 ± 8.8[a] | 22.8 ± 3.0 | 48.4 ± 22.9[b] |
| 5:1 | 40.3 ± 6.1 | 86.6 ± 1.0[a] | 27.6 ± 5.9 | 66.2 ± 7.6[b] |
| 10:1 | 50.2 ± 4.8 | 84.0 ± 1.6[a] | 43.9 ± 9.7 | 73.8 ± 1.3[b] |
| 22 h | | | | |
| 1:1 | 13.0 ± 3.7 | 30.0 ± 5.5[a] | 12.4 ± 3.1 | 26.5 ± 5.8[a] |
| 5:1 | 29.9 ± 5.4 | 52.7 ± 3.6[a] | 28.2 ± 4.9 | 47.3 ± 3.9[a] |

Sonuçlar % harabiyet olarak verilmiştir.




Üst sıra: planktonik hücreler (yeşil), monositler (kırmızı), 0., 30. dk ve 2.saat
Alt sıra biyofilm hücreleri 2 ve 22. saatler (monositler yoğun biyofilm ağında sıkışmış)

*Katragkou A, et al. JID 2010;201:1941*



Biyofilm içindeki *Candida* suşları antifungal ilaçlara dirençlidir.

*Baillie et al. Methods Enzymol 1999;310:644*
*Chandra et al. J Bacteriol 2001;183:5385.*

# Biyofilm, Antifungaller ve Genetik

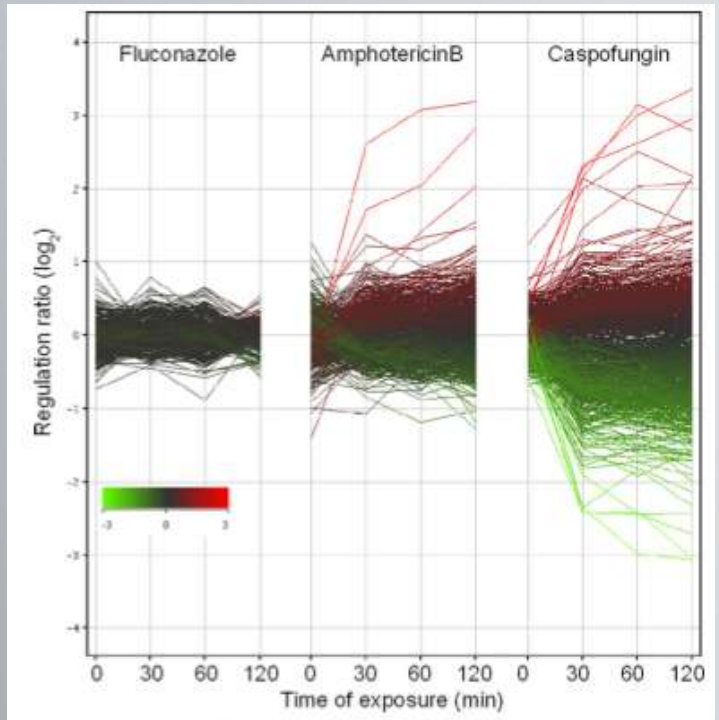



FIG. 1. Differential gene expression in mature antifungal-exposed biofilms. Thirty-hour-old *C. albicans* SC5314 biofilms were exposed to fluconazole (80 μg/ml), amphotericin B (8 μg/ml), or caspofungin (5 μg/ml). Age-matched control biofilms and antifungal-exposed biofilms were collected and subjected to transcript profiling. Data were analyzed and viewed with GeneSpring software. The ratios of gene expression between antifungal-exposed and control biofilms are shown for all *C. albicans* genes. Graphs are colored according to the ratio observed at 120 min postexposure for each antifungal.

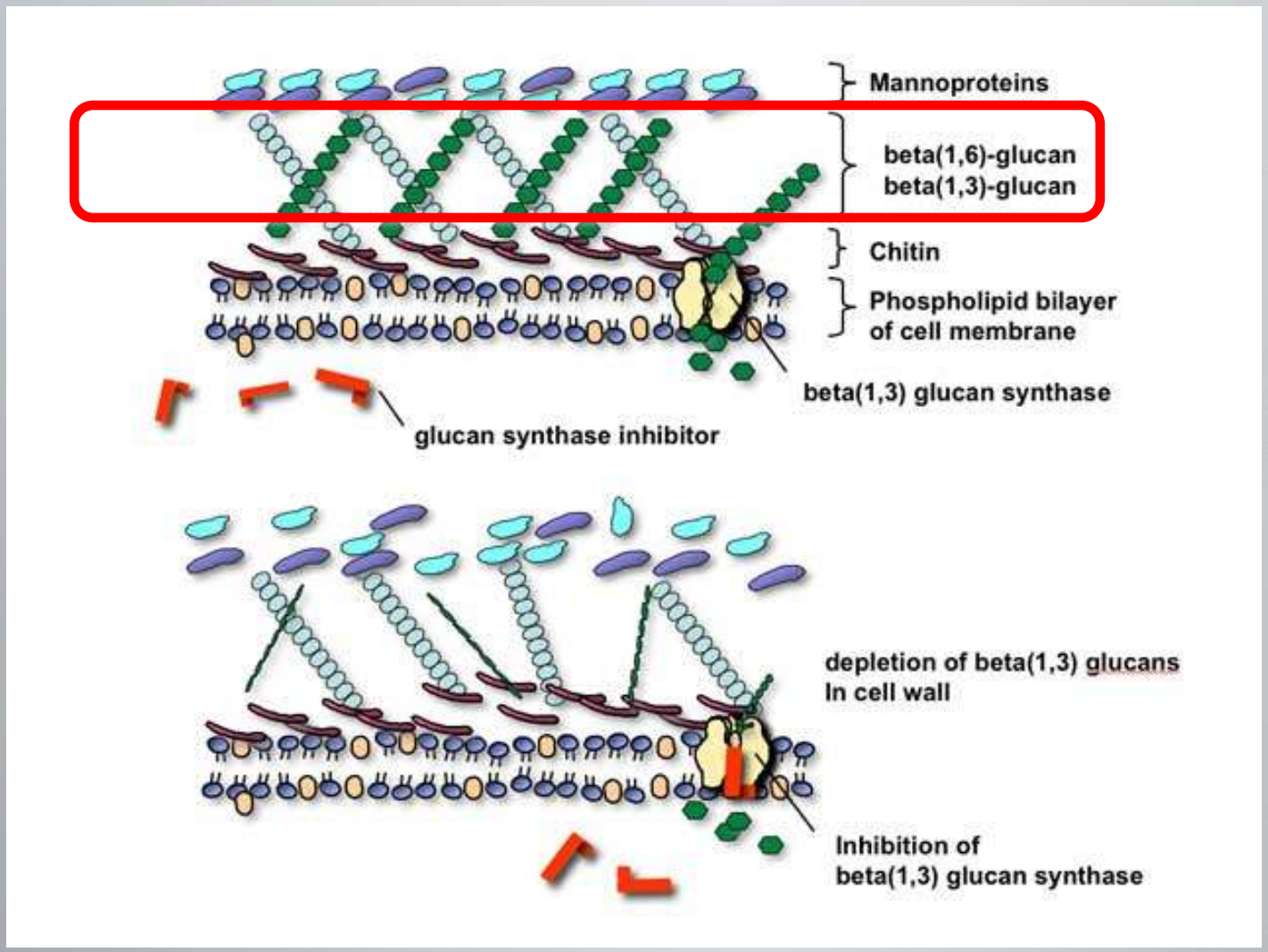
- **Matür *Candida* biyofilmleri planktonik hücreler için letal konsantrasyonda FLU, AMB ve CAS'e 2 saat maruz bırakıldığında letal etki saptanmadı.**

- **FLU ile gen ekspresyonunda bir değişiklik gözlenmez iken AMB ile genlerin %2.7'sinde, CAS ile %13.0'ünde değişiklik saptandı. Özellikle CAS ile biyofilm yapımında rol oynayan ALS3 ve HWP1 gibi hif-spesifik genler ''upregüle'' oldu.**

*Vediyappan G, et al. AAC 2010;54:2096.*

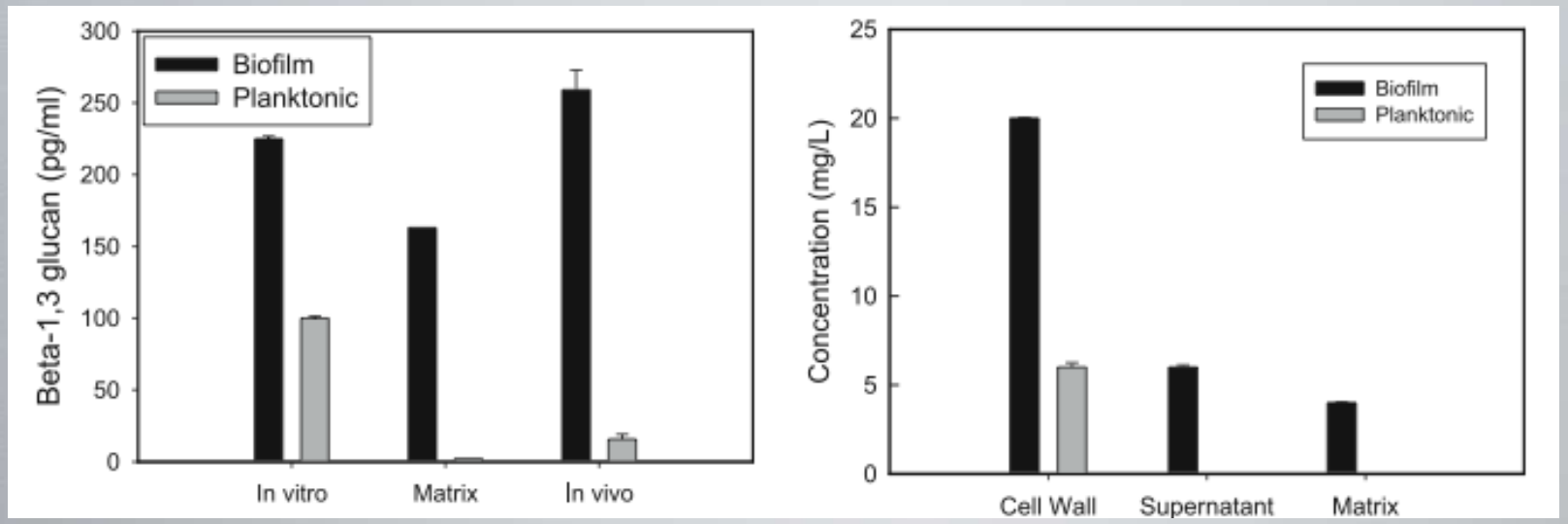
# DİRENÇTEN SORUMLU: BİYOFİLMİN KENDİ YAPISI

# Biyofilmde Direnç Mekanizması?



# Biyofilm Direncinde β-glukan

*Nett J, AAC 2007;51:510*

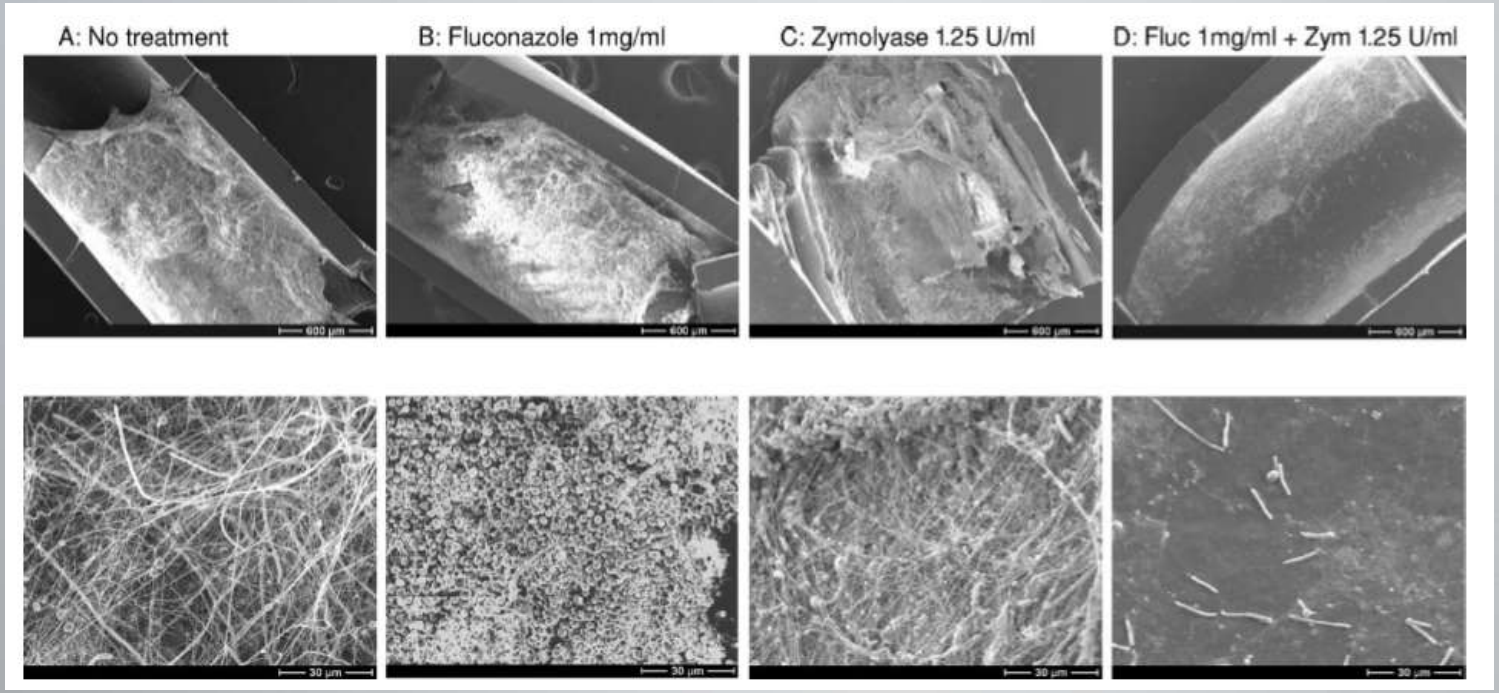



*C.albicans* biyofilm ve planktonik *C.albicans*'dan in vitro ve in vivo elde edilen ekstrasellüler beta-glukan konsantrasyonları.

FLC'un biyofilm ve planktonik hücre duvarı, kültür süpernatanı ve biyofilm matriksine bağlanması

# Biyofilm Direncinde β-glukan

*Nett J, AAC 2007;51:510*




Glukanaz etkisi

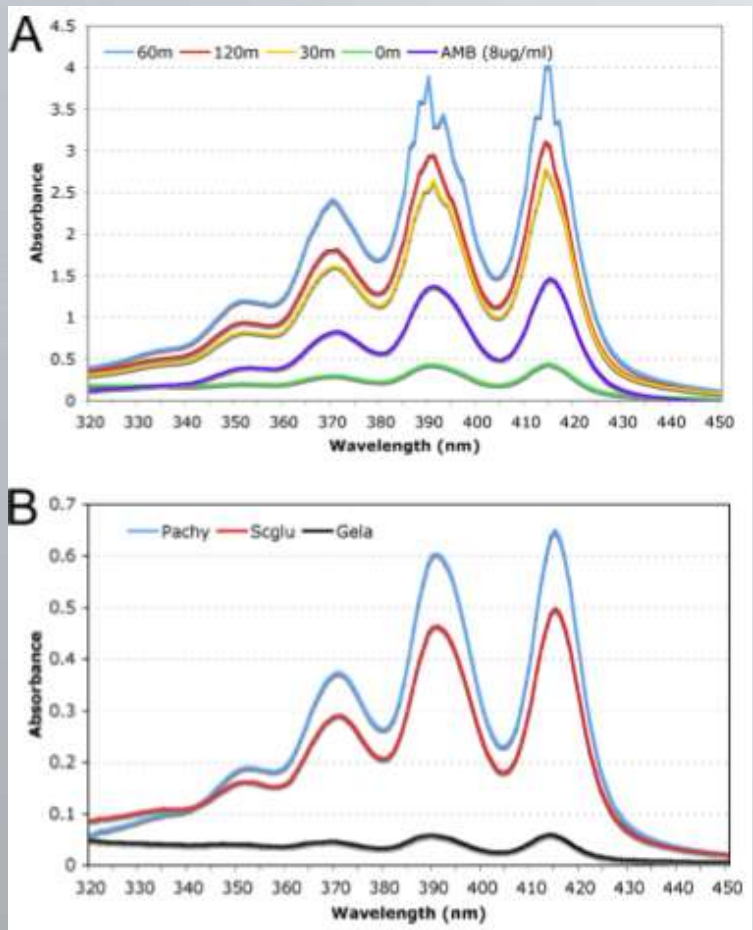


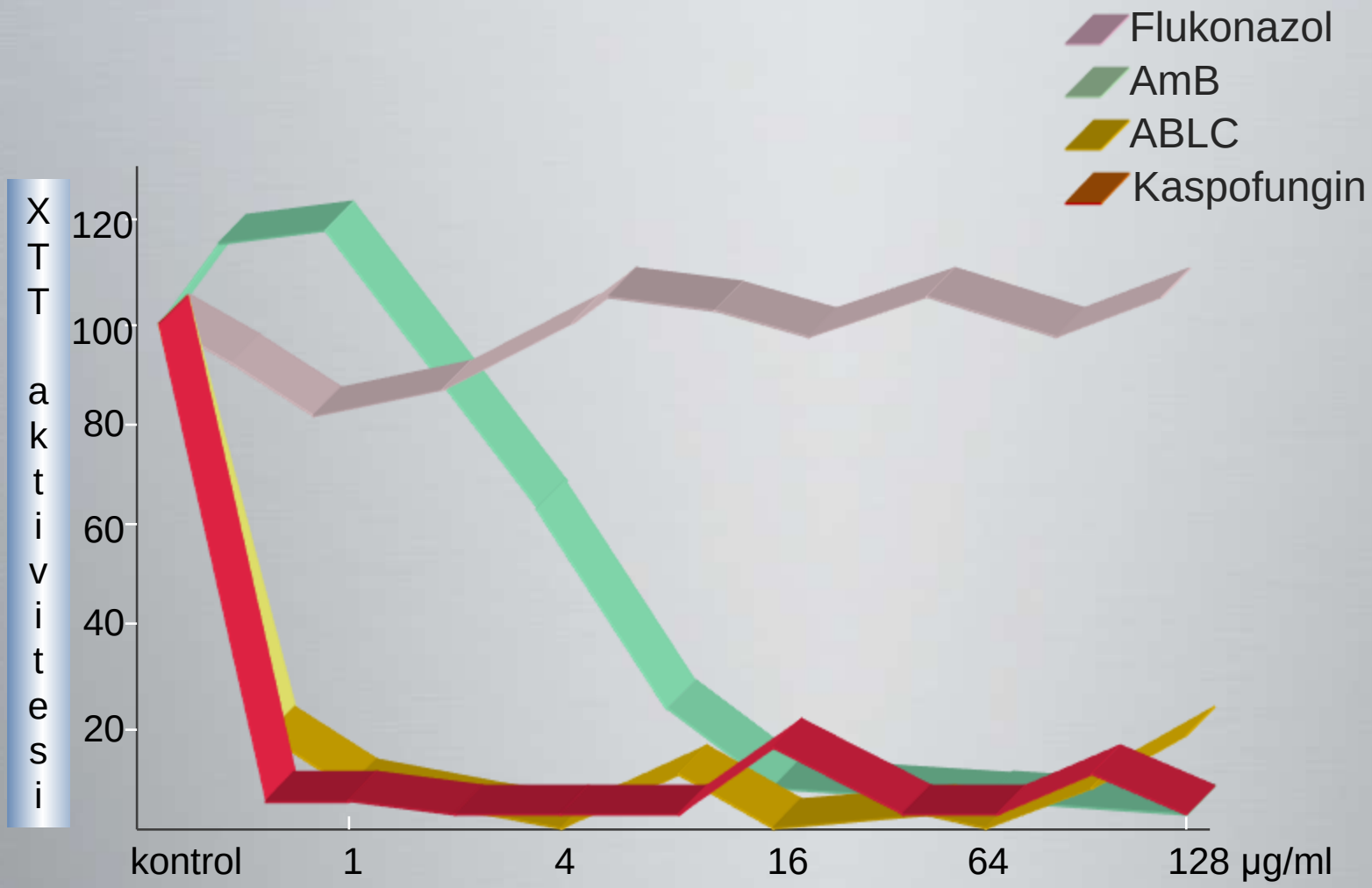

FIG. 2. UV-visible absorption spectra of AMB extracted from biofilms and β-1,3-glucans. (A) One hundred percent DMSO (1 ml) was used to extract AMB-exposed biofilms (0, 30, 60, and 120 min post-AMB exposure), and extracts were scanned for absorption maximum spectra. The biofilm medium containing AMB (8 μg/ml) was also scanned in parallel as a control. (B) Insoluble β-1,3-glucans derived from *Saccharomyces cerevisiae* (Scglu) and *Poria cocos* (Pachy) were incubated with AMB. After removal of unbound AMB, polymer-bound AMB was extracted by 100% DMSO and analyzed as described for panel A. Gelatin (as a nonspecific control) was treated similarly for AMB binding and was analyzed in parallel.

AMB ve FLU ekstrasellüler matriksteki beta-glukana bağlanır, yüksek konsantrasyonuna karşın hücreye ulaşımı engellenir.

*Vediyappan G, et al. AAC 2010;54:2096.*

# Biyofilmdeki *Candida*'ya antifungallerin etkisi

***Kuhn et al. AAC 2002;46:1773.***



# ABLC kateter-"lock""

*Mukherjee PK et al. Int J Antimicrob Agents 2009;33:149*

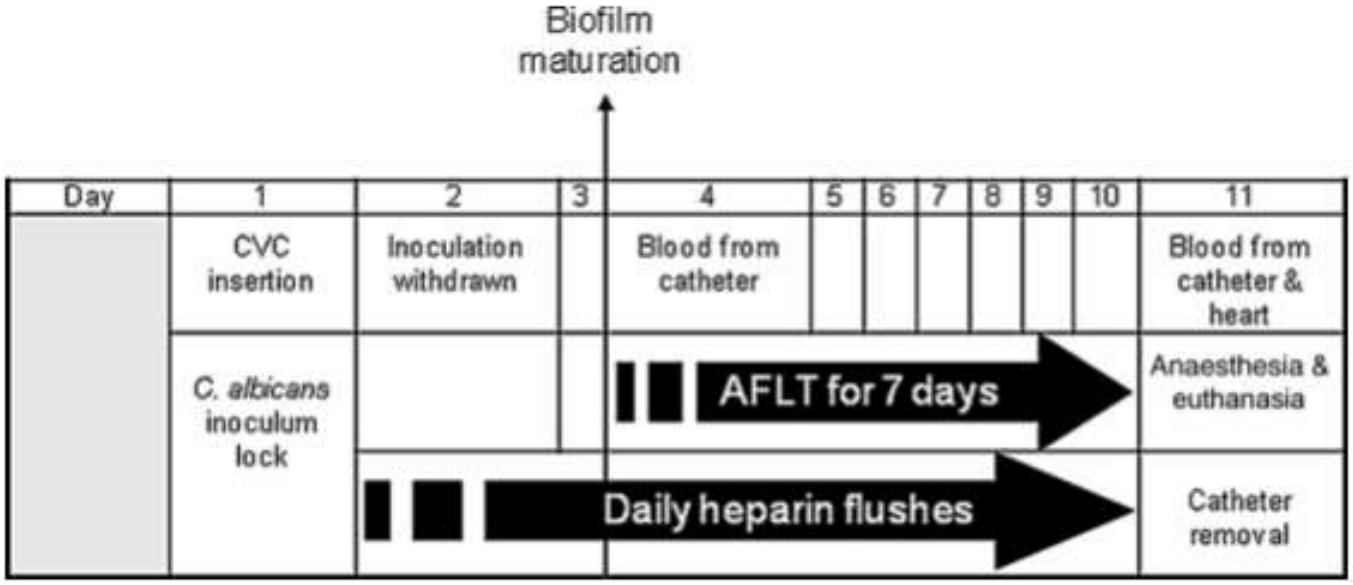
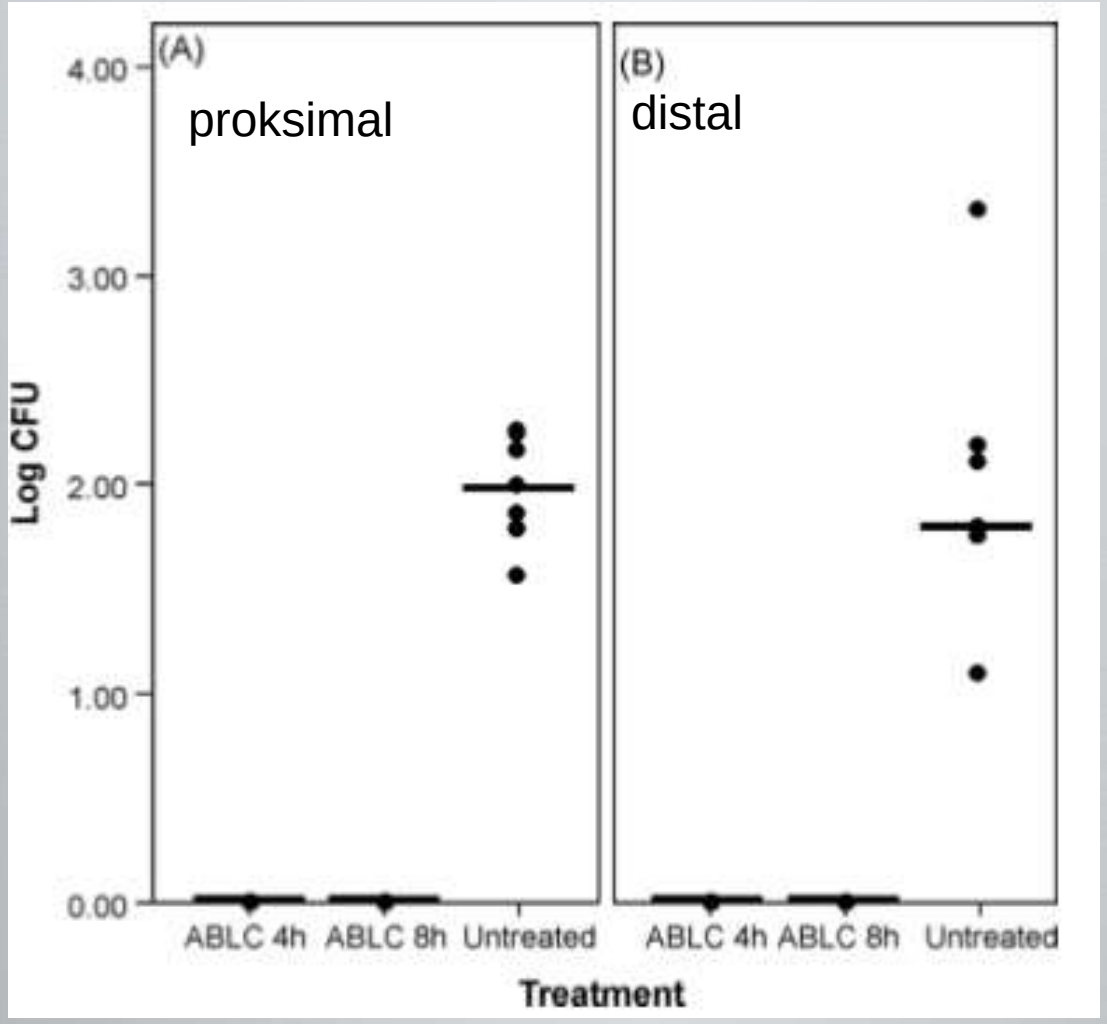



**Fig. 1.** Schedule for biofilm formation and antifungal lock therapy (AFLT) in vivo.

# ABLC kateter-"lock""

*Mukherjee PK et al. Int J Antimicrob Agents 2009;33:149*



# ABLC kateter-”lock””

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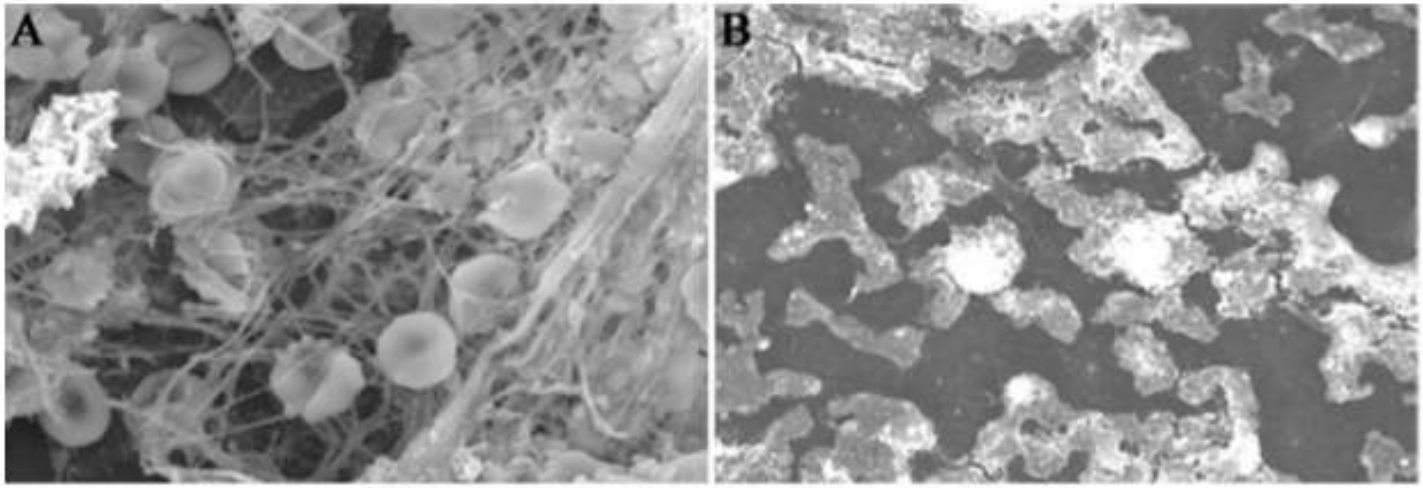


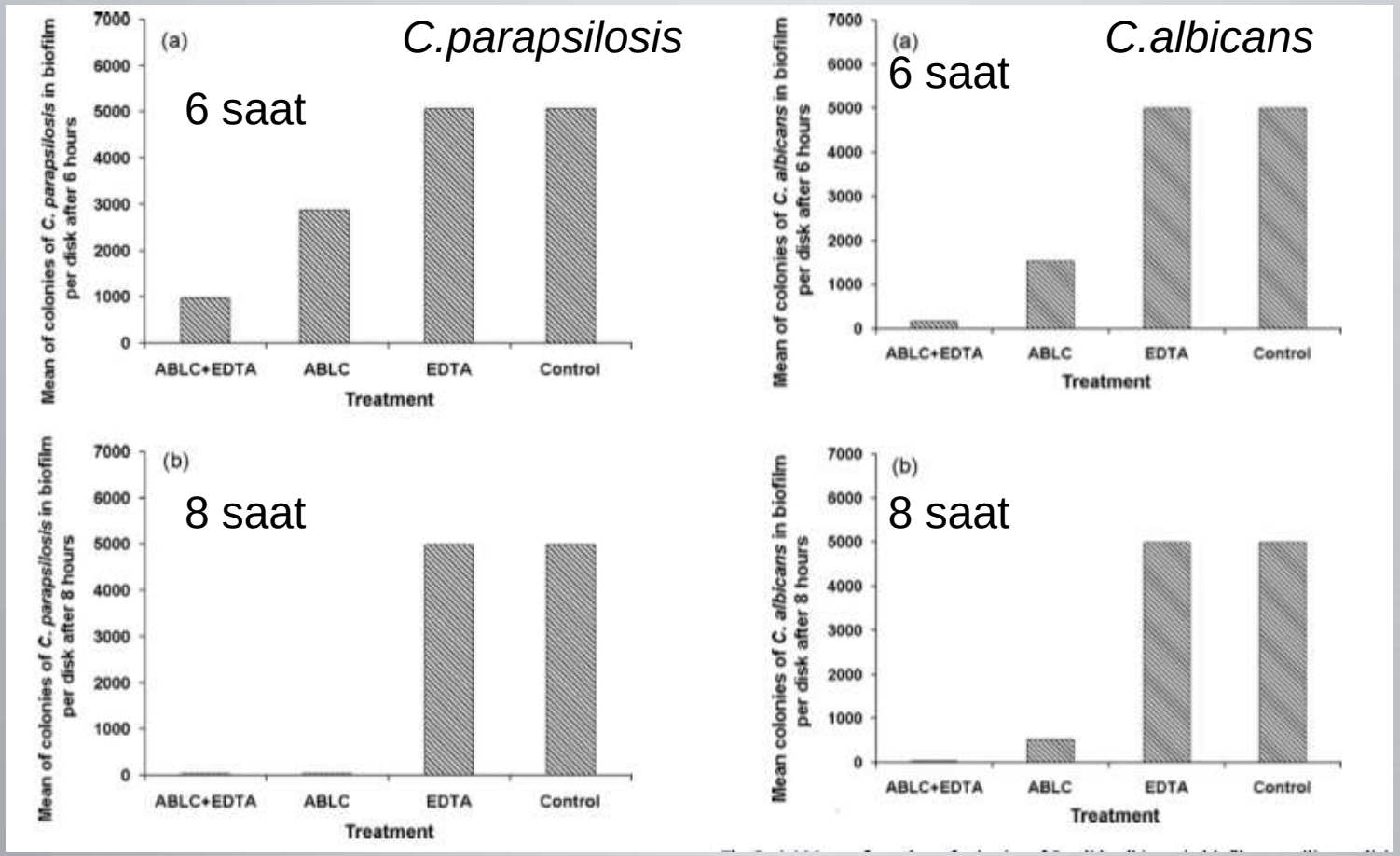

**kontrol**

**4 saat**

**8 saat**

# EDTA ±ABLC kateter-"lock"

Raad II, Int J Antimicrob Agents 2008;32:515.



# *C. albicans* biyofilmine karşı ANI'nin in vivo etkinliği

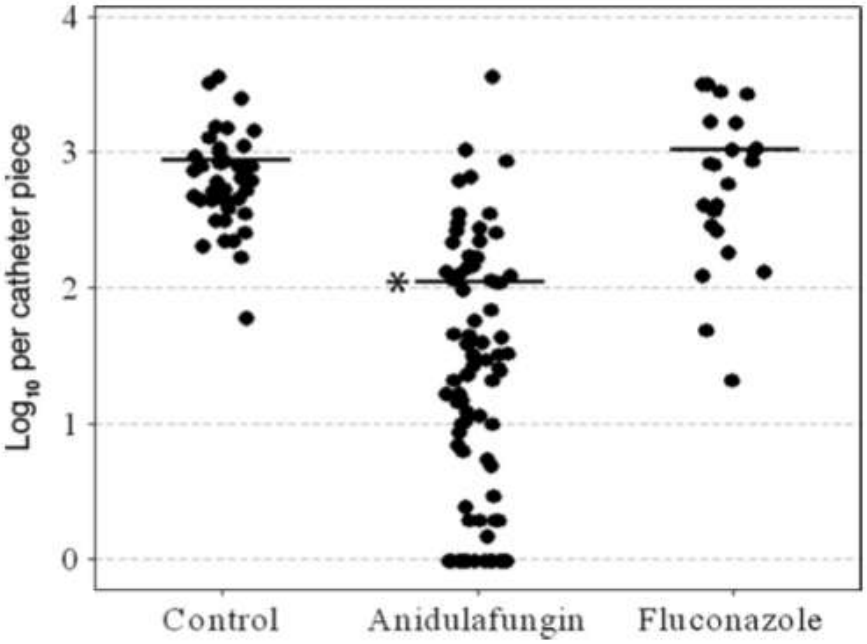
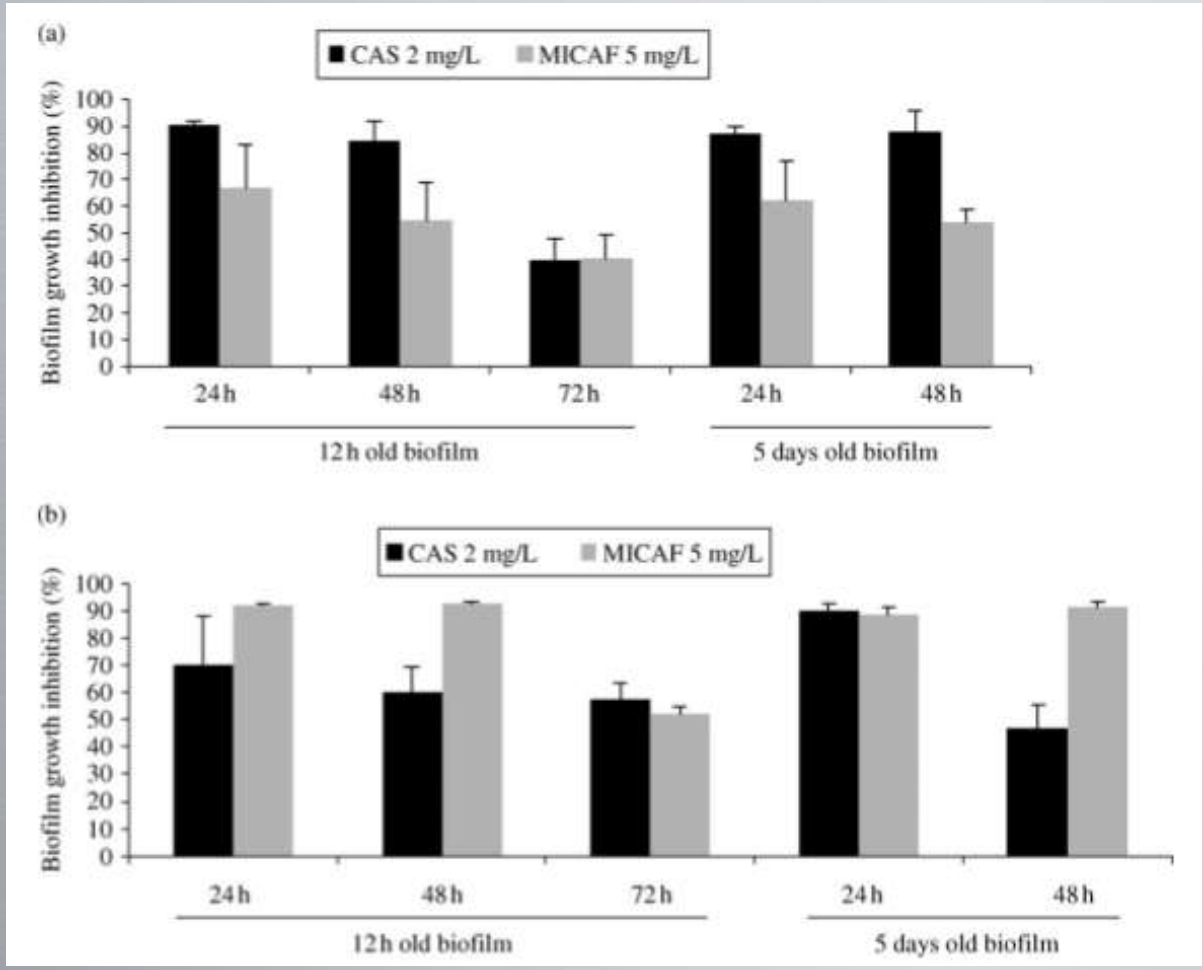



FIG. 1. Effect of antifungal intraperitoneal treatment on mature *Candida* biofilms formed on the catheter's lumen in a rat model. The $\log_{10}$ numbers of CFU of *Candida albicans* cells cultured from each catheter in the control group, the anidulafungin group, and the fluconazole treatment group are shown. The horizontal line shows the median values for $\log_{10}$ number of CFU obtained per catheter fragment. A significant difference was found between the anidulafungin-treated rats and the control group (*, $P < 0.0001$).

*Kucharikova S, et al. AAC 2010;54:4474.*

# Ekinokandin kateter-”lock”

*Cateau E, et al. JAC 2008;62:153*

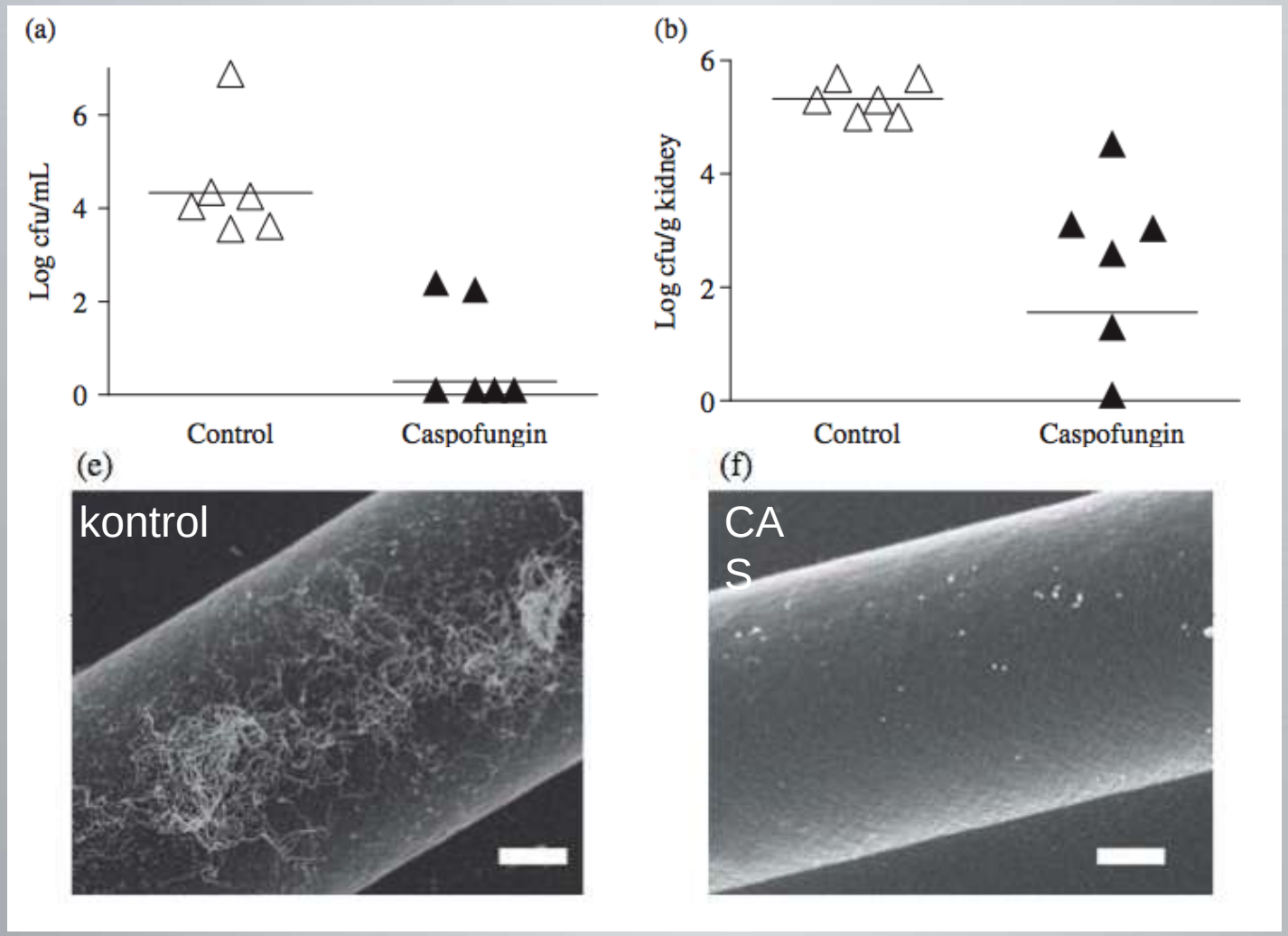



*C.albicans*
ATCC 3153
biyofilm

*C.albicans*
ATCC 66396
biyofilm

# *C. albicans* biyofilmi ve CAS in vivo

***Lazzell AL, et al. JAC 2009;64:567***

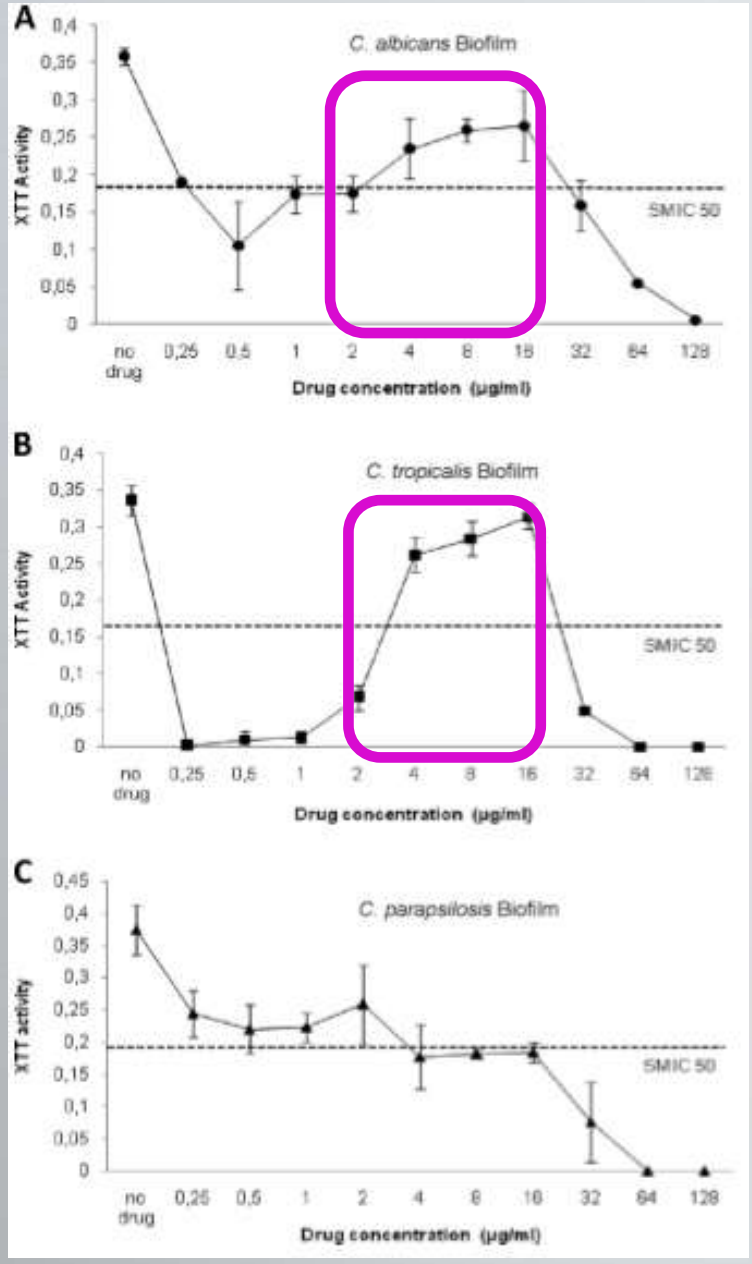


# Biyofilmde paradoksik etki

TABLE 1. Antifungal susceptibilities of different *Candida* sp. isolates under planktonic and biofilm growth conditions as determined using the CLSI and XTT methods[a]

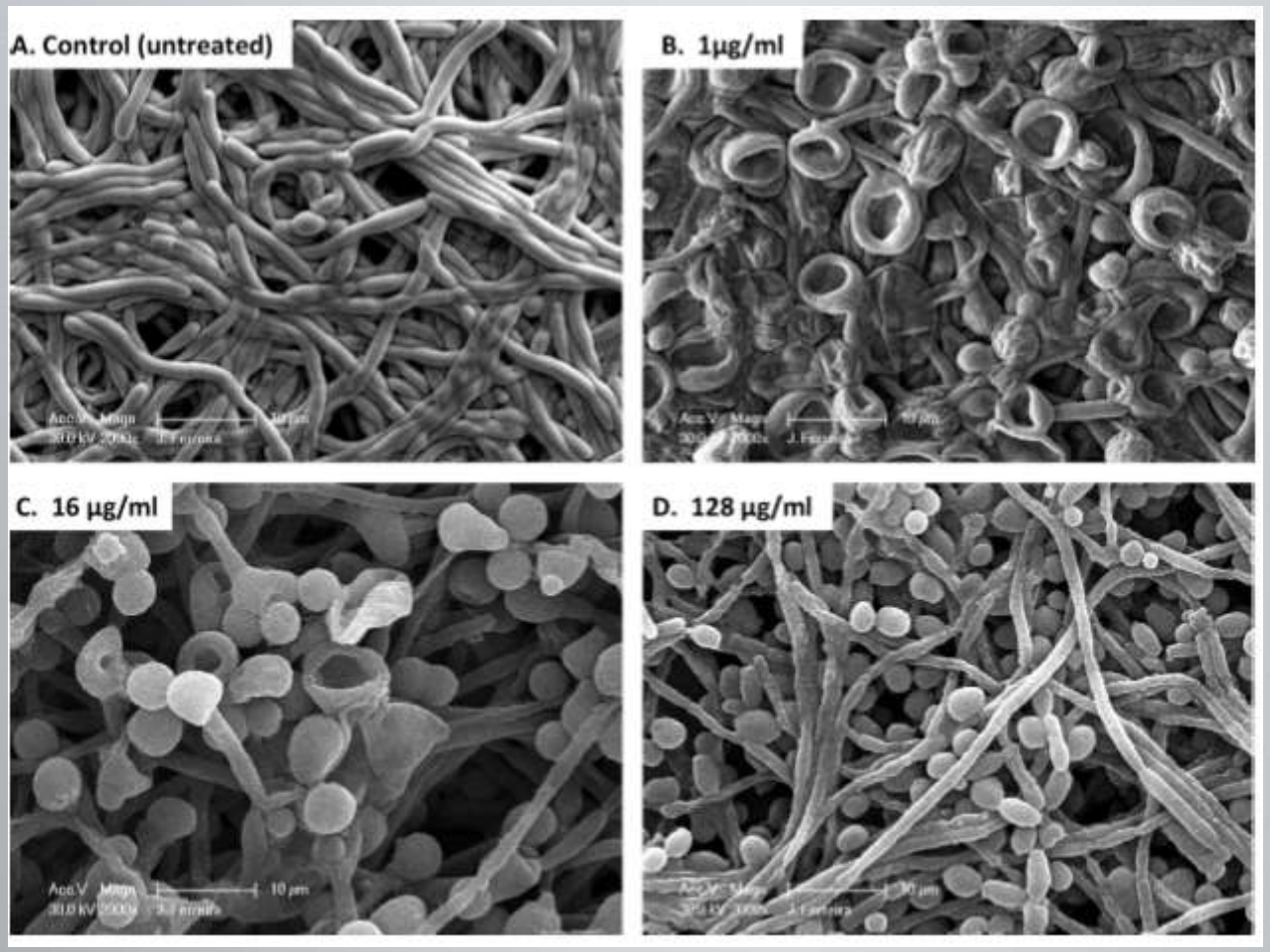
| Isolate | MIC (μg/ml) of CAS for: | |
|---|---|---|
| | Planktonically grown cells[b] | Biofilms at 48 h ($SMIC_{50}$)[c] |
| *C. albicans* (CA4) | 0.0625 | 0.5 |
| *C. parapsilosis* (CP1) | 0.25 | 4 |
| *C. tropicalis* (CT8) | 0.0625 | 0.25 |

*Ferreira JAC, AAC 2009;53:4377.*



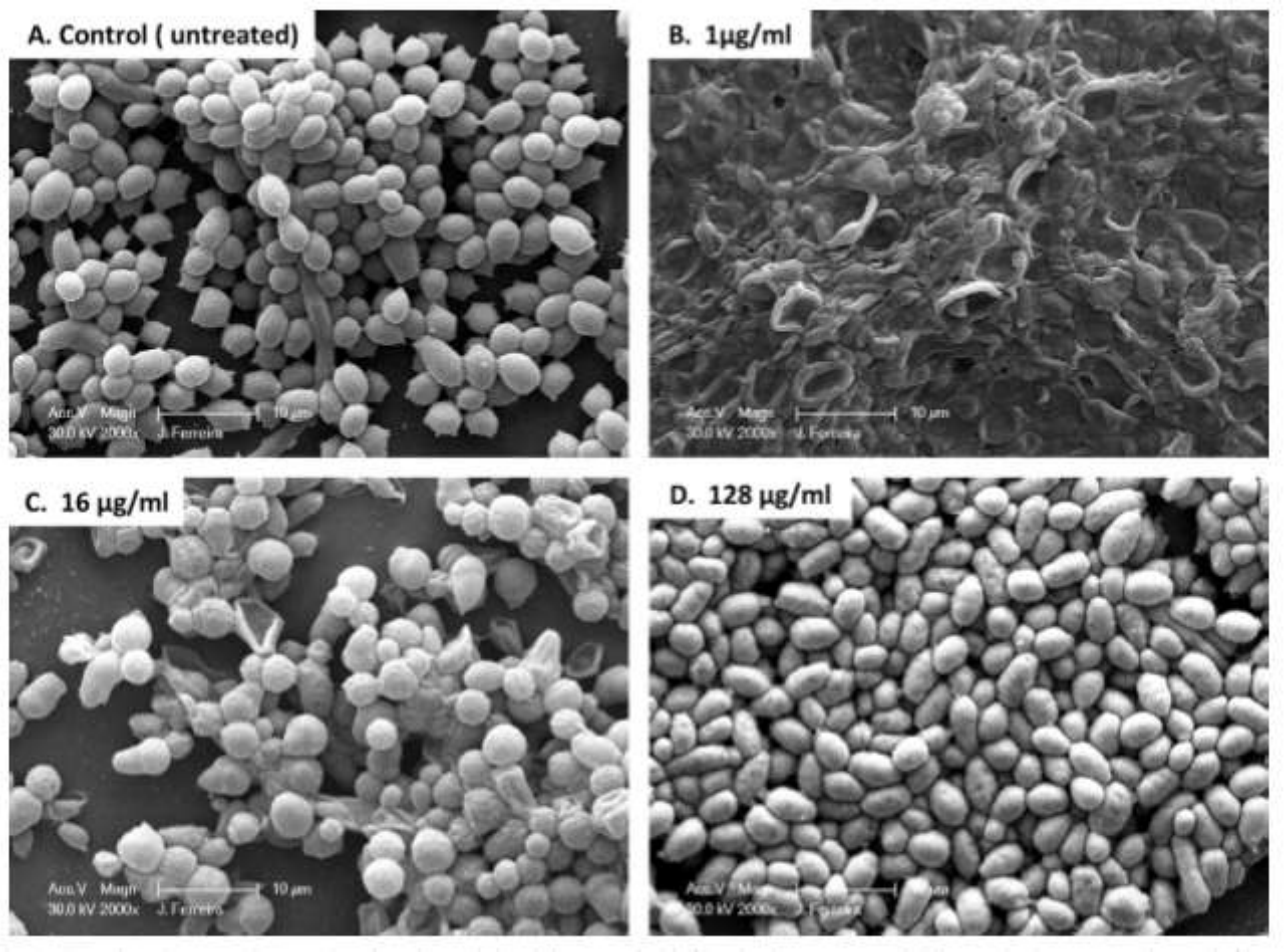

*Ferreira JAC, AAC 2009;53:4377.*

# *C. albicans* - CAS




*Ferreira JAC, AAC 2009;53:4377.*

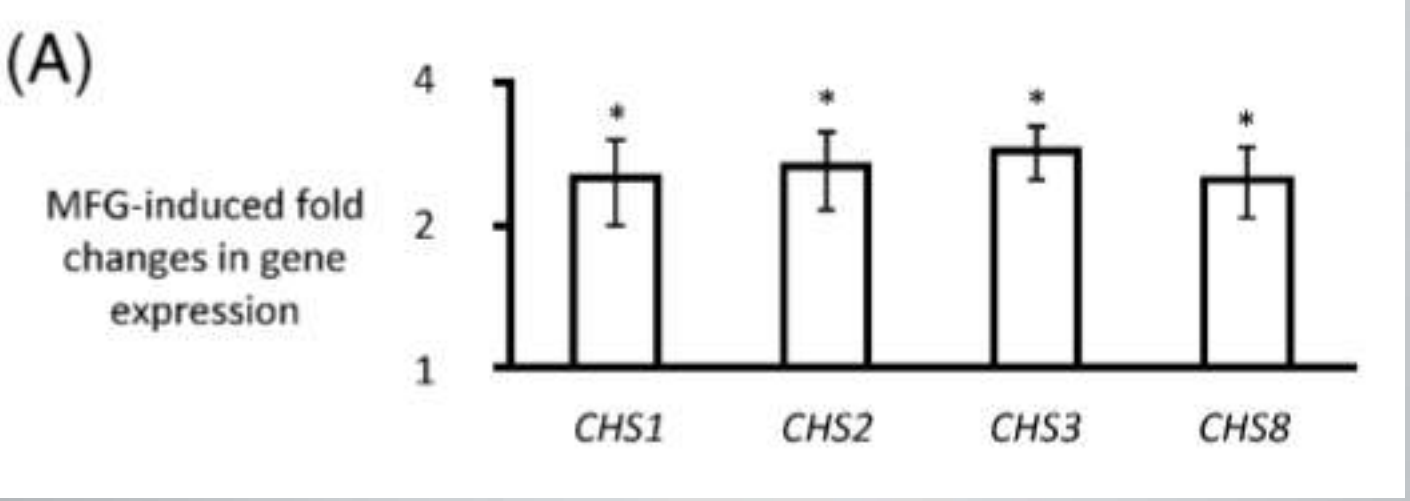
# *C. tropicalis* - CAS




*Ferreira JAC, AAC 2009;53:4377.*

# MICA'nin etkisi

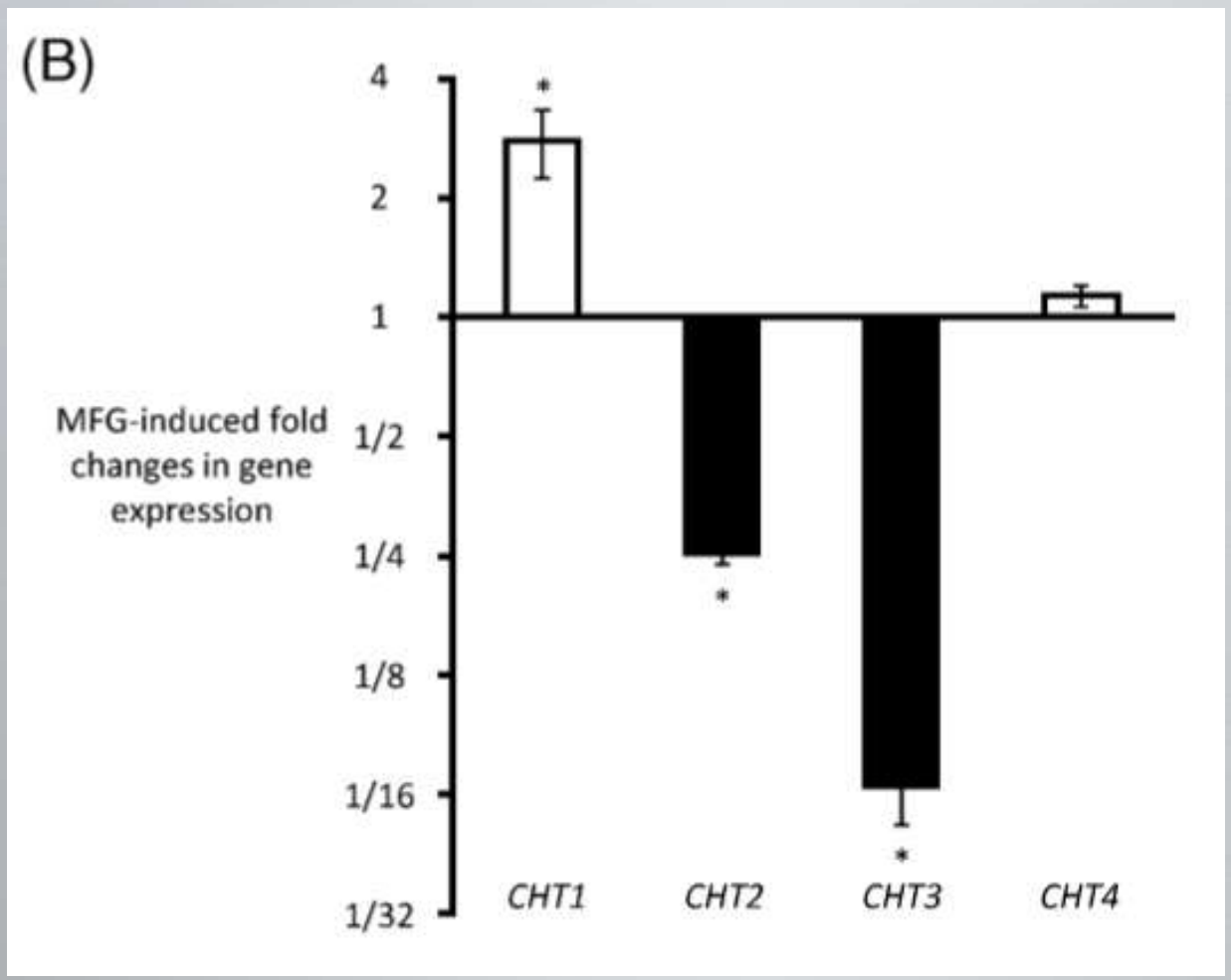
*Kaneko Y, et al. Jpn J Infect Dis 2010;63:355.*




Kitin sentazları kodlayan genler

# MICA'nin etkisi

*Kaneko Y, et al. Jpn J Infect Dis 2010;63:355.*




Kitinazları kodlayan genler

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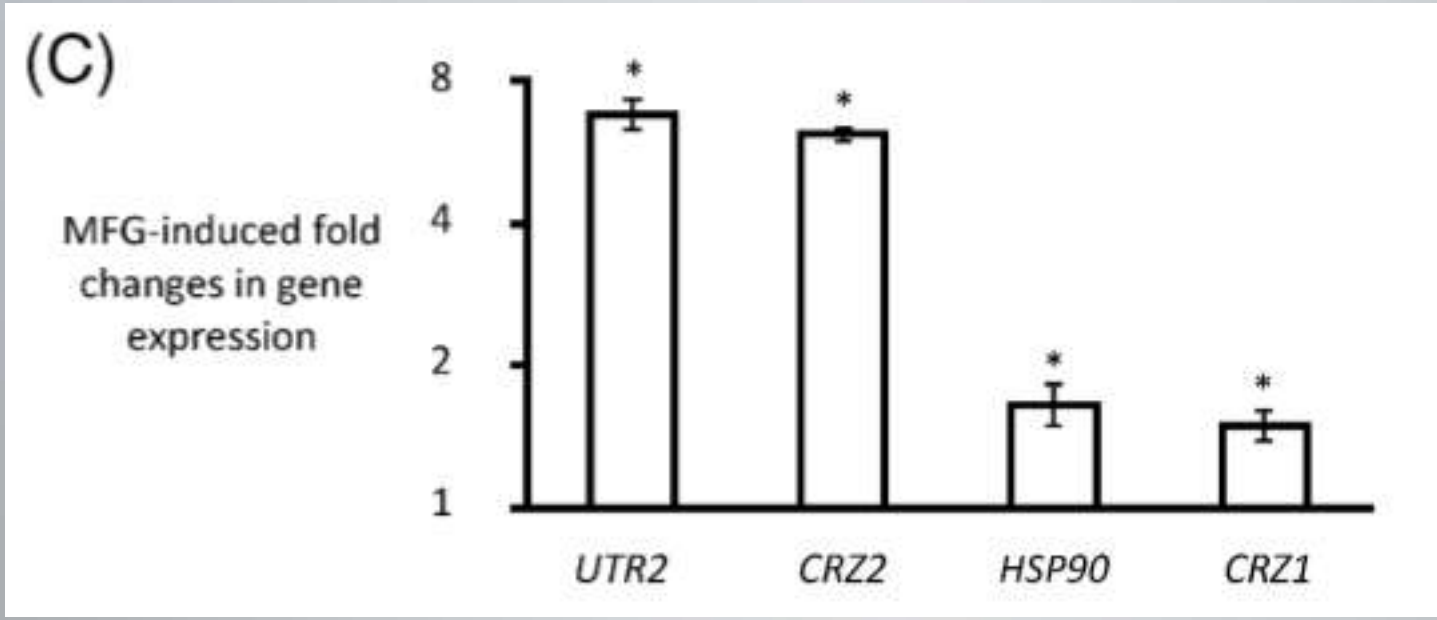
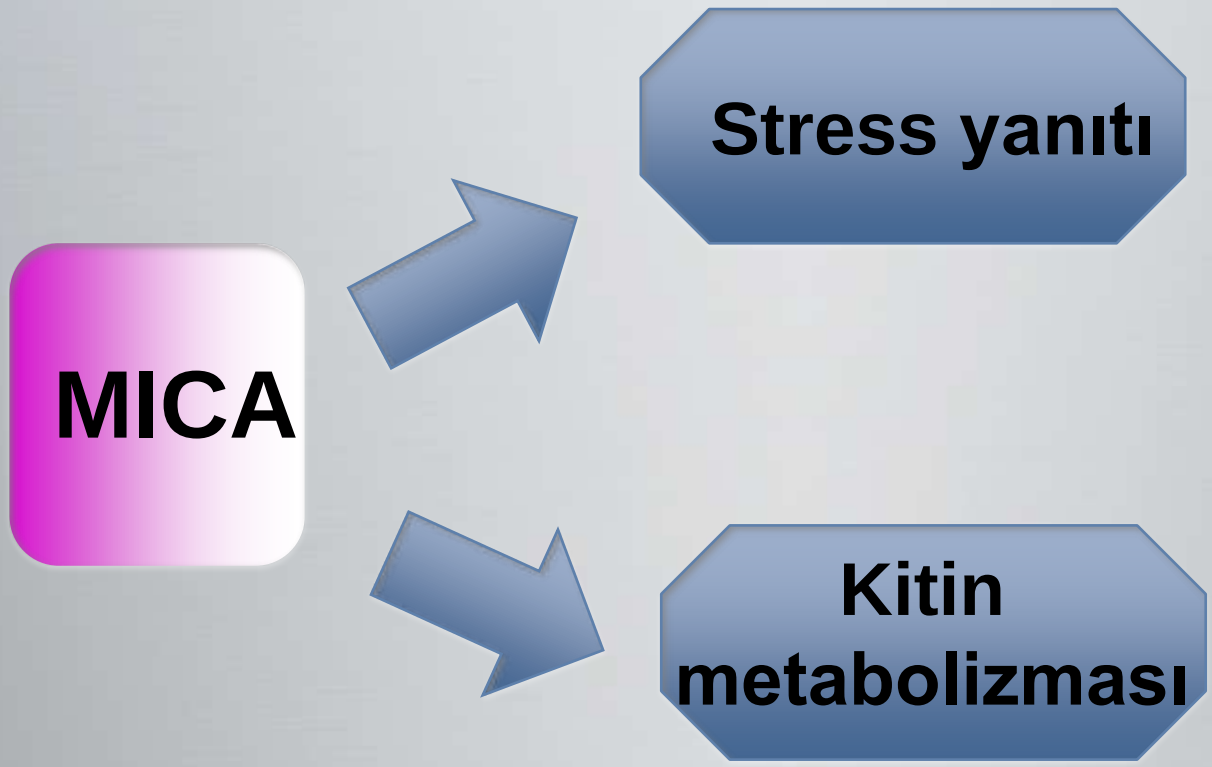



UTR2 calcineurin bağımlı promoter içeren stress yanıtını kodlayan gen
CRZ2: calcineurin-bağımsız promoter içeren stress yanıtını kodlayan gen

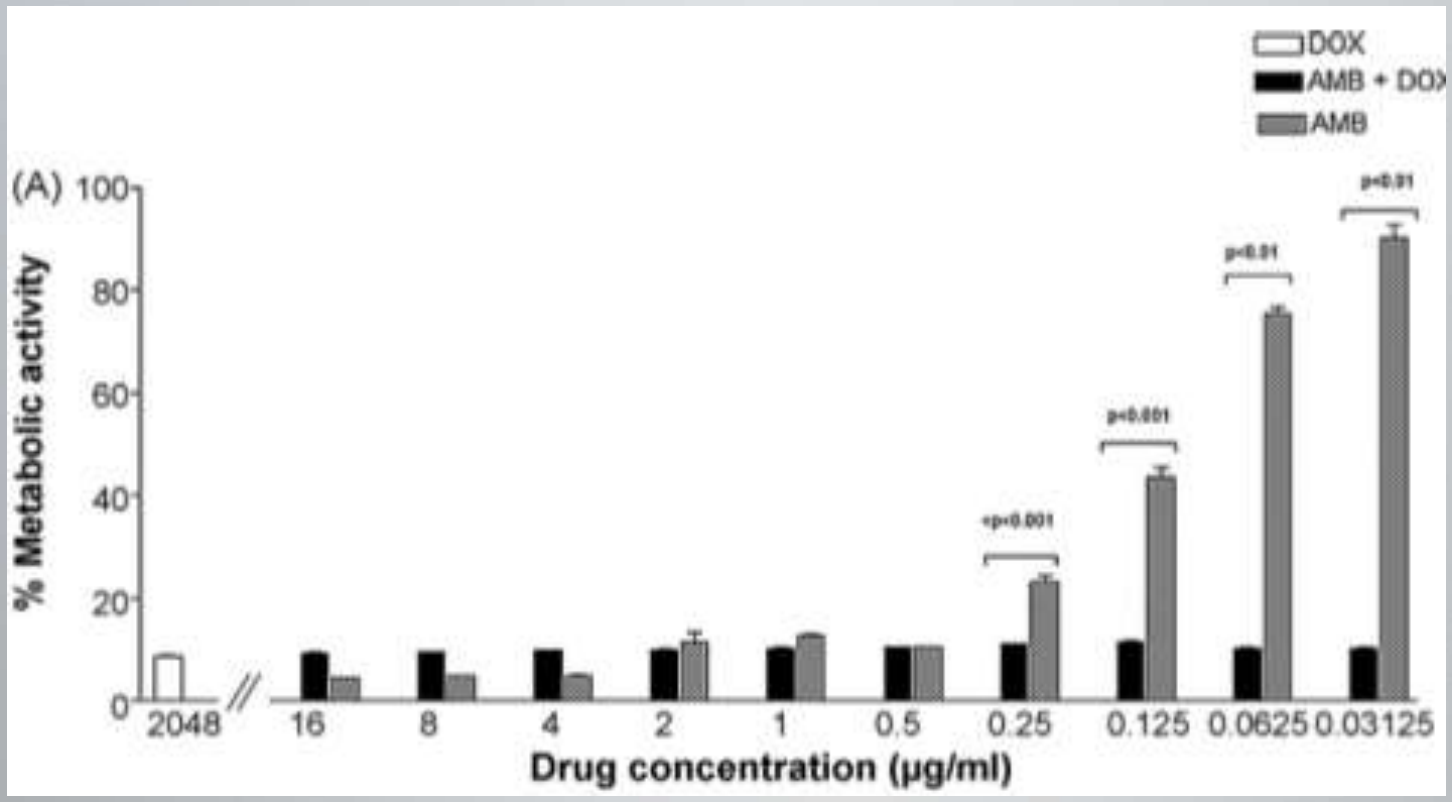
# MICA'nin etkisi

*Kaneko Y, et al. Jpn J Infect Dis 2010;63:355.*



# DOX±AMB kateter-"lock"

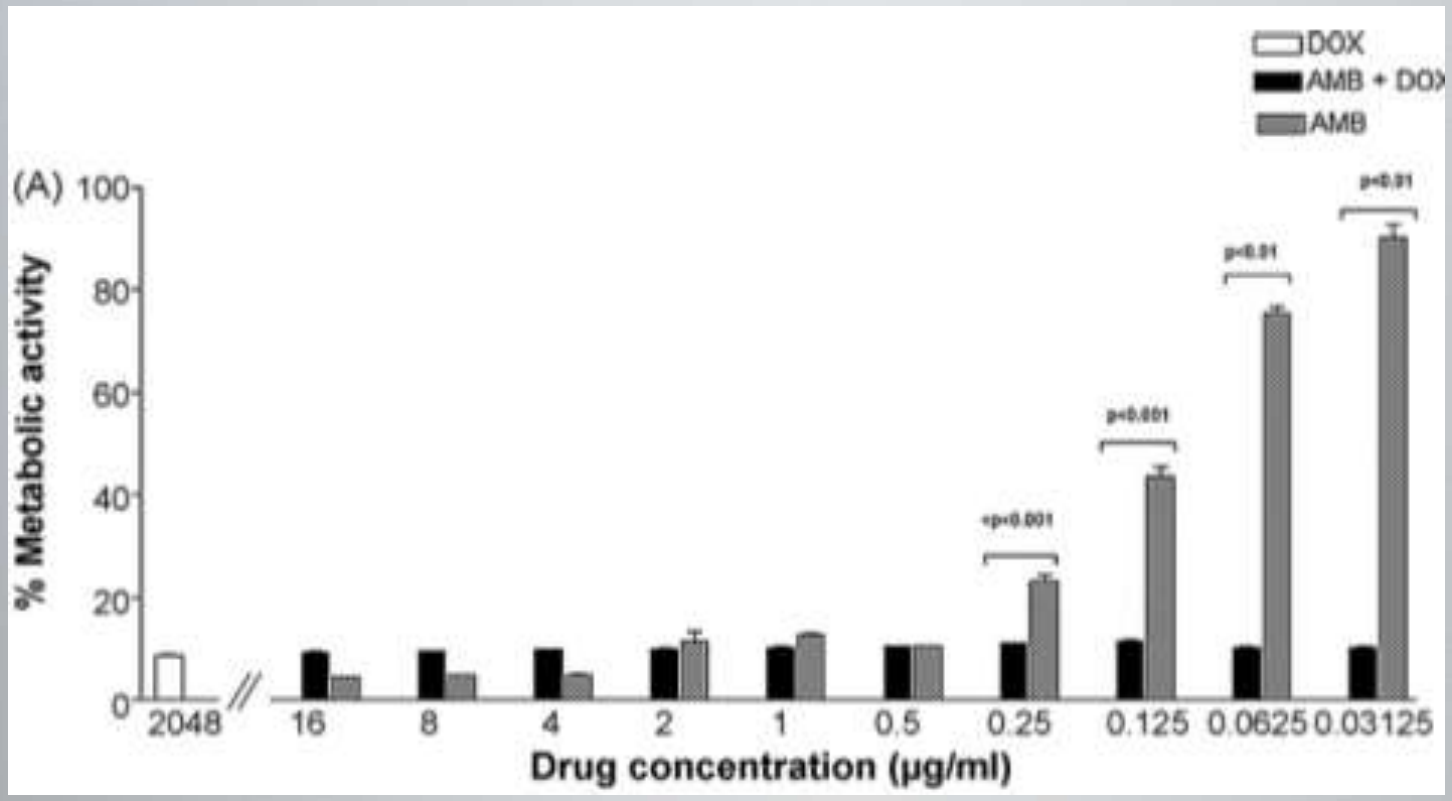
*Miceli MH, et al. Int J Antimicrob Agents 2009;34:326*




A: DOX 2048 mcg/ml

# DOX±AMB kateter-"lock"

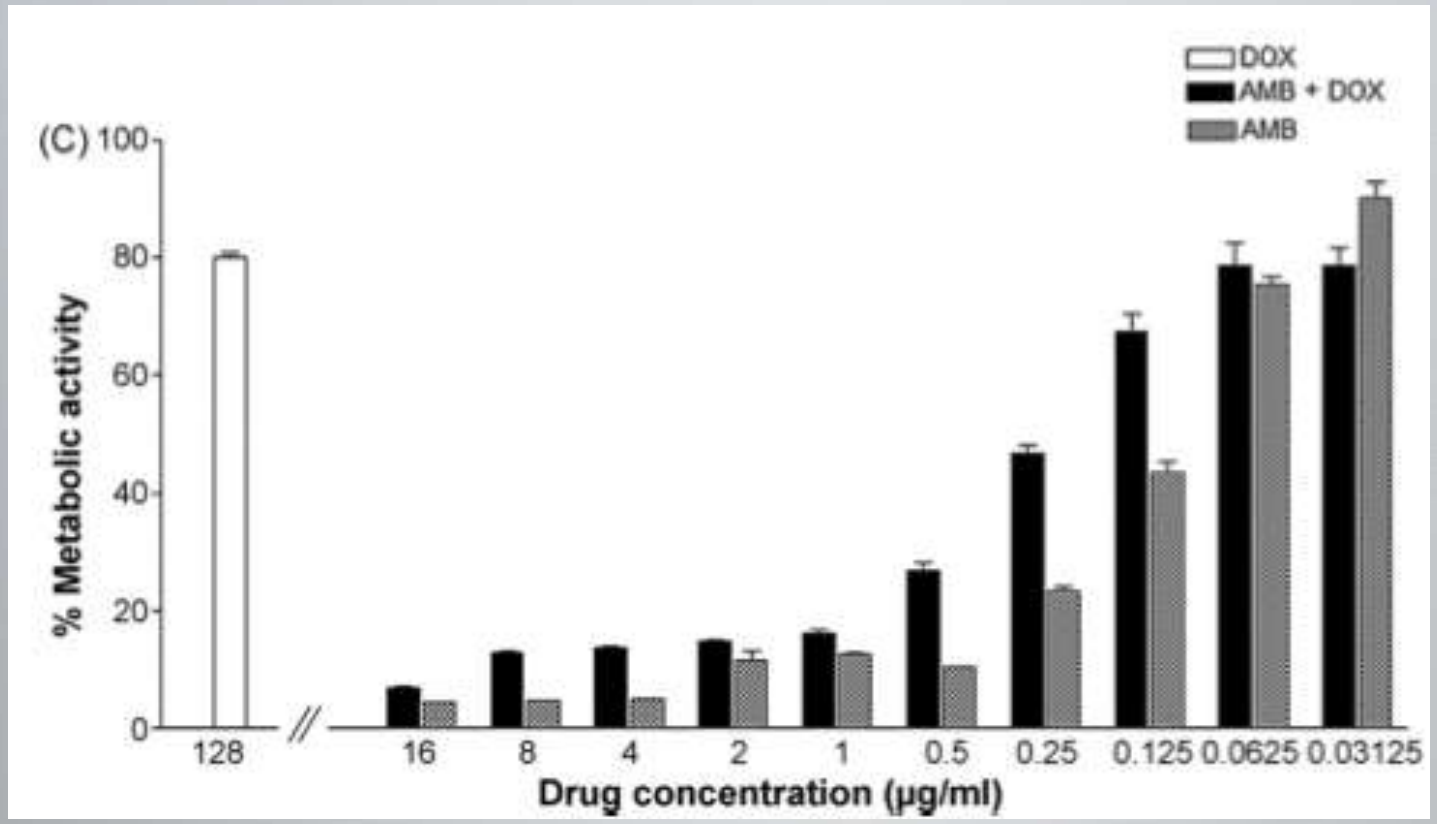
*Miceli MH, et al. Int J Antimicrob Agents 2009;34:326*




B: DOX 512 mcg/ml

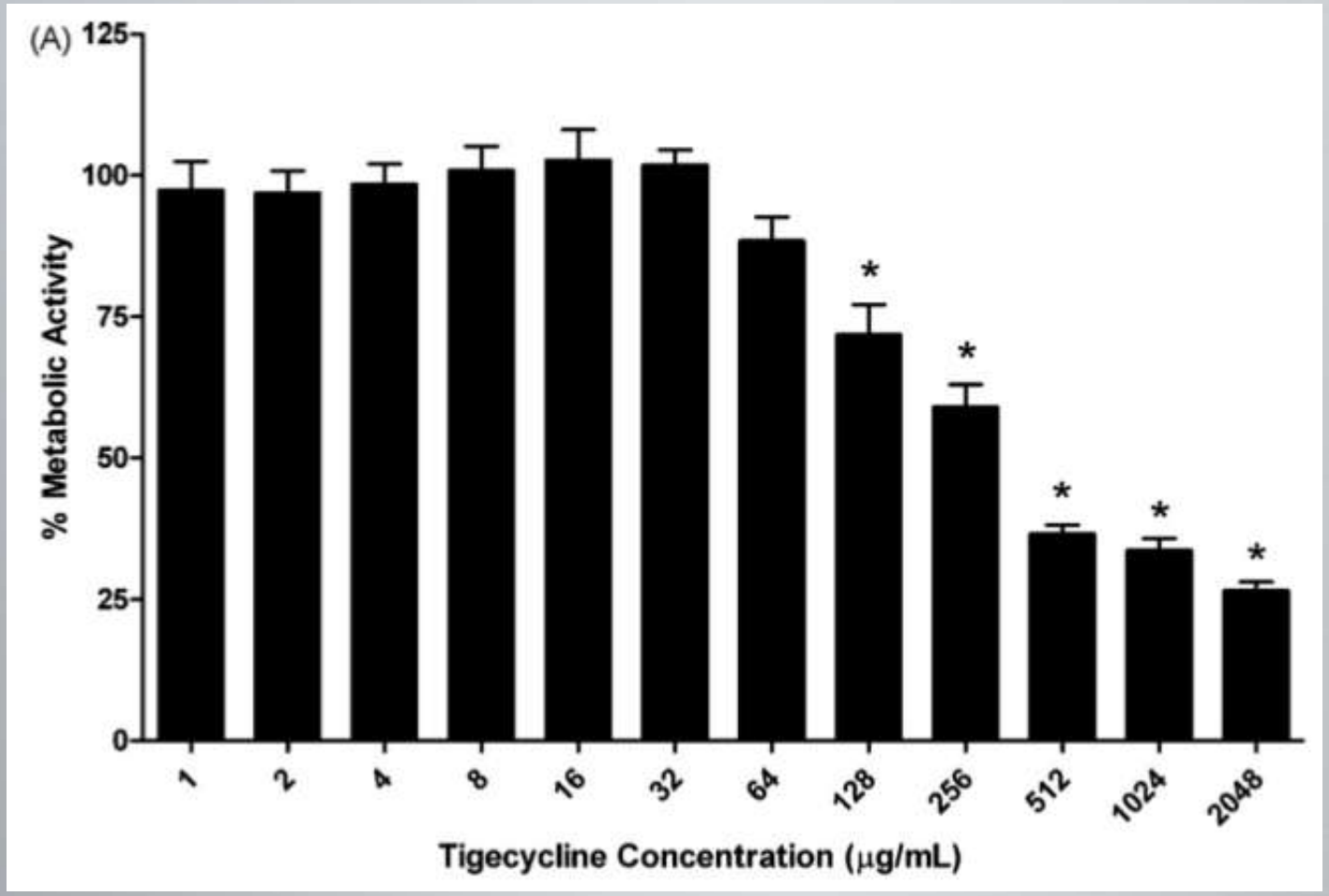
# DOX±AMB kateter-"lock"

*Miceli MH, et al. Int J Antimicrob Agents 2009;34:326*



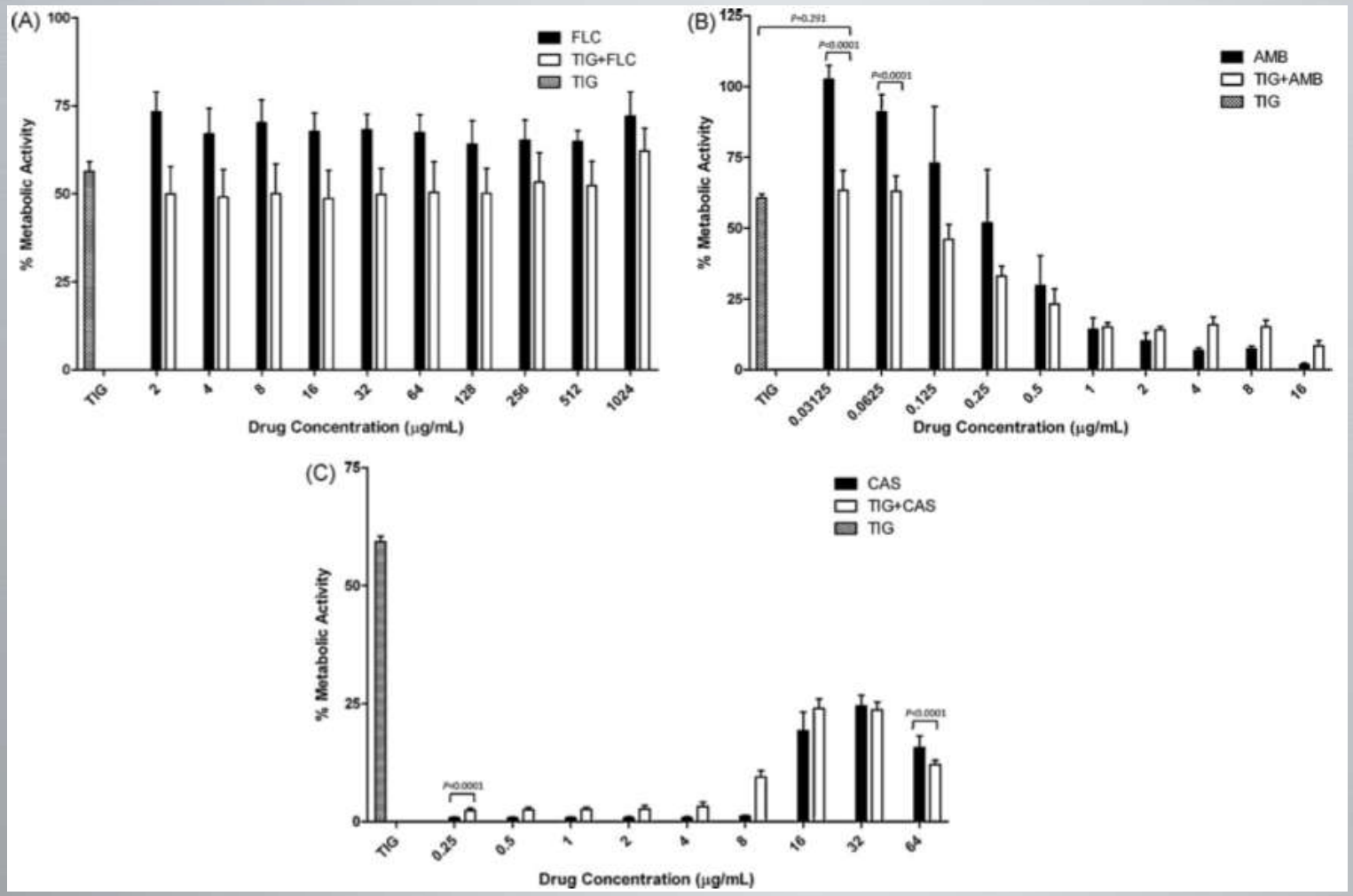

C: DOX128 mcg/ml

# Tigesiklin: *C. albicans*




*Ku TSN et al. Int J Antimicrob Agents 2010;36:441.*

# Tigesiklin: *C. albicans*




*Ku TSN et al. Int J Antimicrob Agents 2010;36:441.*

# Aspirin ± EDTA'nın Etkisi

*Al-Bakri AG, et al. J Applied Microbiol 2009;107:280.*

**Table 1** Minimum inhibitory concentration (MIC; mg $ml^{-1}$) and minimum biocidal concentration (MBC; mg $ml^{-1}$) values of aspirin and EDTA against *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans*. MIC and MBC values are means ($n$ = 3) ± SD

| Microbial strains | MIC (mg $ml^{-1}$) | | MBC (mg $ml^{-1}$) | |
|---|---|---|---|---|
| | Aspirin | EDTA | Aspirin | EDTA |
| *P. aeruginosa* | 2·03 ± 0·45 | 5·6 ± 0 | 4·8 ± 1·1 | NA* |
| *E. coli* | 1·2 ± 0·2 | 11·25 ± 0 | 4·9 ± 1 | NA* |
| *C. albicans* | 2·65 ± 0·0 | 0·085 ± 0·005 | 5·28 ± 0 | NA* |

NA*, not achieved at concentration ≤60 mg $ml^{-1}$.

**Table 2** Fractional inhibitory concentration index values ($n$ = 3) and their interpretation for aspirin-EDTA combination against *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans*

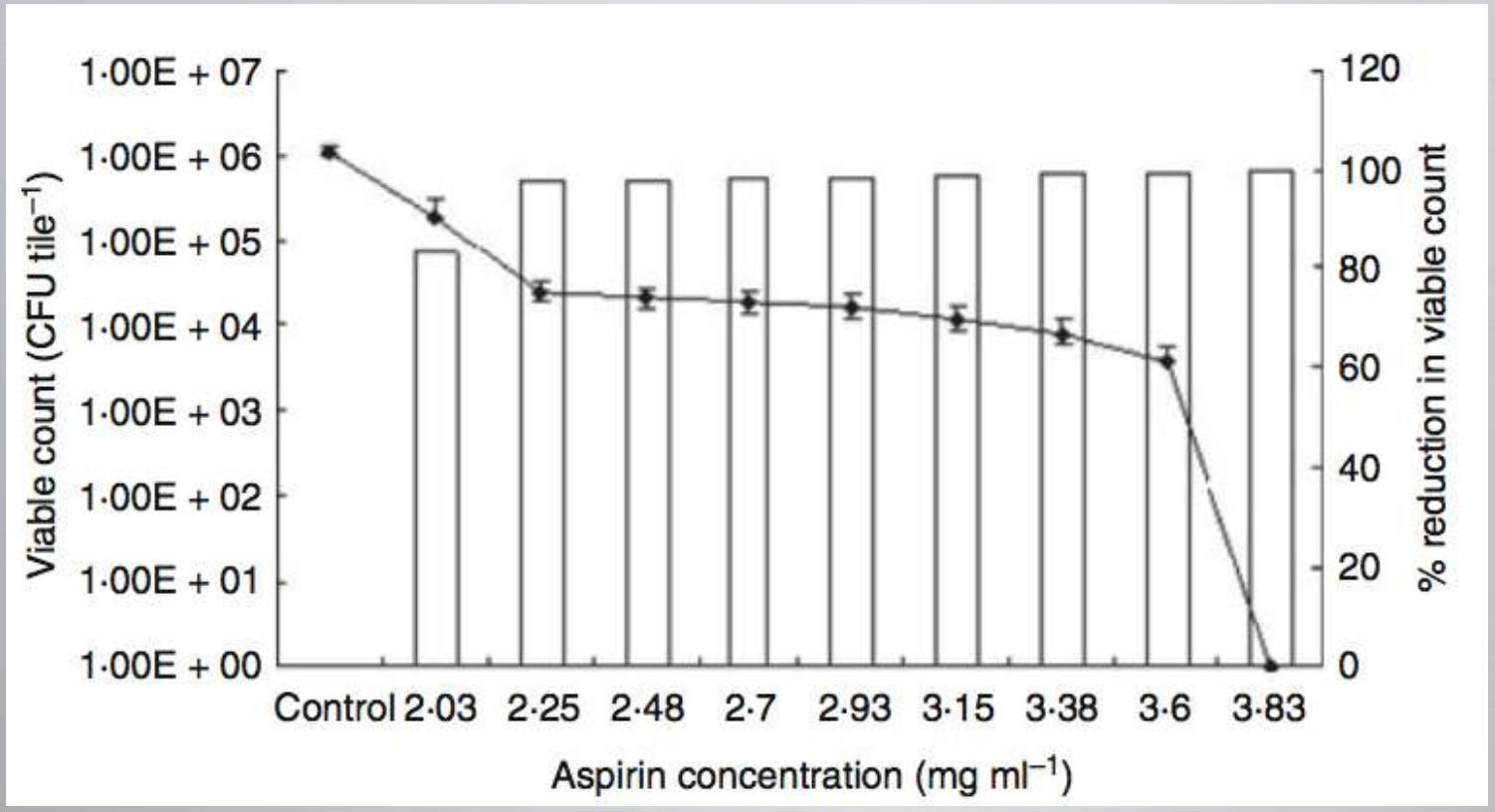
| Micro-organism | FIC index | Interpretation |
|---|---|---|
| *P. aeruginosa* | 0·5 | S* |
| *E. coli* | 0·132 | S* |
| *C. albicans* | 0·0623 | S* |

S*, is equivalent to 'synergy'.

- **Salisilatların antimikrobiyal aktiviteleri membran potansiyeli, virülans faktör yapımındaki değişiklikler, ekstrasellüler polisakkarid ve prostaglandin yapımında azalma ile ilişkilidir.**

# Aspirin ± EDTA'nın Etkisi

*Al-Bakri AG, et al. J Applied Microbiol 2009;107:280.*




24 saat aspirine maruz bırakılan *C.albicans* biyofilmi

# Aspirin ± EDTA'nın Etkisi

*Al-Bakri AG, et al. J Applied Microbiol 2009;107:280.*

**Table 4** Percentage reduction in viable count (%, $n = 3$) of established biofilms of *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans* after 1 h exposure to aspirin (MBEC), EDTA (8 mg $ml^{-1}$) and aspirin-EDTA (MBEC, 8 mg $ml^{-1}$) combination

| | Percentage reduction in viable counting (%) | | |
|---|---|---|---|
| Microbial biofilm | Aspirin | EDTA | Aspirin-EDTA |
| *P. aeruginosa* | 48·5 | 98·98 | 99·83 |
| *E. coli* | 92·4 | 53·18 | 94·1 |
| *C. albicans* | 51·87 | 96·9 | 74·7 |

MBEC, minimal biofilm eradication concentration values.

# Diğerleri…

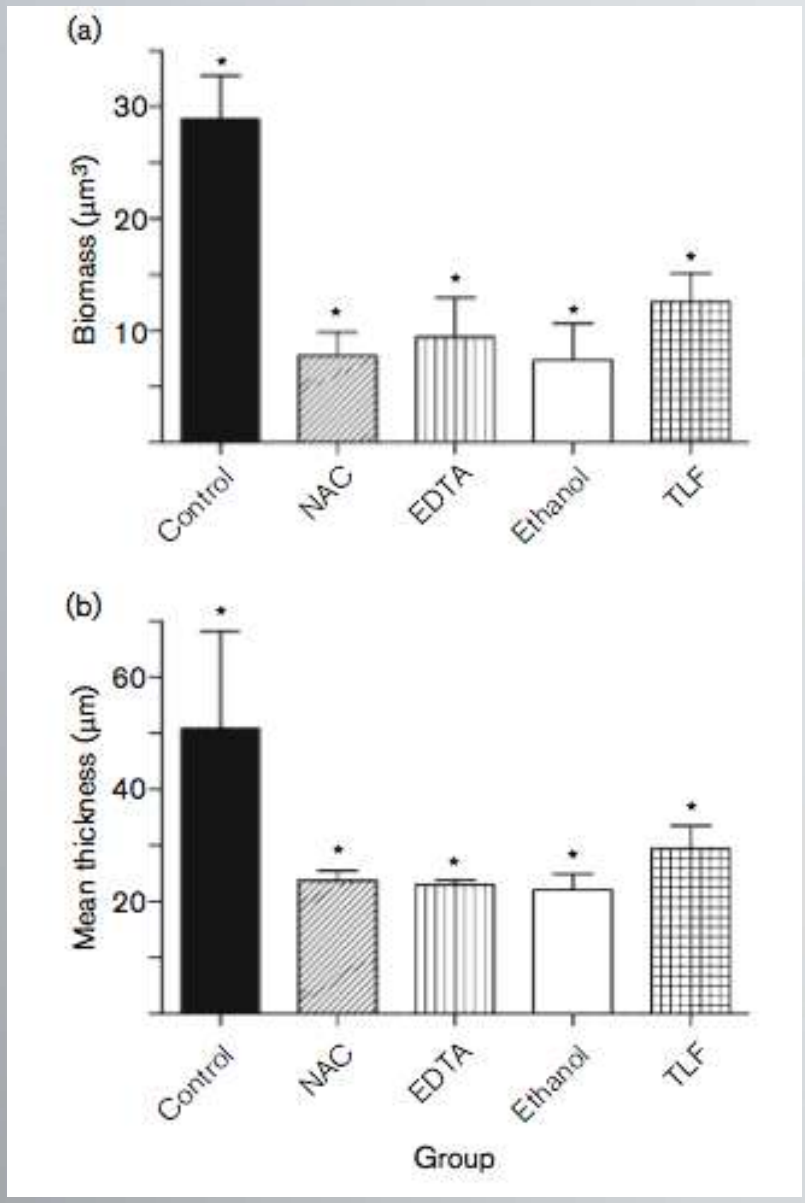
**Table 1.** $MBEC_{50}$ for EDTA, NAC, ethanol and TLF against monomicrobial and polymicrobial biofilms

| Organism | NAF ($\mu g\ ml^{-1}$) | VAN ($\mu g\ ml^{-1}$) | AMB ($\mu g\ ml^{-1}$) | FLC ($\mu g\ ml^{-1}$) | EDTA ($mg\ ml^{-1}$) | NAC ($mg\ ml^{-1}$) | Ethanol (%) | TLF ($mg\ ml^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| *S. epidermidis* ATCC 55133 | 8 | 8 | – | – | 4 | 8 | 12.5 | 4 |
| *S. epidermidis* H100 | 8 | 16 | – | – | 1 | 1 | 12.5 | 8 |
| *S. epidermidis* S101 | 32 | 32 | – | – | 4 | 4 | 12.5 | 8 |
| *C. albicans* ATCC 32354 | – | – | 64 | 64 | 32 | 32 | 12.5 | 33.2 |
| *C. albicans* ATCC MYA 4441 | – | – | 32 | 32 | 16 | 32 | 12.5 | 66.4 |
| Polymicrobial biofilm of *S. epidermidis* ATCC 55133 and *C. albicans* ATCC 32354 | – | – | – | – | 0.25 | 2 | 6 | 4 |

AMB, Amphotericin B; FLC, fluconazole; NAF, nafcillin; VAN, vancomycin.

**NAC: N-asetil sistein**
**TLF: Talaktoferrin**

*Venkatesh M, et al. J Med Microbiol 2009;58:936.*

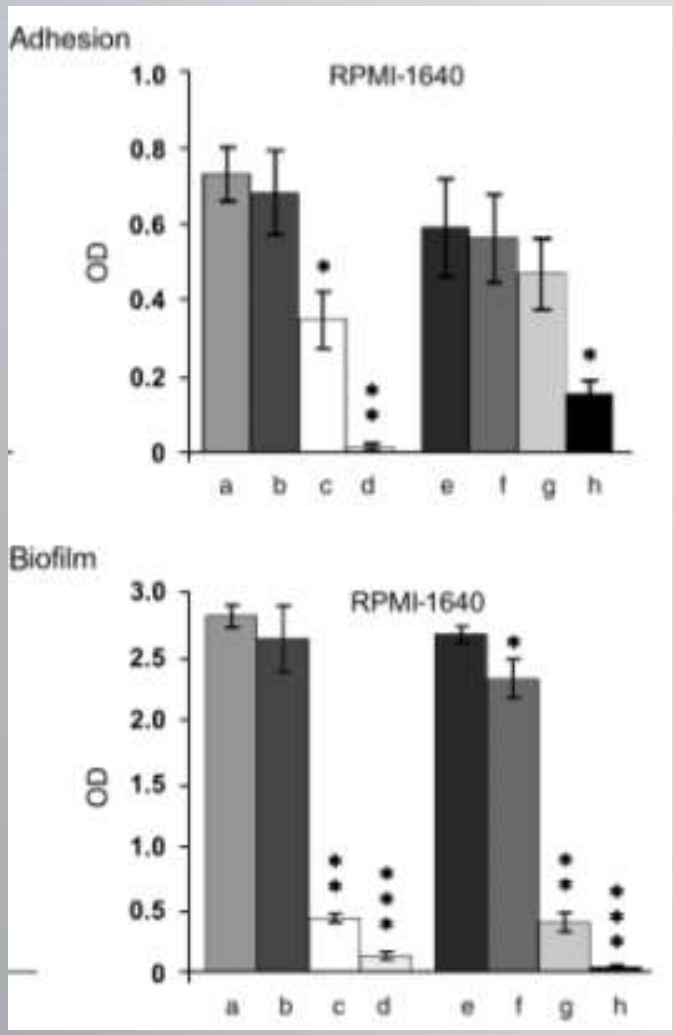



*C. albicans* ATCC 32354

***Venkatesh M, et al. J Med Microbiol 2009;58:936.***

# *S. boulardii* etkisi

***Krasowa A, et al. FEMS Yeast Res 2009;9:1312.***




**a: Ca kontrol**
**b: Ca/Sb 10:1**
**c: Ca/Sb 1:1**
**d: Ca/Sb 1:10**

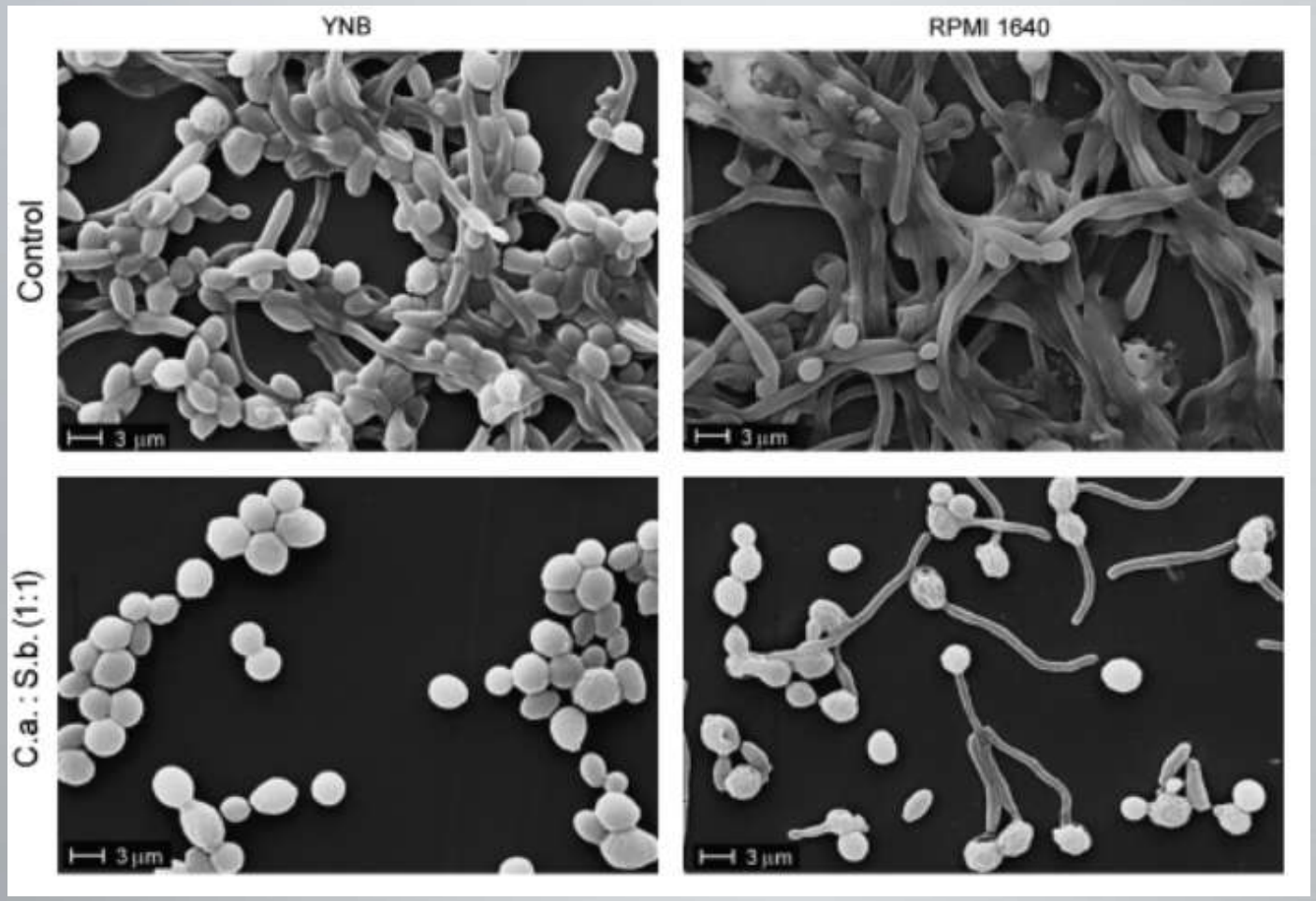
Canlı *S. boulardii* hücreleri

**e: Ca%1 metanol kontrol**
**f: Ca+ Sb ekstre 0.012 mcg /ml**
**g: Ca/Sb ekstre 0.048**
**h: Ca/Sb ekstre =.16**

*S. boulardii* kültür ekstresi

# *S. boulardii* etkisi

*Krasowa A, et al. FEMS Yeast Res 2009;9:1312.*



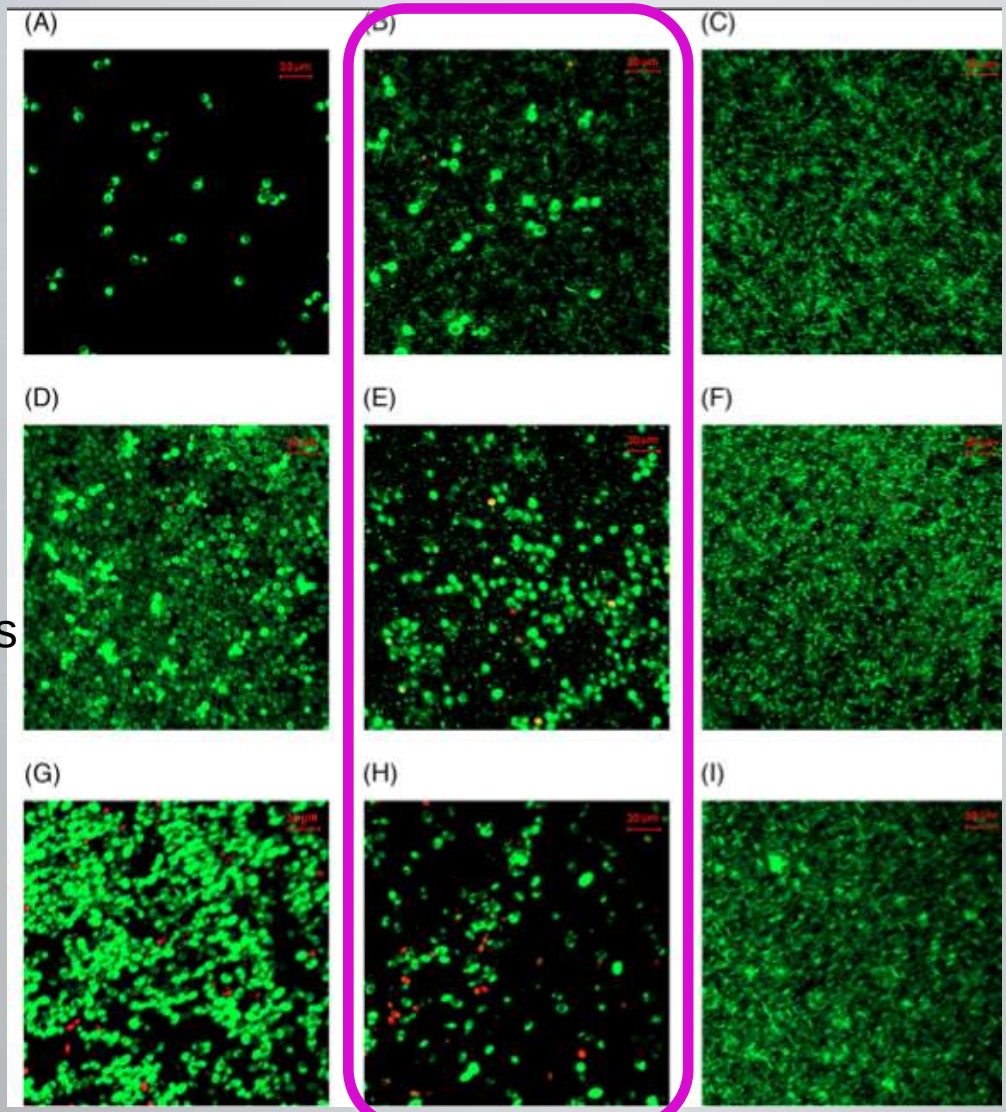
# *P. aeruginosa*: Dost??

Candida | Candida +PA | P.aeruginosa

C.albicans — 90 dk

C.dubliniensis — 24 saat

C.tropicalis — 48 saat




*Bandara HMHN, et al. BMC Microbiol 2010;10:125*

# Antifungal Kombinasyonları?

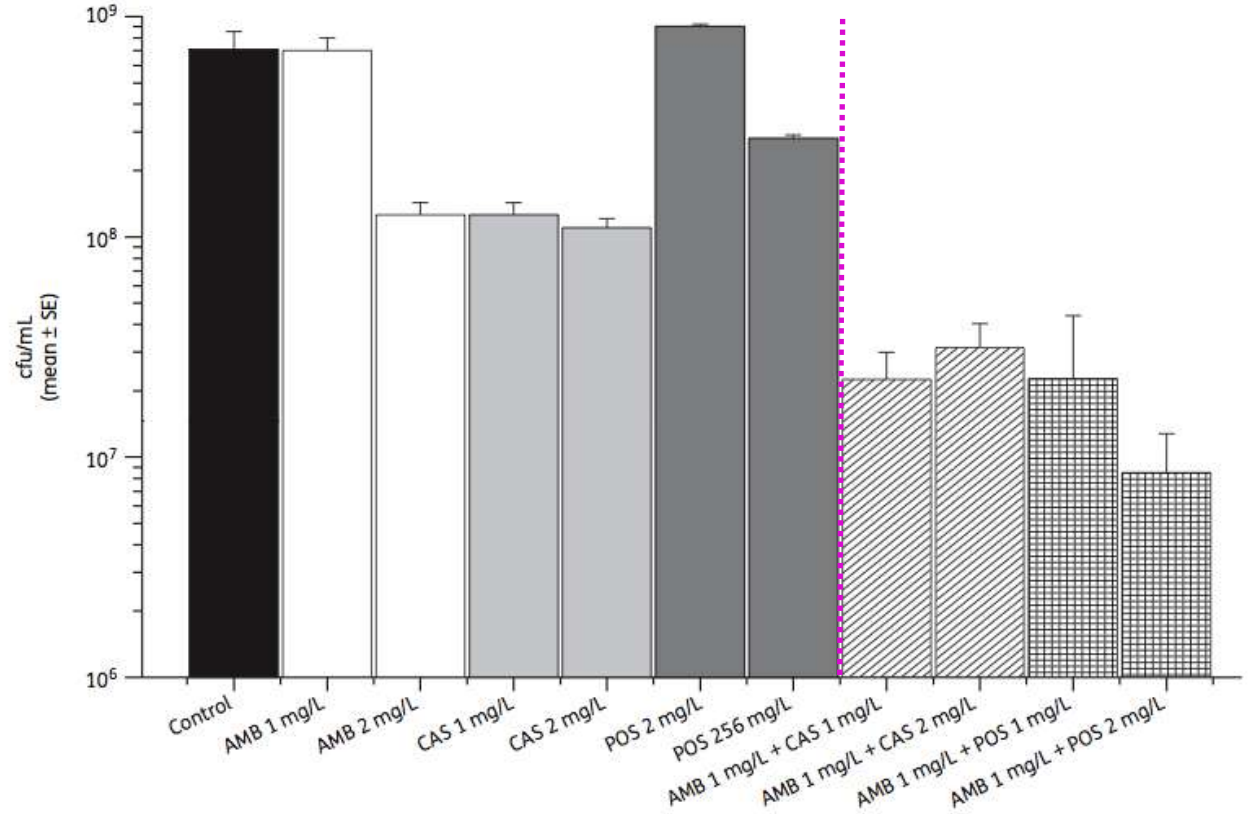



**Figure 1.** Reduction of the fungal colony counts (cfu/mL) in the biofilms of 10 invasive *C. albicans* isolates after incubation with amphotericin B (AMB), caspofungin (CAS) and posaconazole (POS) alone or in combination. The concentrations of antifungal drugs alone or in combination are in accordance with the MICs determined using the XTT assay.

*Tobudic S, et al. JAC 2010;65:271.*

- **Candida biofilmlerinde AMB ve CAS median MIC 4 mg/L,  POS .256 mg/L.**
- **Biofilmde üreyen tüm suşlarda AMB + POS sinerjik (FICI 0.26), AMB + CAS indifferan (FICI, 0.75-1.25).**
- **Planktonik koşullarda 10 suşun 4'ünde AMB+CAS sinerjik, AMB+POS hepsinde indifferan.**

***Tobudic S, et al. JAC 2010;65:271.***

# Biofilmde VRC + MICA

*Kaneko Y, et al. ISHAM Med Mycol 2010;48:606.*

- **Tek başına kullanıldıklarında VRC ve MICA ayrı ayrı planktonik hücrelerinin metabolik aktivitesini azalır.**
- **Biofilm üzerine VRC etkili değildir, MICA etkilidir.**
- **VRC, Candida biofilmi üzerine MICA'nin fungisidal aktivitesini antagonize eder.**
- **Bu etki, ortamdan VRC uzaklaştırıldıktan sonra bile belirgindir.**
- **Buna karşılık, VRC'un 24 saat sonra eklenmesi MICA duyarlılığını azaltmaz.**
- **HSP90 inhibitörleri eklenmesiyle VRC'ün MICA üzerindeki bu etkisi ortadan kalkar.**

# Yüzey Tasarımı: Antifungal β-peptid İçeren Polielektrolit Çoklu Tabakalar

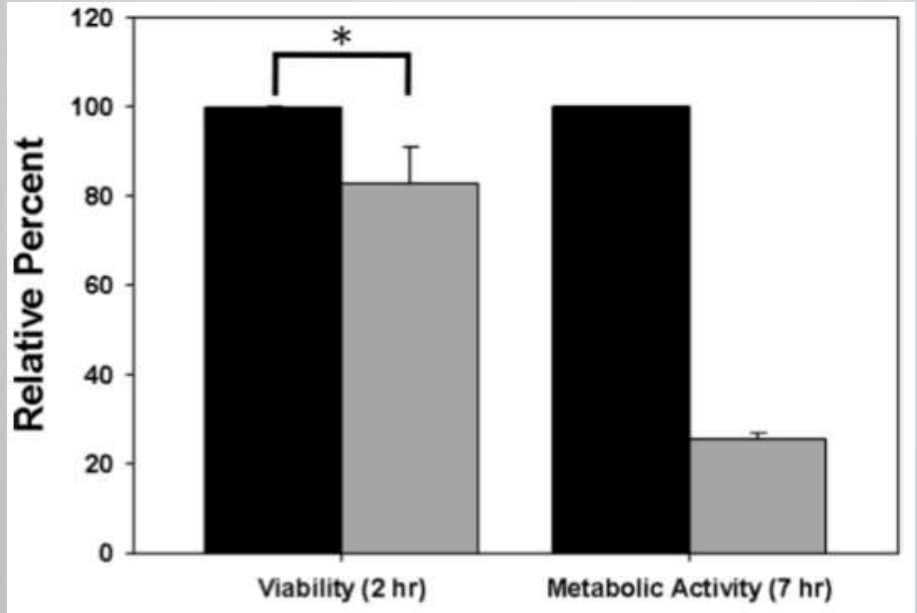



**Figure 5.** Percent of viable *C. albicans* and the relative metabolic activity of *C. albicans* cells grown on $(PGA/PLL)_{20}$ films (black bars) and $(PGA/\beta\text{-peptide/PLL})_{20}$ films (gray bars). Percent viability was determined by PI exclusion 2 h after cell seeding, and metabolic activity was determined by XTT reduction 7 h after cell seeding. The viability data represent an average of three experiments, each consisting of three control and three $\beta$-peptide-functionalized films. The metabolic activity data are from a single representative experiment consisting of three of each type of coated substrate; $^*p < 0.001$.

*Karlsson AJ, et al. Biomacromolecules 2010;11:2321.*

# Yüzey Tasarımı: Antifungal β-peptid İçeren Polielektrolit Çoklu Tabakalar

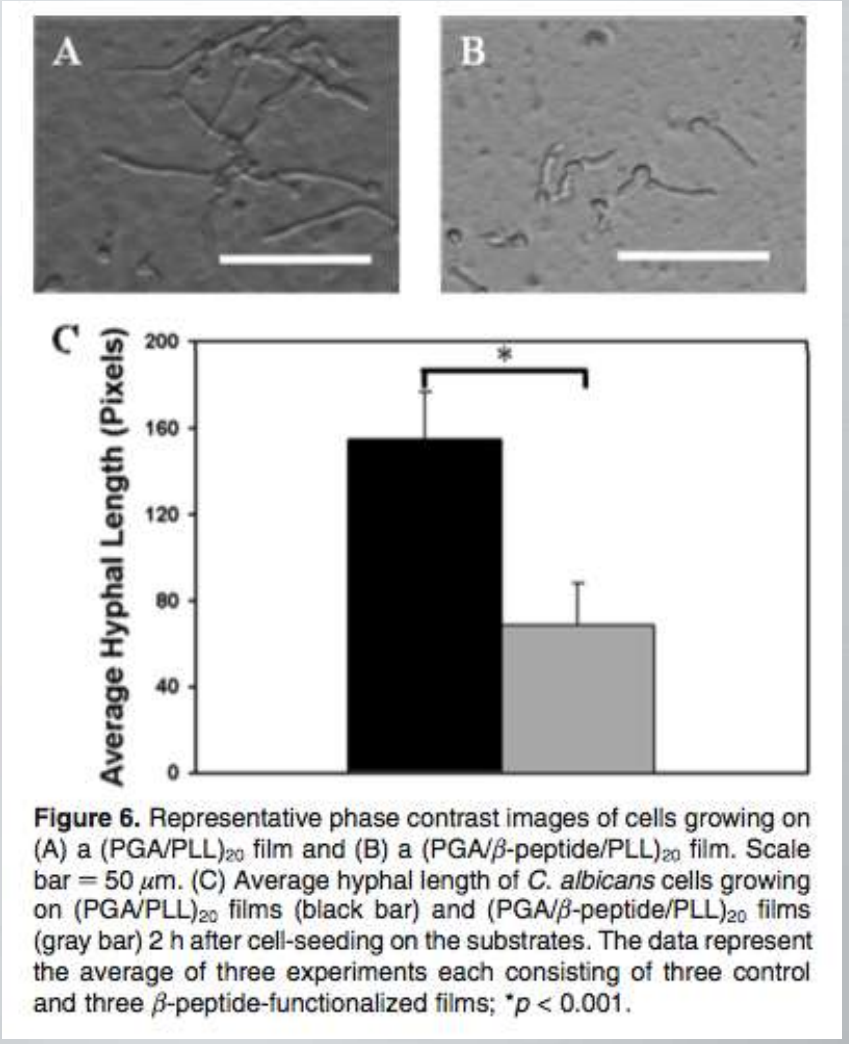



**Figure 6.** Representative phase contrast images of cells growing on (A) a $(PGA/PLL)_{20}$ film and (B) a $(PGA/\beta\text{-peptide/PLL})_{20}$ film. Scale bar = 50 $\mu$m. (C) Average hyphal length of *C. albicans* cells growing on $(PGA/PLL)_{20}$ films (black bar) and $(PGA/\beta\text{-peptide/PLL})_{20}$ films (gray bar) 2 h after cell-seeding on the substrates. The data represent the average of three experiments each consisting of three control and three $\beta$-peptide-functionalized films; $^*p < 0.001$.

*Karlsson AJ, et al. Biomacromolecules 2010;11:2321.*

# Poliklonal anti-*Candida albicans* Ab (IgY)

***Fujibayashi T, et al. Jpn J Infect Dis 2009;62:337.***

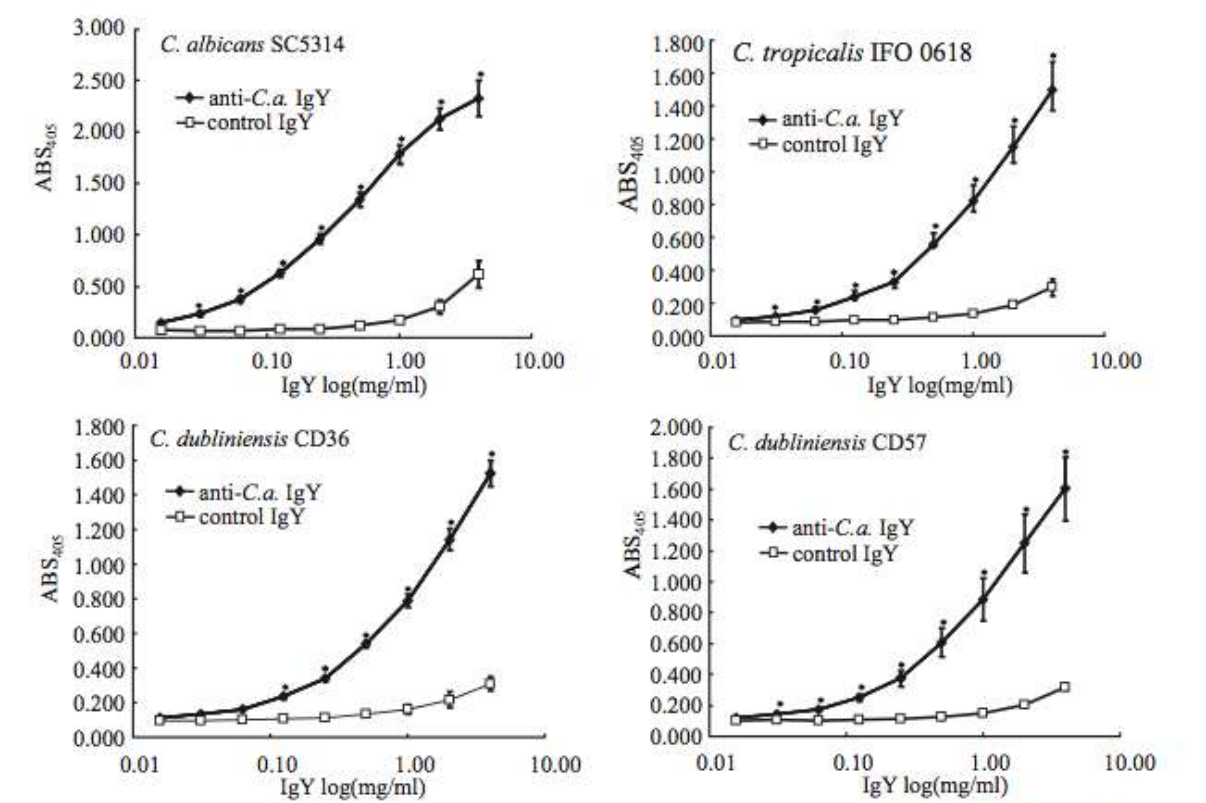



Fig. 1. ELISA antiboby titer of anti-*C. albicans* IgY. Various protein concentration (0.0, 0.063, 0.0125, 0.25, 0.5, 1.0, 2.0 and 4.0 mg/ml) of anti-*C. albicans* IgY or control IgY were applied to 96-well microtiter plates coated with *C. albicans* SC5314, *C. tropicalis* IFO0618, *C. dubliniensis* CD36 and CD57. The titers were determined using a microplate reader at 405 nm. Results are the mean ± standard deviation of three independent experiments each performed using triplicate assays; and compared to control IgY (*$P < 0.01$).

# Poliklonal anti-*Candida albicans* Ab (IgY)

***Fujibayashi T, et al. Jpn J Infect Dis 2009;62:337.***

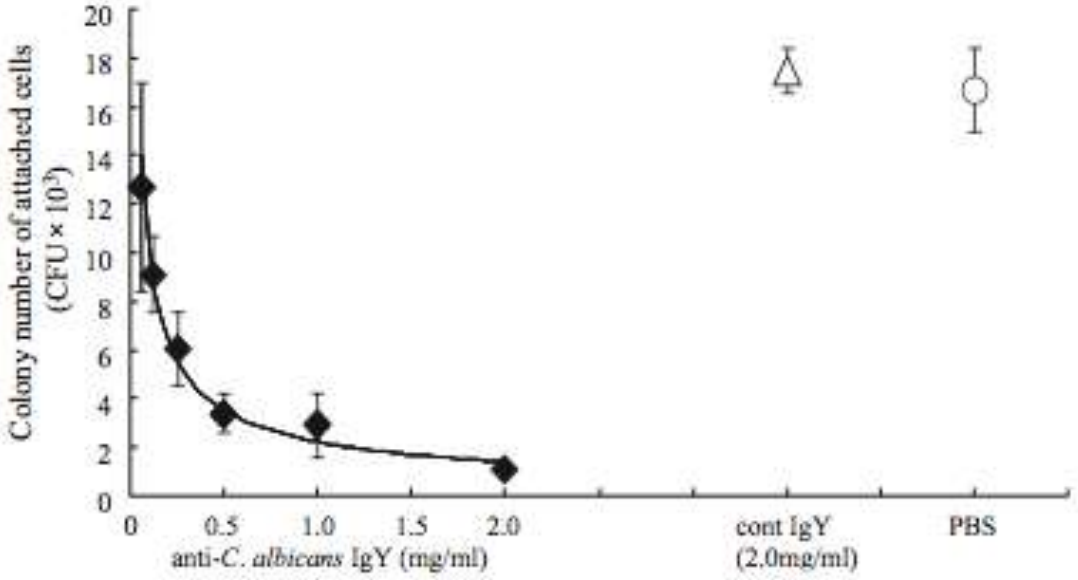
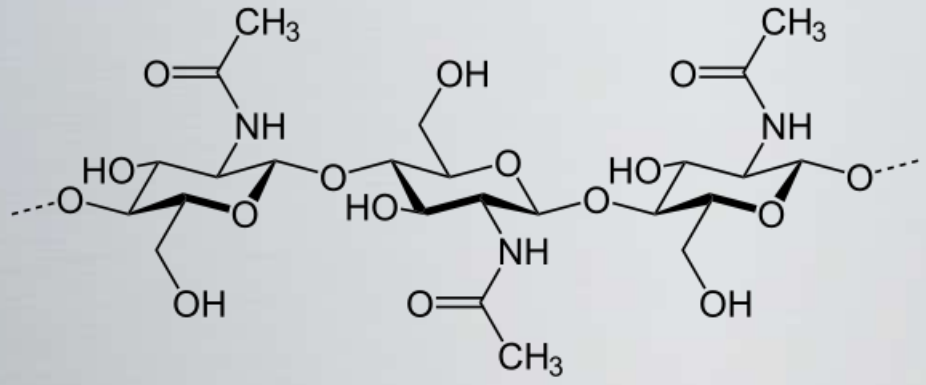
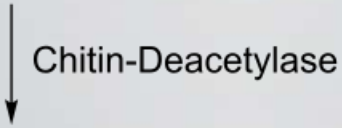
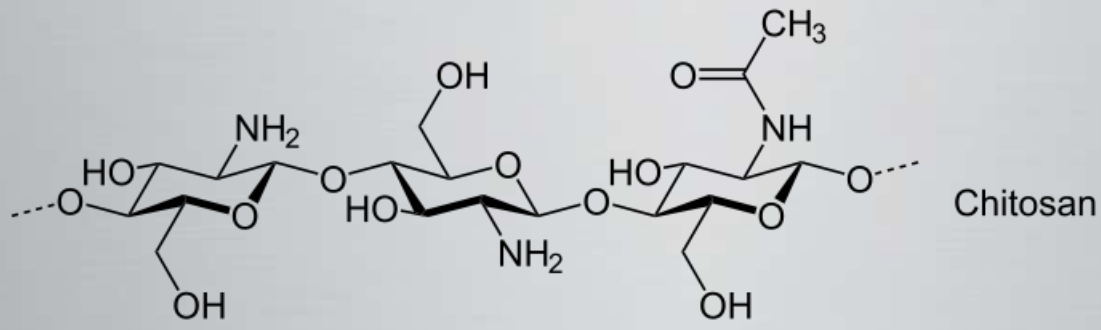



Fig. 4. Effects of anti-*C. albicans* IgY on *C. albicans* adherence. *C. albicans* SC5314 was mixed with 0.0, 0.063, 0.125, 0.25, 0.5, 1.0 and 2.0 mg/ml anti-*C. albicans* IgY, 2.0 mg/ml control IgY or PBS; and applied onto the epithelial cells (Ca9-22). The cell suspension detached using 0.05% trypsin-EDTA were spread on YPD agar plates. After incubation for 24 h, the numbers of colonies on the plates were counted. Results are the mean ± standard deviation of three independent experiments each performed using triplicate assays.

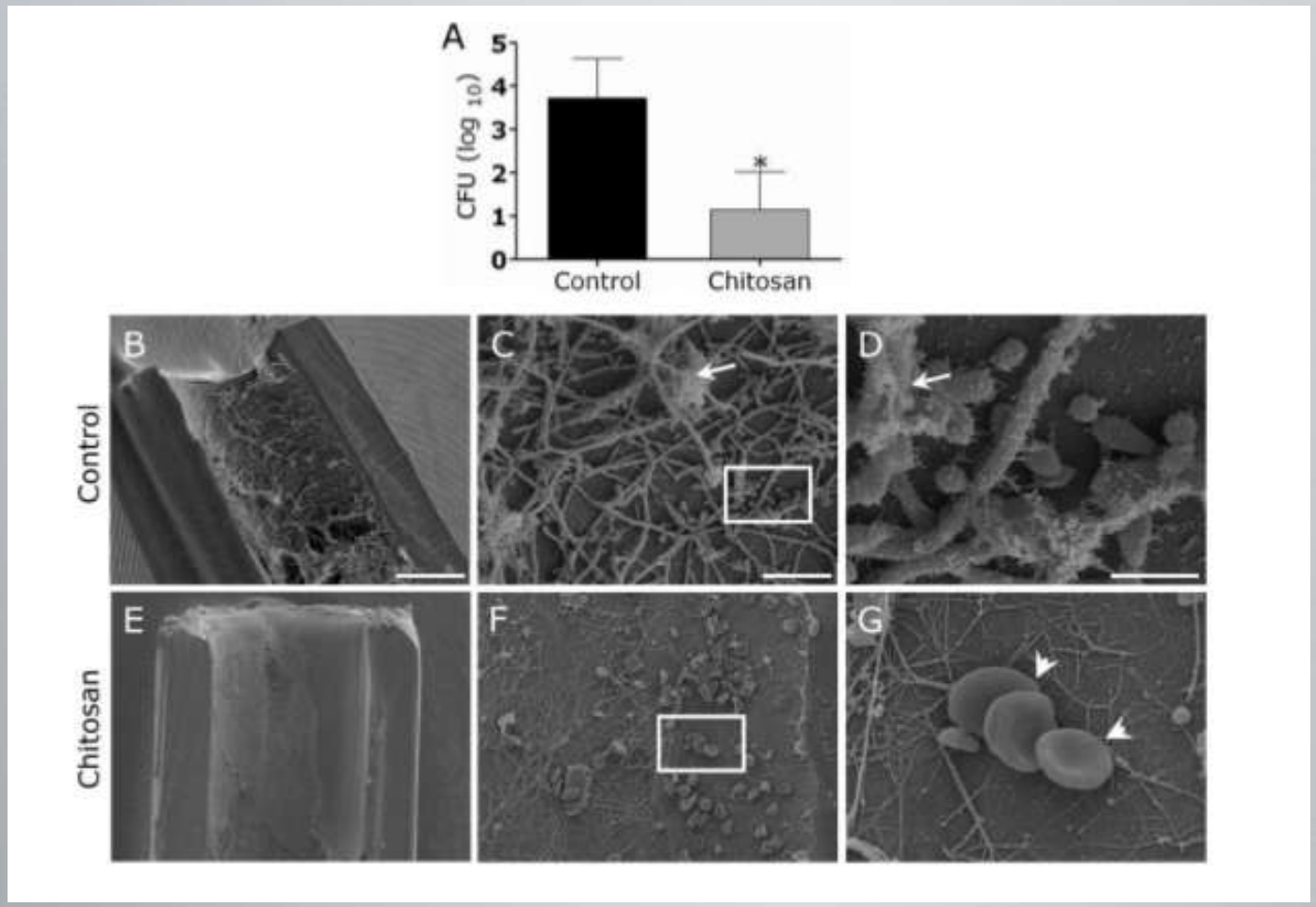

CH3
O
NH
HO
O
OH
O
HO
NH
O
CH3
Chitin


Chitin-Deacetylase


CH3
O
NH
NH2
HO
O
OH
O
HO
NH2
Chitosan

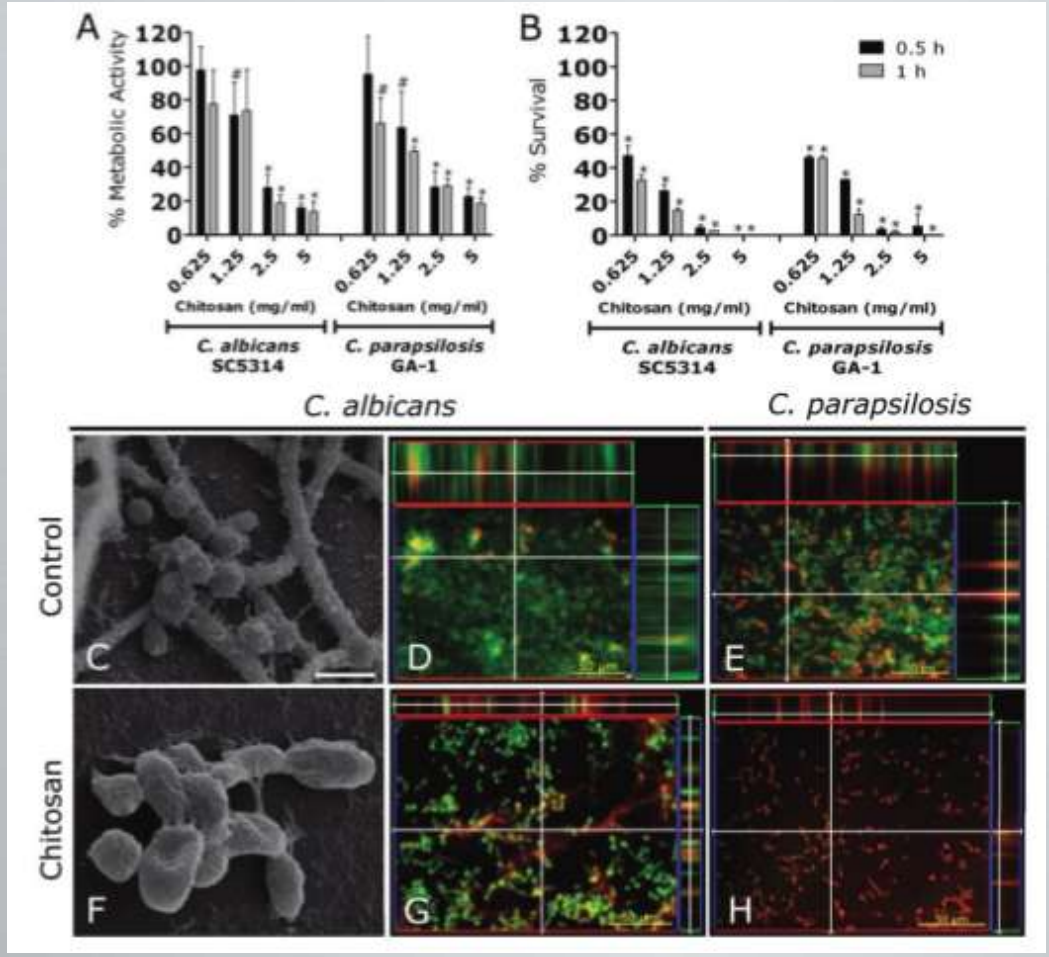
# Chitosan-kaplı Kateter

*Martinez LR, et al. JID 2010;201:1436.*



# Chitosan-kaplı Kateter

***Martinez LR, et al. JID 2010;201:1436.***




Yeşil: Polisakkarid ekstrasellüler materyel
Kırmızı: Metabolik olarak aktif hücreler
Sarı-kahverengi: Ölü veya inaktif hücreler