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Examination of mechanism of action of the antibiotic primycin on *Candida albicans* yeast cells

PhD thesis

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INTRODUCTION

In recent decades, there has been a significant increase in the number of opportunistic and antifungal antibiotic-resistant *Candida albicans* and non-*albicans Candida* fungal infections. The antibiotic primycin that exhibits a broad antimicrobial spectrum and specific mechanism of effect could be a resolution for the prevention against infections caused by polyresistant strains.

It is effective against human pathogen Gram-positive (from 0.02 μg ml^{-1}) and Gram-negative bacteria (from 10 μg ml^{-1}), including polyresistant strains, and pathogen yeasts and filamentous fungi (from 5 μg ml^{-1}). Primycin in combination with various statins exhibits synergistic and additive effects *in vivo* against some human pathogenic yeasts and mould species. The primycin sulphate-containing Ebrimycin® gel has been successfully applied to prevent the bacterial infection of surface traumas and burned tissues because resistance against this drug has not been observed. Experimental data proved that primycin had a lytic effect on human erythrocytes. Since primycin posses an effect on varied kind of cells, the investigation of its mechanism of action seemed to be a promising challenge.

The attack point of primycin has been determined as the first step of my work. It was based on some previous experimental data according to that changes in the membrane permeability and losing of alkali cations (K^+, Rb^+, Cs^+) were described by primycin treatment on human erythrocytes. Based on these results and the varied cell types, we supposed that primycin had a plasma-membrane attack point that worth to analyze. However, only the ion-efflux does not mean an attack point of plasma membrane. For example the efflux of ions in the case of azole treatment is the consequence of the inhibition of a demethylase-enzyme of the biosynthesis of ergosterol. It leads to the accumulation of methylated sterols in the plasma membranes consequently the biological features and biophysical parameters change that process cause a gradually intracellular ion-losing.

The other part of my theses is concerned with the mode of action and biological consequence of primycin. For the experiment a parental clinical isolate strain of *C. albicans* and its ergosterol-less mutant was applied. These model organisms allowed of the investigation of disorganization and structural alteration of plasma membrane. Knowing the exact composition of membrane of model organisms we had the possibility to analyze the primycin attack-point in molecular level.

Since primycin is an antibiotic complex that consists of three main components, investigation of the effect of individual components on the plasma membrane –in comparison with primycin complex- formed part of my work. These data could provide important

information of the possible synthesis of the drug for the manufacturer. The knowledge of the attack point and mechanism of action of an antibiotic has an essential role in the licensing requirements and play a role in the development of its hemi synthetic variants.

AIMS

To clear up the attack point and mechanism of action of primycin, the following experiments were projected. The aim was to determine the effect of primycin on the plasma membrane of yeast cells applying *in vivo* biophysical methods. For the experiments the wellcharacterized ergosterol producing strain (*33erg*$^+$) and its plasma membrane mutant (*erg*-2) were chosen, because applying these strains we had the possibility to get information on that the different membrane composition how could influence the primycin-membrane interaction.

Since primycin is a mixture of a few components, we considered to analyze the effect of the main components on the plasma membrane. Furthermore, we investigated the interaction between primycin and the main membrane components of *C. albicans* such as ergosterol (that is 71 % of the sterol components), phosphatidylcholine (that is 53.9 % of phospholipids) and oleic acid (53% of fatty acids).

According to the results of the above mentioned examinations, the biological consequences of the interaction of primycin-plasma membrane, the effect on the barrier-function and cell-morphology were studied by the following set of experiments:

1. Investigation of the antifungal activity of primycin on the parental strain (*33erg*$^+$) and on its ergosterol-less mutant strain (*erg*-2). Determination of the minimal inhibitory concentration of primycin using broth-macrodilution and -microdilution and grow inhibition methods.
2. Investigation of the effect of primycin on the plasma membrane of strains *33erg*$^+$ and *erg*-2 by *in vivo „steady-state"* fluorescence and EPR spectroscopy.
3. Investigation of plasma membrane-effect of A1, A2 and C1 components of primycin using EPR spectroscopy on the strain *33erg*$^+$.
4. Investigation of the interaction between primycin and the main membrane-constituent compounds (oleic acid, ergosterol, phosphatidylcholine) using *in vitro „steady-state"* fluorescence spectroscopy.
5. Investigation of the direct biological effect of primycin on the plasma-membrane and its indirect effect on the cell-morphology.

METHODS

For the biological activity of primycin two microorganism-strains were applied. The strain *erg-2* is an ergosterol-deficient mutant that was produced by treatment of nitrosoguanidine from the adenine-auxotroph, ergosterol-producing wild-type strain *33erg*$^{+}$.

Investigation of minimal inhibitory concentration (MIC)

Broth-macrodilution method: Inoculated cells of fresh- culture were suspended in 5 ml physiological salt solution. The final concentrations of primycin in the suspensions were 0, 1, 2, 3, 4, 6, 8, 12 or 16 µg ml^{-1}. The cell number was 2 x 10^3 cells ml^{-1} and the incubation temperature was 30 °C. To determine the growth inhibition, OD was measured at 595 nm with a spectrophotometer (Spectronic® Genesys^{TM}2).

Broth-microdilution technique: primycin were diluted with RPMI 1640 medium. Aliquots of 0.1 ml of drug solution were dispensed into each well of 96-well microtitre plates (Costar, Corning Incorporated, Corning, NY). The inoculum size, ranging from 1 to 5 x 10^3 cell ml^{-1}, was prepared by using the bürker counting chamber. A 0.1 ml volume of the adjusted solution of inoculum and medium was dispensed into each well of a microtitre plate containing 0.1 ml of a 2 x antifungal agent concentration. The final concentration of the antibiotic ranged from 0.25 to 128 µg ml^{-1}. The MICs were determined after incubation for 48 h at 37 °C, and the optical density (OD) was measured at 595 nm with a microtitre plate reader (Multiskan EX, Thermo). Uninoculated medium was used as the background for the spectrophotometric calibration; the growth control wells contained inoculum suspension in the drug-free medium. The MIC was defined as the lowest concentration causing a 90% decrease in turbidity as compared with the growth in the control well.

Growth inhibition by primycin was measured at 30 °C in 20 ml shaken liquid media containing 0,8- 25,6 µg ml^{-1}primycin, with initially 1 x 10^6 cells ml^{-1} Cells multiplication was followed spectrophotometrically at 595 nm in every 3 hours. The values of optical density were plotted in function of time.

Examinations of plasma membrane-dynamic using „steady-state" fluorescence spectroscopy

For the measurements protoplasts were formed (10^8 cell ml^{-1}). The protoplast suspension was treated with 128 µg ml^{-1} primycin for 15 min. The samples were washed twice and labeled with TMA-DPH flourophore for 5 min in dark. The excitation and emission wavelength were λ=340 and λ=430. The fluorescence anisotropy was determined between 0 and 35 °C in 5 °C steps.

Examinations of plasma membrane-dynamic using electron paramagnetic resonance (EPR) spectroscopy

Conventional EPR measurements: EPR spectra were recorded with an ESP 300E spectrometer (Bruker BioSpin, Germany) equipped with a diTC 2007 temperature regulator. The accuracy of temperature measurement was ± 0.2 °C. The EPR spectra of the membrane-incorporated fatty acid spin probe 5-SASL were registered in the temperature range 0 to 30 °C (Belágyi *et al.*, 1999). The conventional EPR spectra were obtained at a microwave power of 10 mW and a field modulation of 100 kHz with an amplitude of 2 G. The spectra were scaled to the same peak-to-peak amplitude or were normalized to an identical double integral. The spectra were characterized with the hyperfine splitting constant ($2A'_{zz}$), obtained from the conventional EPR spectra, as the distance between the outermost extremes. Spectra were analysed in terms of $2A'_{zz}$ against reciprocal absolute temperature ($1/T \times 10^3$, $^oK^{-1}$). A computer-assisted procedure was used to derive the breakpoint and to calculate the statistical significance of the difference between the slopes of the straight lines. For all samples, our fitting resulted in a better approximation to two straight lines. This supports the suggestion of a phase change in the membrane with increasing temperature.

Saturation transfer (ST) -EPR measurements: Second harmonic, 90° out-of-phase absorption spectra were recorded at a microwave power of 63 mW and a field modulation of 50 kHz, with an amplitude of 5 G, the signals being detected at 100 kHz out-of-phase. The microwave power of 63 mW corresponds to an average microwave field amplitude of 0.25 G in the central region of a standard cavity. The ratio of the central-field diagnostic peaks C'/C and L"/L were used to obtain an estimate of the rotational correlation time τ_c in the range 10^{-7} to 10^{-3} s of the incorporated probe molecules.

Examination of the interaction between primycin and components of plasma membrane: *In vitro* fluorescence measurements

Increscent concentration of primycin was added to 10^{-3}M oleic acid. Both compound were diluted in DMSO (dimethyl sulfoxide). "Steady-state" fluorescence measurements were carried out. The emission photoluminescence spectra were recorded between 16 and 36 °C in 2 °C steps. The integration time was 0.2 s.

Examination of biological effect of primycin

Detection of the leakage of substances absorbing at 260 nm: Mid-log phase cells of both strains (10^8 cells ml^{-1}) in Sorensen phosphate buffer at pH 6.5 were exposed to 64 µg ml^{-1} primycin at 30 °C. Untreated and treated cell suspensions were centrifuged at 0, 2, 5 and 9 h, and the OD_{260} values of the supernatants were than measured with a Spectronic Genesis *TM 2*

spectrophotometer to determine the leakage of nucleotides, nucleosides and free bases which appear in the extracellular space.

Scanning electron microscopy (SEM): Two-day-old stock cultures were used to inoculate YPD broth at a starting cell concentration of 2 x 10^6 cells ml^{-1}. Cultures containing 0, 32 or 64 μg ml^{-1} primycin were shaken at 30 °C for 24 h. After treatment, the cell suspensions were washed twice by centrifugation in 0.1 M sodium phosphate buffer and the resuspended pellet was kept in 2.5% glutaraldehyde solution at 22 ^oC for 1 h. The twice-washed cells were then maintained in 2% OsO_4 for 1 h. Dehydration of the samples was performed by dilution in an ethanol series (30, 50, 70, 80, 90, 95 and 100%), for 5 min in each case. For the gold plating of samples, a JEOL fine coat ion sputter JFC 1100 instrument was used. The SEM records were made with the use of a JEOL JSM 6300 electron microscope.

RESULTS

Investigation of minimal inhibitory concentrations

The MIC values of primycin (using macro- and microdilution methods) were significantly different for the strains *C. albicans 33erg*$^+$ and *erg-2*. According to the examinations the parental strain (*33erg*$^+$) was more sensitive than its ergosterol-less mutant (*erg-2*) (MIC were 8 μg ml^{-1} and 12 μg ml^{-1} by macrodilution, 12 and 16 μg ml^{-1} by microdilution technique).

According to the growth inhibitory method the parental strain *C. albicans 33erg*$^+$ proved to be more sensitive, than its mutant *erg-2* (the multiplication of cells was completely blocked by 64 μg ml^{-1} and 128 μg ml^{-1} primycin treatments in the case of parental and mutant strains).

Data shows that the composition of plasma membrane is strongly influence the sensitivity of strains to the antibiotic primycin. The most significant differences between the plasma membrane of two strains werethe absence of ergosterol in the mutant and the different ratio of unsaturated fatty acids.

Examinations of plasma membrane-dynamic using „steady-state" fluorescence spectroscopy

To analyze the alterations of plasma membrane structure, firstly *"steady-state"* fluorescence anisotropy measurements were carried out. During the investigations significant differences were detected in membrane dynamic for both strains. The accuracy of the measurements was supported by the decreasing tendency caused by increasing temperature (since at higher temperature the membrane become more fluid, consequently the correlation time of the probe molecule become faster that consequence is the increase of anisotropy).

Since the applied membrane label TMA-DPH characterizes molecular movements of the level of near-surface hydrophobic region of phospholipid bilayer and significantly lower anisotropy was detected by treated samples at each temperature, data allowed concluding that the anisotropic movements of the fluorescence probe increased. Consequently, viscosity of this region decreased alluding the presence of less-compact membrane structure caused by primycin treatment. The increased anisotropy of the treated, mutant strain at increasing temperature indicated the decreased flexibility of plasma membrane in the analyzed region. The contradiction of membrane-dynamic change of the strain parental and mutant could be interpreted by the following correspondences: in the strain *C. albicans erg*-2 the ratio of sterol/ phospholipid decreased and because the increase of synthesis of unsaturated fatty acids its plasma membrane was more rigid, originally. To summarize, there was a different reaction of altered plasma membrane composed-strains to the primycin treatment. The sensitivity of this method seemed to be lower because the high coefficient of variation (that may proceed from the internal light-scattering of living cells). On further investigations EPR spectroscopy was applied to examine the structural alterations of plasma membrane dynamic.

Examinations of plasma membrane-dynamic using electron paramagnetic resonance (EPR) spectroscopy

The conventional EPR and the ST-EPR techniques cover the whole range of interesting molecular motions in lipid and membrane systems. In the conventional EPR measurements, the hyperfine splitting constant $2A'_{zz}$ is one of the parameters most commonly used to evaluate changes in membranes; it can be derived from the EPR spectra. From the $2A'_{zz}$ values, the correlation time of the probe molecules can be estimated in the ns order of magnitude. It characterizes the tumbling motion of the incorporated molecules, and thereby reflects the „fluidity" of the analyzed region. In our experiments, the increase in $2A'_{zz}$ as a function of increasing primycin concentration indicated that the probe mobility was decreasing. The data are the means of the results of three independent measurements, with the standard deviations. Differences were detected in the angular offset of the curves in the exponential phase for the two strains. Saturation of the curves of *33erg*$^{+}$ and *erg-2* started at primycin concentrations of 64 µg ml^{-1} and 256 µg ml^{-1}, respectively, at which concentrations the growth of these strains was fully blocked. The increase in $2A'_{zz}$ indicates that the mobility of the incorporated spin label molecules decreased in the monitored region of the plasma membrane. This "rigidizing" effect of the primycin proved to be concentration-dependent. The plot of $2A'_{zz}$ against reciprocal temperature exhibited a non-linear dependence; accordingly, a well-defined phase-transition temperature could be deduced. Treatment of the

protoplasts with 128 µg ml^{-1} primycin resulted in 5 °C increases of the breakpoints of both strains *33erg*$^{+}$ and *erg-2*. These results showed that primycin molecules are able to interact with the components of the plasma membrane, and thereby to decrease the flexibility of the lipid chains. The above data allowed the conclusion that the target molecules of primycin are located in the cell membrane and since the plasma membrane of strain *erg-2* didn't contain ergosterol consequently the main target of primycin is not the membrane-localized ergosterol.

ST-EPR measurements were carried out to characterize the "very small" molecular motions in the lipid bilayer as a consequence of primycin treatment. In the ST-EPR spectra of 5-SASL, the ratio C'/C was analysed at 20 °C so as to estimate the value of τ_c As a result of primycin treatment, the anisotropic rotation of the spin label was changed. In the control plasma membrane, ST-EPR spectroscopy indicated angular motion ($\tau_c \approx 10^{-6}$-10^{-8} s) and long axis rotation ($\tau_c \approx 10^{-8}$-10^{-9} s) of the spin label. Primycin addition eliminated this motion and the molecule displayed much slower motion. The data demonstrated the interesting phenomenon that the correlation time for the liquid crystalline phase at room temperature was of the order of magnitude of 10^{-9} s, as compared with 10^{-5} s for the gel phase of the investigated plasma membrane systems. The retardation of τ_c by approximately 1-1.5 orders of magnitude appears to indicate the disorganization of the structure of the plasma membrane.

Examination of the effect of primycin components A1, A2 and C1 on membrane dynamic of strain *C. albicans 33erg*$^{+}$ using EPR spectroscopy

Since the plasma membrane disorganizing effect of primycin complex was proved, the same effect of individual components was presumed. Hence, examinations of plasma membrane dynamic were carried out in the case of primycin component A1, A2 and C1. According to Tm of treated membranes the fluidity of microenvironment of spin label decreased in the following sequence: (12.5 °C) < A2 (14.3 °C) < C1 (15 °C) < primycin complex (17.5 °C) < A1 (21 °C). According to ratio of L"/L the following rotation correlation times were estimated: A2 (1 x10^{-4} s) < primycin complex (6 x10^{-5}s) < C1 (5 x10^{-5}) < A1 = untreated (10^{-7} s). In the axial rotation movement (τ_c) of the spin probe significant differences were estimated by the ratio C'/C. The compounds blocked this anisotropic rotation in the following order: primycin complex (4 x10^{-6}s) > C_1 (1x10^{-6} s) > A_2 (5 x 10^{-7}) = A_1 (5 x 10^{-7}) > untreated (2 x 10^{-8}). Comparing with untreated samples, it can be established that the primycin components separately or in complex decreased the both estimated correlation times of 5-SASL molecule in the surface-near hydrophobic region of plasma membrane.

Examination of the interaction between primycin and plasma membrane components: *In vitro* fluorescence measurements

To obtain quantitative results on the possible forms of the oleic acid–primycin complexes the emission spectra were decomposed to Gauss-functions. Then the area associated to the individual Gauss-functions was plotted against the temperature. Results suggested the presence of two species in the solutions: according to the change of their equilibrium with the temperature, the concentration of one species increased while the concentration of the other species decreased at elevated temperatures. To obtain quantitative data on the thermodynamics of the possible chemical equilibriums present in this system the equilibrium constant was determined first by the Benesi-Hildebrand method using the two series of data associated to the two different temperature dependences as mentioned above.

The temperature dependence of the equilibrium constants were applied to determine the enthalpy and entropy change of the association reaction using the van't Hoff theory. The enthalpy change (ΔH) can be obtained from the slope, while the entropy change (ΔS) can be determined from the intercept of the line fitted to the experimental data.

Results highlight presence of two chemical equilibriums between the oleic acid and primycin. The strength of these interactions (ΔH~23-24 kJ $mol^{-}1$) suggests participation of hydrogen bonds in the complex formation. The oleic acid reaches the primycin molecules by its carboxyl group and this carboxyl group interacts with the -OH groups of the primycin molecules. As a result two situations can be formed: i) single hydrogen bond formed between the –OH group of the carboxyl group of the oleic acid and ii) hydrogen bond formed between the OH bond of the primycin and O atom of the carboxyl group of the oleic acid in addition to the bond formed already in the former i) case.

Biological effects of primycin

The losing of barrier function of plasma membrane was proved during the examinations. The amount of 260 nm absorbing materials was increased in the extracellular media as a consequence of 64 µg ml^{-1} primycin treatment. Morphological changes were described by the dimorphic *C. albicans* cells as a consequence of the change of interior milieu of cells and regulation of cell morphology caused by primycin. On the SEM records there is a diagnostic distortion of cell-surface that is the consequence of the losing of barrier function of plasma membrane. The pseudohyphae cell-type detected in the case of strain *33erg*$^{+}$ after treatment of 64 µg ml^{-1} primycin indicated some alterations in the signal transduction systems, supposedly.

SUMMARY

1. In the series of experiments the MICs of primycin by micro- and macrodilution methods and the primycin induced growth inhibition in shaken liquid medium for the strains of parental *C. albicans 33erg*$^{+}$ and its ergostrol mutant *erg-2* have been determined. The determination of MIC was necessary to continue the work with biologically relevant concentrations of primycin during the membrane- biophysical measurements and investigations of the biological consequences of primycin-plasma membrane interaction. We have been demonstrated significantly different MICs between the two investigated strains. In the micro- and macrodilution testing the parental strain proved to be more sensitive (MICs: 12 and 8 μg ml^{-1}) than its membrane mutant *erg-2* (16 and 12 μg ml^{-1}). The MIC concentrations of growth inhibition in shaken liquid medium were 64 μg ml^{-1} for the strain *33erg*$^{+}$ and 128 μg ml^{-1} for *erg-2*. These data suggested that the composition of plasma membrane influenced the mechanism of action of the antibiotic primycin.
2. Alterations in the dynamic of plasma membrane, as a consequence of 128 μg ml^{-1} primycin treatment have been demonstrated by measurements of *"steady-state"* fluorescence anisotropy. More accurate characterization of the structural and dynamical changes of the plasma membrane was carried out by EPR spectroscopy. We proved a significantly higher phase transition temperature in the case of both treated strains than untreated. The phase transition temperatures of untreated strain *C. albicans 33erg*$^{+}$ and its mutant *erg-2* were 11 °C and 12.5 °C, respectively. After 128 μg ml^{-1} primycin treatment, these values increased to 16 °C and 17.5 °C, revealing a significant reduction in the phospholipid flexibility. The saturation transfer EPR measurements demonstrated, that the rotational correlation times of the spin label molecule were changed after the treatment. The molecular motions, detected in the plasma membrane of untreated samples of strain *33erg*$^{+}$ and *erg-2*, were 60 ns and 100 ns. These correlation times decreased gradually by addition of increasing primycin concentrations, reaching 8 μs and 1 μs.
3. The effect on the membrane dynamic of the main components (A1, A2 and C1) of primycin was investigated by conventional and ST-EPR methods. Highest phase transition temperature has been demonstrated in the case of component A1 (21 °C) after 128 μg ml^{-1} primycin treatment, indicating that it was the most effective primycin compound. The phase transition temperatures of A2 and C1 derivates were 14.3 °C and 15 °C. Describing different molecular movements-calculated from the ST-EPR spectra-, the existence of a (gel) membrane-phase in the plasma membrane of the strain *C. albicans 33erg*$^{+}$ was demonstrated, which was formed by the contribution of the molecules of primycin.

Thereby we have proved that primycin reorganized the lipid bilayer leading to conformational changes of the membrane-bound transport- and channel-forming proteins, supposedly that biological consequence is the losing of physiological barrier function and disturbance in the signal transduction systems.

4. The *in vitro* fluorescence experiments of membrane-forming compounds and primycin interaction showed a primycin-oleic acid complex formation, stabilized by one or two hydrogen bonds that can be formed together (ΔH: -27,77) or separately (ΔH: -8,08). The above described series of experiments proved that the attack points of the antibiotic primycin were the membrane-forming fatty acids.
5. We have demonstrated, that the direct biological consequences of the biophysical changes of plasma membrane were: (i) the loss of high molecular weight cell-constituents, such as the 260 nm absorbing nucleotides, nucleosides and free bases caused by 64 μg ml^{-1} for 2 hours primycin treatment (~ 62-76 % loss in the *C. albicans 33erg*$^{+}$ and *erg-2* strains); (ii) the loss of barrier function of cells that caused indirectly the unipolar pseudohypha formation and cell-surface distortion after a treatment of 64 μg ml^{-1} primycin. In the case of *C. albicans*, these changes may be induced by the alteration of interior milieu of cells, the loss of essential ions and nucleic acids and by the alteration of cAMP signaling pathway, supposedly.

In our work the primycin - plasma membrane direct interaction was firstly demonstrated with biophysical methods. We explained that the antibiotic primycin how can be effective against almost all cell-types. We clarified the apparently contradictory results of previous researches about the mode of action of primycin.

Novel findings are: (i) rigidizing effect of primycin on the plasma membrane, (ii) inducer effect of primycin on the membrane phase rearrangement, (iii) primycin - fatty acid complex formation in the plasma membrane, (iv) alteration of plasma membrane dynamic caused by the main components (A1, A2 and C1) of primycin.

PUBLICATIONS

1. Publications related to the thesis

Virág, E., Pesti, M., Kunsági-Máté, S. Competitive hydrogen bonds associated with the effect of primycin antibiotic on oleic acid as a building block of plasma membranes, J. Antibiot. 63 (2010) 113-117. IF: 1.628

Virág, E., Juhász, Ákos., Kardos, Roland., Gazdag, Z., Papp, G., Pénzes, Á., Nyitrai, M., Vágvölgyi, Cs. and Pesti, M. *In vivo* direct interaction of the antibiotic primycin on a *Candida albicans* clinical isolate and its ergosterol-less mutant. Acta Biol. Hung. (1) 63 (2012) 42-55. IF: 0.793

Virág, E., Belagyi, J., Gazdag, Z., Vágvölgyi, Cs. and Pesti, M. *In vivo* direct interaction of primycin antibiotic with the plasma membrane of *Candida albicans*: an EPR study. Biochim. Biophys. Acta 1818 (2012) 42-48. IF: 4.647

Virág, E., Pesti, M., Kunsági-Máté, S. Complex formation between primycin and ergosterol. Entropy - driven initiation of modification of the fungal plasma membrane structure. J. Antibiot. DOI: 10.1038/JA.2011.140. IF: 1. 628

2. Forthcoming publications related to the thesis

Virág, E., Belagyi, J., Kocsubé, S., Vágvölgyi, Cs. and Pesti, M., Antifungal activity of primycin complex main components A1, A2, C1 on a *Candida albicans* clinical isolate and their effects on the dynamical change of plasma membrane. **(in manuscript)**

Virág, E., Pesti, M., Kunsági-Máté, S. Complex formation between the antibiotic primycin and phosphatidylcholine as a target compound in plasma membrane. **(in manuscript)**

3. Conference abstracts related to the thesis

Virág, E., Gazdag, Z., Belágyi, J., Kardos, R., Nyitrai, M., Pesti, M. The mode of action of primycin antibiotic: membrane dynamics examinations by EPR. Magyar Mikrobiológiai Társaság 2008. évi Nagygyűlése és XI. Fermentációs Kollokvium, Keszthely, 2008, október 15-17.

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Virág, E., Pesti, M., Kunsági-Máté, S. Olajsav-primycin kölcsönhatás modell. A Magyar Biofizikai Társaság XIII. kongresszusa, Pécs, 2009, augusztus 23-26.