GDAŃSK UNIVERSITY OF TECHNOLOGY

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Scientific discipline: Chemical Sciences

DOCTORAL DISSERTATION

Title of doctoral dissertation: Enzymes of the L-methionine biosynthesis pathway in *Candida albicans* as potential novel targets for antifungal chemotherapy.

Title of doctoral dissertation (in Polish): Enzymy szlaku biosyntezy L-metioniny z *Candida albicans* jako potencjalne nowe cele molekularne dla chemoterapii przeciwgrzybowej.

Supervisor *signature*	Auxiliary supervisor *signature*
Prof. Sławomir Milewski, PhD, DSc, Eng.	Kamila Rząd, PhD, Eng.

Gdańsk, year 2024

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The author of the doctoral dissertation: mgr inż. Aleksandra Kuplińska

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DESCRIPTION OF DOCTORAL DISSERTATION

The Author of the doctoral dissertation: Aleksandra Kuplińska

Title of doctoral dissertation: Enzymes of the L-methionine biosynthesis pathway in *Candida albicans* as potential novel targets for antifungal chemotherapy.

Title of doctoral dissertation in Polish: Enzymy szlaku biosyntezy L-metioniny z *Candida albicans* jako potencjalne nowe cele molekularne dla chemoterapii przeciwgrzybowej.

Language of doctoral dissertation: English

Supervisor: Prof. Sławomir Milewski, PhD, DSc, Eng.

Auxiliary supervisor: Kamila Rząd, PhD, Eng.

Date of doctoral defense:

Keywords of doctoral dissertation in English: *Candida albicans*, L-methionine biosynthesis pathway, antifungal compounds, enzyme inhibitors.

Keywords of doctoral dissertation in Polish: *Candida albicans*, szlak biosyntezy L-metioniny, związki przeciwgrzybowe, inhibitory enzymów.

Summary of doctoral dissertation in English:

Fungal pathways of essential amino acids biosynthesis provide an abundant source of molecular targets for new antifungal compounds, among which the L-methionine biosynthesis pathway (MBP) may be promising. In this dissertation I characterized three *C. albicans* enzymes involved in MBP: homoserine *O*-acetyltransferase (CaMet2p), *O*-acetyl-L-homoserine sulfhydrylase (CaMet15p), and cystathionine-*γ*-synthase (CaStr2p). I performed crystallization trials of analyzed proteins, which led to the formation of a CaMet15p crystal of 7 Å resolution. Additionally, I proved that the inhibition of CaMet2p, CaMet15p, and CaStr2p induces fungal growth deficiency dependent on L-methionine (L-Met) presence. Among variety of examined potential inhibitors, I selected L-penicillamine (L-PEN) as the most effective against CaMet2p enzyme, and β-(5-Oxo-3-isoxazolin-2-yl)-L-alanine as an inhibitor of CaMet15p and CaStr2p. Moreover, I have assessed antifungal effect induced by combination of inhibitors, which showed a synergistic effect between L-PEN and other selected potential inhibitors of MBP. I discovered that the growth of fungal cells treated with L-PEN could be rescued by the supplementation of media with L-Met concentration 10-fold higher than that found in human serum. Presented results confirm the significance of MBP in the development of novel antifungal compounds.

Summary of doctoral dissertation in Polish:

Źródła poszukiwań potencjalnych celów molekularnych dla nowych terapii przeciwgrzybiczych stanowią m.in. szlaki biosyntezy aminokwasów egzogennych, pośród których szlak biosyntezy L-metioniny (MBP) wydaję się być najbardziej obiecujący. W ramach pracy doktorskiej scharakteryzowałam trzy enzymy uczestniczące w MBP w komórkach *C. albicans:* transacetylazę homoserynową (CaMet2p), sulfhydrylazę *O*-acetylo-L-homoserynową (CaMet15p) oraz syntazę-*γ*-cystationiny (CaStr2p). Przeprowadziłam próby krystalizacji badanych białek i otrzymałam kryształ białka CaMet15p o rozdzielczości 7 Å. Dodatkowo, wykazałam, że inhibicja badanych enzymów powoduje zahamowanie wzrostu komórek grzybowych, zależne od obecności L-metioniny (L-Met). Wytypowałam L-penicilaminę (L-PEN) jako najbardziej skuteczny inhibitor CaMet2p, oraz β-(5-Oxo-3-isoxazolin-2-yl)-L-alaninę jako inhibitor CaMet15p i CaStr2p. Wykonałam badania wpływu kombinacji inhibitorów na wzrost komórek grzybowych i wykazywałam, że L-PEN wykazuje działanie synergistycznie z innymi, badanymi inhibitorami MBP. Udowodniłam, że zahamowanie wzrostu komórek grzybowych traktowanych L-PEN może być odwrócone przez dodanie do pożywki L-Met o stężeniu dziesięciokrotnie przewyższającym to w ludzkiej surowicy krwi. Przedstawione wyniki potwierdzają znaczenie MBP w poszukiwaniu nowych związków o działaniu przeciwgrzybowym.