Title: *Novel microbial hydrolases for the exploitation of plant-derived residues: production, biochemical and functional characterization*
PI: Dr. Miklós Takó
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The main objective of the project was to investigate the biological-enzymological requirements of the utilization of plant-derived byproducts and waste materials, and identify and characterize new hydrolase, mainly β-glucosidase and lipase, producing fungal strains/enzymes, which can serve as a basis for further basic and applied researches. In this context, our efforts concentrated on the detailed analysis of filamentous fungal enzyme activities, especially from zygomycetes strains. We aimed the identification and characterization of new enzyme sources, analysis of β-glucosidase and lipase activities in submerged and solid-state fungal fermentation systems, and purification and biochemical characterization of the enzyme activities considering their properties relevant for practical applications. Furthermore, we planned to examine enzymatic synthesis processes catalyzed by fungal β-glucosidases and lipases with the aim to optimize the reaction conditions. In parallel to the planned tasks mentioned above, our goal was to investigate enzymological and biological conditions to improve the efficiency of the antioxidative phenolic liberation from glycosides of plant-derived residues.

The following main research tasks were planned to achieve these objectives:

1) Analysis of hydrolase activities of zygomycetes fungi, which covers the screening of zygomycetes for enzyme production, fermentation on plant residues, and selection of enzymes for further basic studies.
2) Characterization of selected hydrolases, which covers the isolation of the enzymes, and biochemical characterization of their hydrolytic activities.
3) Characterization of hydrolase-catalyzed synthetic reactions, which covers the analysis of product yield (e.g. alkyl-esters) under various reaction conditions, and development and optimization of analytical protocols to identify of the synthetic compounds.
4) Analysis of bioactive phenolic liberation from plant residues, which covers *in vivo* fungal fermentation and *in vitro* enzymatic treatment assays to enrich free phenolic compounds from various plant byproducts, and optimization of HPLC methods for rapid detection of phenolic compounds.

Accordingly, the results of the assays performed by the objectives are follows:

1) New hydrolase sources have been identified and characterized among fungi.

In this objective, screening assays were conducted to isolate cellulolytic and lipolytic fungi from inductive surroundings. Fungal samples were isolated and analyzed for their hydrolase activities from different fatty (e.g. chilled animal fat and meat samples) and compost sources. Since these media have high lipid and/or cellulose content, they are excellent substrates for microorganisms able to produce cellulolytic and lipolytic enzymes in high amount. Under laboratory conditions, *Cladosporium*, *Penicillium* and *Fusarium* strains were isolated from chilled fatty samples, and *Rhizopus*, *Mucor*, *Penicillium* and *Fusarium* strains were collected from samples derived from different part of a compost bed. The isolates were subjected to morphological analysis. The ITS sequence based identification was conducted for the

Cladosporium isolate; this strain has been deposited to the Szeged Microbiology Collection (SZMC, http://www.szmc.hu). Although some of these isolates are not belonging to the Mucoromycotina group, their exohydrolases could express valuable properties potentially useful in future enzymological studies and in practical processes. Among these fungal samples, lipolytic activity of the *Cladosporium* isolate was examined in detail using submerged and solid-state fermentation conditions. Fermentation results showed active lipase under low temperature conditions (5 °C) as well, which indicates that the enzyme would be a promising candidate in several practical applications (e.g. fermentation procedures in food processing, and environmental bioremediations and biotransformations). Lipolytic activity of the *Cladosporium* isolate was also examined in minimal liquid media contained tween 80 and soybean, sunflower, olive, extra virgin olive, wheat germ, corn germ, sesame seed, pumpkin seed, cottonseed and cedar oil as a sole carbon source. Tween 80 and olive oils proved to be good inductors for lipase production by this strain, which agrees with our previous test carried out for other filamentous fungi. These results are a valuable part of an MSc diploma work (A. Sója) defended in 2015.

Several cellulolytic and lipolytic isolates were identified from our strain collection (SZMC) during screening studies prior to this project. Because these isolates proved to be excellent candidates for successful completion of the project tasks, we didn't plan further experiments to isolate cellulolytic and lipolytic fungal strains in this project. Instead, we have carried out detailed investigations on the properties of their exohydrolases and on their use for production of valuable natural products in fermentation assays. These studies are in accordance with the additional tasks aimed in this project. In this context, we have started a very interesting study regarding to the investigation of chitinolytic, cellulolytic and lipolytic activities of *Smittium* isolates (Harpellales, Kickxellomycotina). *Smittium* fungi are living in the digestive tract of various freshwater insect larvae. They have many interesting structural and functional features due to their adaptation to living in the gut of arthropods but their physiology and enzyme production have not been investigated so far. Similarly, their relationship with the hosts is largely unknown. Within the frame of the present project, we have continued the exohydrolase producing tests of these fungi, in which a highly chitinolytic *Smittium simulii* isolate has been identified and analyzed. The chitinase of this fungus was purified by chromatography. The molecular mass of the enzyme was estimated to be about 60 kDa, and its chitinase activity was identified by zymography. The optimum temperature and pH for the activity, and the substrate specificity of the enzyme were also determined. More importantly, the fungal cell wall-degrading activity of the chitinase was also analyzed with the plant pathogenic fungus *Sclerotinia sclerotiorum* under light-microscope. The purified enzyme was added to the mycelia-buffer mixture, and increased vacuole formation could be observed after 24-h incubation. This can make the enzyme promising for practical applications. It is important to mention that this is the first chitinase from Kickxellomycotina that has been isolated and characterized. From the results so far, BSc and MSc diploma works of V. Bense were defended in 2015 and 2017, respectively. Additionally, her work entitled "*Analysis of extracellular chitinase activities in Smittium fungi: production, isolation, biochemical and functional characterization*" has been awarded in the local student conference (TDK) of the University of Szeged in 2016 (3rd prize), and in the Biological Section of the corresponding national student conference (OTDK) (3rd prize). These results were presented at conference as well:

Takó M, Bense V, Kotogán A, Nagy G, Csernetics Á, Vágvölgyi C, Papp T (2017) **Analysis of chitinase activities in *Smittium* fungi: production, isolation, biochemical and functional characterization.** 7th Congress of European Microbiologists (FEMS 2017). Valencia, Spain, 2017.07.09-2017.07.13. Paper FEMS7-0944.

2) Cellulase and lipase production of several filamentous fungi have been analyzed in different fermentation systems.

Enzyme production of previously identified cellulolytic and lipolytic fungal sources was studied under different conditions. Besides the abovementioned lipase production tests for the isolated *Cladosporium*, we have investigated the cellulase and xylanase production of a *Mucor corticolus* strain on corncob products with high cellulose and hemicellulose content, in conjunction with the examination of the applicability of the samples for fermentation under solid-state conditions. These different particle-size granules were produced from the woody and spongy part of the cob by the Cobex Hungária Ltd., and can be utilized for many industrial purposes like absorbents, carriers and cleaning components. During the fermentation tests, we monitored the total cellulase, endoglucanase, cellobiohydrolase, β-glucosidase and xylanase activities using standard procedures appropriate for detection. Results showed intensive cellulase production both on spongy and woody substrates, especially on which having average particle size of 180 and 560-710 μm, respectively. Spongy granules were better substrates for β-glucosidase production presenting at least three times higher activity than measured on the woody medium. Some corncob granules proved to be suitable also for xylanase production. A BSc diploma work (M. Tóth) has been defended from this topic in 2015. And the results of these researches were presented at a conference:

Vágvölgyi C, Tóth M, Das A, Mondal KC, Papp T, Takó M (2015) **Production of *Mucor corticolus* hydrolases using corncob granules as substrate.** 17th Danube-Kris-Mures-Tisa (DKMT) Euroregional Conference on Environment and Health. Szeged, Hungary, 2015.06.05-2015.06.06., Program and Abstracts. p. 74. (ISBN:978-963-306-374-3).

Fermentation studies on plant residues were also conducted in the frame of a joint research with the group of K.C. Mondal (Vidyasagar University, India). These experiments focused on the cellulase production of filamentous fungi, and on the utilization of prepared cellulase mixtures to enhance fermentable sugar yield from plant-wastes. Although not only zygomycetes but other filamentous fungal strains were involved in these investigations, we could exploit our experience and knowledge about a set of solid-state fermentation systems in these studies. In a co-culture fermentation system using wheat-bran and rice straw as substrates, a cellulolytic cocktail with 265.4 U/g substrate β-glucosidase activity was yielded from the *Aspergillus fumigatus* ABK9 and *Trichoderma reesei* SAF3 strains. Then, the enzymatic treatment of NaOH pretreated rice straw by this cocktail resulted in maximum reducing sugar of 24.9 g/l. Finally, maximal ethanol yield of 40.1 g/l was produced from this sugar in an optimized co-culture system of *Saccharomyces cerevisiae* MTCC 173 and *Zymomonas mobilis* MTCC 2428. In further studies, filter paper, carboxymethylcellulase and xylanase activities of *A. fumigatus* SKH2 was investigated using wheat bran as a substrate. The fermentation time, medium pH and incubation temperature were optimized for high yield enzyme production. Enzyme activities in the obtained crude enzyme cocktail were assayed at different pH and temperatures to optimize the subsequent saccharification of different plant waste substrates. Alkaline (NaOH), acidic (H_2SO_4) and oxidizing (H_2O_2) agent mediated pretreatments were carried out to make the substrate more accessible to enzymatic saccharification. These investigations validate eco-friendly and cost-effective production of a cellulolytic enzyme cocktail on agricultural waste materials, and subsequent application of this cellulase mixture for efficient saccharification of lignocellulosic biomass. Results so far of this cooperative work have been presented at international conference and a journal paper:

Das A, Takó M, Vágvölgyi C, Mondal KC (2015) **Ethanol from rice straw: Optimization of process protocols using two stage microbial systems.** 2nd International Conference on Frontiers in Biological Sciences - InCoFIBS-2015. Rourkela, India, 2015.01.22-2015.01.24., Book of Abstracts. p. 33.

Jena H, Halder SK, Soren JP, Takó M, Mondal KC (2016) **Valorization of wheat bran for cost-effective production of cellulolytic enzymes by *Aspergillus fumigatus* SKH2 and utilization of the enzyme cocktail for saccharification of lignocellulosic biomass.** ACTA BIOLOGICA SZEGEDIENSIS 60:(2) pp. 129-137.

Accepting a Reviewer's suggestion made for our project plan, we examined the polyunsaturated fatty acid (PUFA) production and profile of Mucoromycotina strains identified as good lipase producer prior to this project. These assays would provide information on the correlation of the fatty acid profile and the lipase production. Lipolytic *Mortierella*, *Umbelopsis*, *Mucor*, *Rhizomucor* and *Rhizopus* strains were tested on malt extract, yeast extract-glucose, minimal broth with yeast nitrogen base (YNB) and tween-80 supplemented YNB broth that are suitable for both fatty acid and lipase production. Fatty acids were extracted from dried fungal mycelia with a potassium hydroxide-methanol-chloroform treatment, and then the fluorescent PUFA derivatives were separated with analytical HPLC. Lipolytic activities in each ferment broth were determined spectrophotometrically by using *p*-nitrophenyl palmitate (*p*NPP) as substrate. PUFA production of the tested strains showed high variability, and the *Mortierella* isolates exhibited the highest yield. However, their overall lipolytic activity was lower than other zygomycetes isolates. There were significant differences in the PUFA composition of the isolates as well, since *Mortierella* isolates produced mainly arachidonic acid, whilst other zygomycetes produced mostly linoleic-, oleic and γ-linoleic acid. A BSc student (A. Tari) has defended her diploma work in 2016 from this topic. Results of these studies were also presented at conference:

Kotogán A, Tari AR, Bencsik O, Papp T, Vágvölgyi Cs, Nyilasi I, Mondal KC, Takó M (2016) **Analysis of fatty acid production of lipolytic zygomycetes fungi.** 18th Danube-Kris-Mures-Tisa (DKMT) Euroregional Conference on Environment and Health. Novi Sad, Serbia, 2016.06.02-2016.06.04., Book of abstracts. pp. 93-94. (ISBN:978-86-6253-059-2)

3) Many fungal hydrolases have been purified and characterized. Immobilization studies of some purified enzymes have also been performed.

Characterization of new exohydrolases (mainly cellulases and lipases) potentially utilizable in various practical processes was an important part of our researches. Besides the purification of the enzymes by sequential chromatographic processes, assays regarding to substrate specificity, temperature, pH, metal ion and organic solvent tolerance have been involved in these studies. During the project period, the *Rhizomucor miehei* NRRL 5282, *Rhizopus oryzae* NRRL 1526, *Rhizopus stolonifer* SZMC 13609, *M. corticolus* SZMC 12031 and *Mortierella echinosphaera* CBS 575.75 lipases were investigated in detail. These isolates were identified as lipolytic fungi prior to this project by our research team (Kotogán et al. 2014. *Food Technology and Biotechnology* 52(1): 73-82.). Based on our previous studies, wheat bran-based solid-state or submerged fermentation systems were selected to produce the lipases in high yield. Ammonium-sulfate precipitation, size-exclusion and ion-exchange separations were combined for the enzyme purification.

Results obtained for the *R. miehei* and *Rh. oryzae* lipases have been published in an open-access journal:

Takó M, Kotogán A, Papp T, Kadaikunnan S, Alharbi NS, Vágvölgyi Cs (2017) **Purification and properties of extracellular lipases with transesterification activity and 1,3-regioselectivity from *Rhizomucor miehei* and *Rhizopus oryzae*.** JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY 27:(2) pp. 277-288. *IF: 1.750*

Briefly, properties of the additional three lipases are follows:

The *Rh. stolonifer*, *M. corticolus* and *Mo. echinosphaera* lipases have molecular weight of 28, 20 and 30 kDa, respectively, as estimated by SDS-PAGE. During the project, we have optimized a fast and easy to perform zymography method to analyze the lipase activities under native-PAGE conditions. This assay showed active purified lipases stained with 4-

methylumbelliferyl nonanoate and α-naphthyl acetate. In addition, it has also been proven that the purified lipases can catalyze the transesterification between ethanol and *p*NPP. Specific transesterification activities of 8.15, 13.62 and 18.67 U/mg were detected for the *Rh. stolonifer*, *M. corticolus* and *Mo. echinospharea* lipases, respectively. Temperature optimum for maximal lipolytic activity were 25 °C, 30 °C and 50 °C for the *Mo. echinosphaera*, *M. corticolus* and *Rh. stolonifer* enzymes, respectively. The *Rh. stolonifer* lipase can be considered as thermotolerant because it was stable up to 50 °C. The *M. corticolus* and *Mo. echinosphaera* enzymes proved to be stable up to 40 °C. The *Rh. stolonifer* and *M. corticolus* lipases exhibited remarkable residual activity at 5 °C. The *M. corticolus* enzyme had a pH optimum at neutral pH values from 7.0 to 7.4, and was stable between pH 6.2 to 7.4. Activity of the *Rh. stolonifer* lipase was maximal at pH values from 4.6 to 5.0, and the enzyme retained most of its initial activity between pH 4.2 and 5.4. The *Mo. echinosphaera* lipase had the pH optimum between pH 6.6 and 7.0, and was stable between pH 4.6 and 8.0. The *Rh. stolonifer* lipase had highest specificity for *p*NP esters with C8-C12 acids, while the *Mo. echinosphaera* and *M. corticolus* lipases could effectively hydrolyze the substrates with C3-C10 and C4-C12 acids, respectively. The studied lipases have 1,3-regioselectivity according to triolein hydrolysis. Kinetic parameters of the *Rh. stolonifer*, *M. corticolus* and *Mo. echinosphaera* lipases indicated almost equal affinity and hydrolysis rate for the *p*NPP substrate. During the enzyme activity inhibition tests, the *Rh. stolonifer* and *Mo. echinosphaera* lipases showed high stability in most of the metal salts tested. The Hg^{2+}, N-bromosuccinimide and sodium dodecyl sulfate significantly inactivated the enzymes; however, the Na^{+} and K^{+} in 5 mM concentration enhanced their activity, which may be due to their enzyme conformation stabilizing effect. The *Rh. stolonifer* and *M. corticolus* lipases proved to be stable in methanol, ethanol and isopropanol at concentrations up to 20% (v/v). The *Mo. echinosphaera* enzyme was highly stable in 20% (v/v) isopropanol, and 15% (v/v) methanol and ethanol. Butanol and hexanol inactivated the *p*NPP-hydrolyzing activity of the enzymes, but they remained active and stable in the presence of *n*-hexane, cyclohexane, *n*-heptane and isooctane at concentrations up to 20% (v/v). In addition, enhancing effect of certain alcohols on the activity was observed for some enzymes. A cause of this effect could be the transesterification of the *p*NPP due to the reduced water activity. Transesterification activity of the studied enzymes was also investigated in this project (see Section 4).

Furthermore, we also focused on the entrapment or absorption of the purified lipases to a support. Immobilization allows the reusability, and improves the activity and thermal and solvent stability of the enzymes. In the frame of these studies, we have successfully absorbed the purified *Mo. echinosphaera* lipase to a polypropylene hydrophobic support (Accurel MP1000). The immobilization efficiency was 91.5% indicating a high absorption affinity of the lipase to the hydrophobic matrix used. The immobilized enzyme showed activity yield of 29.9%, and 0.2 and 0.07 U/mg of support specific activities for *p*NPP hydrolysis and transesterification reactions, respectively. The immobilized enzyme was highly stable up to five cycles retaining 83% and 59% of its *p*NPP-hydrolyzing and transesterification activities, respectively, at the fifth recycle step. The relatively high residual activity in transesterification reaction assumes a stable *Mo. echinosphaera* lipase-carrier complex also in non-aqueous surrounding. In addition, the immobilized lipase maintained 60% of its initial hydrolytic activity after two months storage at 5 °C, compared to the free enzyme that almost completely lost its activity at that condition. It is worth emphasizing that we carried out first time the successful immobilization of a *Mortierella* lipase to a support, and the absorbed enzyme was stable and active in both aqueous and water-free environment. Presently, a manuscript containing the results of *Mo. echinosphaera* lipase is under preparation:

Kotogán A, Zambrano C, Kecskeméti A, Szekeres A, Papp T, Vágvölgyi Cs, Takó M (2018) **An organic solvent-tolerant lipase with both hydrolytic and synthetic activities from the oleaginous fungus *Mortierella echinosphaera*.** (under preparation)

Properties identified so far for the *Rh. stolonifer* lipase were documented in an MSc diploma work in 2015 (A. Sója), and were presented at a conference:

Takó M, Kotogán A, Sója A, Kecskeméti A, Mondal KC, Papp T, Vágvölgyi C (2015) **Purification and characterization of a lipase with high synthetic activity from *Rhizopus stolonifer*.** 6th Congress of European Microbiologists (FEMS 2015). Maastricht, The Netherlands, 2015.06.07-2015.06.11. Paper FEMS-1006.

Immobilization studies with different carriers were also started to entrap or absorb the *R. miehei* NRRL 5282 β-glucosidase characterized prior to this project by our research team. This enzyme is promising candidate for various biotechnological applications, such as the synthesis of different oligosaccharides and liberation of free antioxidative phenolics from their glycosides in plant substrates. The enzyme protein has successfully been entrapped to alginate and chitosan beads and, uniquely, to corncob granules having particle sizes about 1000 μm and 560/710 μm. The granules, GM12 and GM20, were obtained from the Cobex Hungária Ltd. Those are characterized with high density and absorption capacity, and rich in cellulosic fibers that are good support for enzyme immobilization; additionally, the cost of these substrates is negligible. During these researches, several reaction parameters (e.g. support concentration, incubation time for entrapping/cross-linking, incubation temperature) were tested to optimize the immobilization and activity yields. Entrapment to alginate resulted higher immobilization yield than that determined for the corncob granule, but very convincing activity yields were obtained in case of the enzyme-corncob granule complex. Since the above tests are the most recent in the topic, we planned to present them at conferences in 2018, and of course, the outcome will be a manuscript describing the comparative analysis of the different immobilization conditions. However, an MSc diploma work (M. Tóth) has been defended in 2017 from the results achieved with alginate beads and corncob granules.

In close connection to the abovementioned studies, a book chapter containing valuable data and new knowledge about the hydrolase production of Mucoromycotina fungi was published:

Papp T, Nyilasi I, Csernetics Á, Nagy G, Takó M, Vágvölgyi C (2016) **Improvement of Industrially Relevant Biological Activities in Mucoromycotina Fungi.** In: Schmoll M, Dattenböck C (Eds.) Gene Expression Systems in Fungi. Springer, pp. 97-118. (ISBN:978-3-319-27949-7)

Additionally, the protein purification processes developed during the implementation of this topic provided methodological support to the high-yield purification of an antifungal protein from *Neosartorya fischeri*. From these results, a research paper and conference abstracts were published:

Tóth L, Kele Z, Borics A, Nagy LG, Váradi Gy, Virágh M, Takó M, Vágvölgyi Cs, Galgóczy L (2016) **NFAP2, a novel cysteine-rich anti-yeast protein from *Neosartorya fischeri* NRRL 181: isolation and characterization.** AMB EXPRESS 6: Paper 75. *IF: 1.825*

Tóth L, Kele Z, Nagy LG, Virágh M, Takó M, Vágvölgyi Cs, Galgóczy L (2016) **NFAP2, an anti-yeast protein from *Neosartorya fischeri* NRRL 181.** A Magyar Mikrobiológiai Társaság 2016. évi nagygyűlése. Keszthely, Magyarország, 2016.10.19-2016.10.21., Abstracts p. 61.

Galgóczy L, Tóth L, Kele Z, Nagy LG, Virágh M, Takó M, Vágvölgyi Cs (2016) **Isolation of a novel, cysteine-rich antifungal protein with anti-yeast activity from *Neosartorya fischeri* NRRL 181.** IV International Conference on Antimicrobial Research (ICAR 2016). Torremolinos, Spain, 2016.06.29-2016.07.01., Book of Abstracts p. 21.

4) Synthetic activity of some selected fungal hydrolases has been characterized in detail.

Our researches in this topic focused mainly on the transesterification and esterification processes operated by *Mucor*, *Rhizomucor*, *Rhizopus*, *Mortierella* and *Umbelopsis* lipases. In these assays, formation of various alkyl ester compounds was analyzed using a gas chromatography method developed by our group. Many representatives of alkyl esters are utilizable in the food and cosmetic industries as flavor, fragrance and surfactant additives.

Through these assays, we provided data about several biocatalysts that have not yet been investigated in organic synthesis processes, and potentially useful in practical applications as well. At first, the transesterification-catalyzing properties of crude lipases were analyzed and compared under various reaction conditions. We examined the effect of various parameters (e.g. reaction medium, incubation time, temperature, acceptor concentration) on the reactions of 11 crude enzymes identified previously as excellent biocatalysts of transesterification. In addition, the effect of acyl acceptors and donors with various chain-lengths on the reaction was also determined. These results were presented at conferences and in a journal paper:

Kotogán A, Kecskeméti A, Papp T, Szekeres A, Vágvölgyi C, Takó M (2015) **Investigation of the transesterification and esterification properties of extracellular lipase enzymes from Mucoromycotina fungi.** 6th Congress of European Microbiologists (FEMS 2015). Maastricht, The Netherlands, 2015.06.07-2015.06.11. Paper FEMS-1012.

Kotogán A, Kecskeméti A, Papp T, Szekeres A, Vágvölgyi Cs, Takó M (2016) **Synthetic reactions of lipase enzymes from Mucoromycotina fungi.** Power of Microbes in Industry and Environment 2016. Krk, Croatia, 2016.09.28-2016.10.01. Abstracts p. 36. (ISBN:978-953-7778-14-9)

Kotogán A, Kecskeméti A, Szekeres A, Papp T, Chandrasekaran M, Kadaikunnan S, Alharbi NS, Vágvölgyi C, Takó M (2016) **Characterization of transesterification reactions by Mucoromycotina lipases in non-aqueous media.** JOURNAL OF MOLECULAR CATALYSIS B-ENZYMATIC 127: pp. 47-55. *IF: 2.269*

Esterification activities and fatty acid preference of *R. miehei* NRRL 5282, *Rh. oryzae* NRRL 1526, *Rh. stolonifer* SZMC 13609, *M. corticolus* SZMC 12031 and *Mo. echinosphaera* CBS 575.75 crude lipases were also investigated in organic solvent surrounding. In these studies, ester synthesis between different carbon chain-length fatty acids (from C4 to C18, i.e., butyric, hexanoic, octanoic, decanoic, dodecanoic, tetradecanoic, hexadecenoic or octadecanoic acids) and methanol was tested at 40 °C using 72-hours incubation. Similar to that of the transesterification analysis, synthetic esters produced were detected by gas chromatography technique. Results indicated lower esterification activities than those detected for transesterification reactions (Table 1). Among the selected enzymes, the *R. miehei* and *Mo. echinosphaera* lipases exhibited the highest specific esterification activities (0.36 U/mg and 0.29 U/mg of protein, respectively).

Table 1. Comparison of the transesterification and esterification activities of Mucoromycota crude lipase biocatalysts.

Source of crude lipase	Transesterification activity (U/mg of protein)	Esterification activity (U/mg of protein)
R. miehei NRRL 5282	4.84 ± 0.44	0.36 ± 0.03
Rh. oryzae NRRL 1526	1.98 ± 0.24	0.20 ± 0.04
Rh. stolonifer SZMC 13609	3.55 ± 0.53	0.23 ± 0.03
M. corticolus SZMC 12031	1.65 ± 0.34	0.25 ± 0.02
Mo. echinosphaera CBS 575.75	2.35 ± 0.34	0.29 ± 0.02

Further study of esterification revealed high affinity for long-chain fatty acids from C16 to C18 by the tested enzymes (Fig. 1). Methyl ester formation was not observed when butyric acid (C4) was used as acyl donor compound. The *M. corticolus* and *Mo. echinosphaera* lipases could catalyze the hexanoic acid (C6) esterification as well, indicating a wide substrate specificity for these enzymes. Methyl octadecanoate (C18) was formed in the highest amount achieving 64.33, 26.53, 50.21, 46.96, and 52.84 μmol/l yields for *R. miehei*, *Rh. oryzae*, *Rh. stolonifer*, *M. corticolus* and *Mo. echinosphaera* lipases, respectively (Table 2). The above results were presented at a conference:

Kotogán A, Kecskeméti A, Papp T, Szekeres A, Vágvölgyi Cs, Takó M (2017) **Examination of esterification reactions catalysed by lipase enzymes from Mucoromycota fungi.** MIKOLÓGIAI KÖZLEMÉNYEK-CLUSIANA 56:(1) pp. 110-112. (6th Hungarian Mycological Conference, Szeged, Hungary: 2017.07.03 -2017.07.05.)

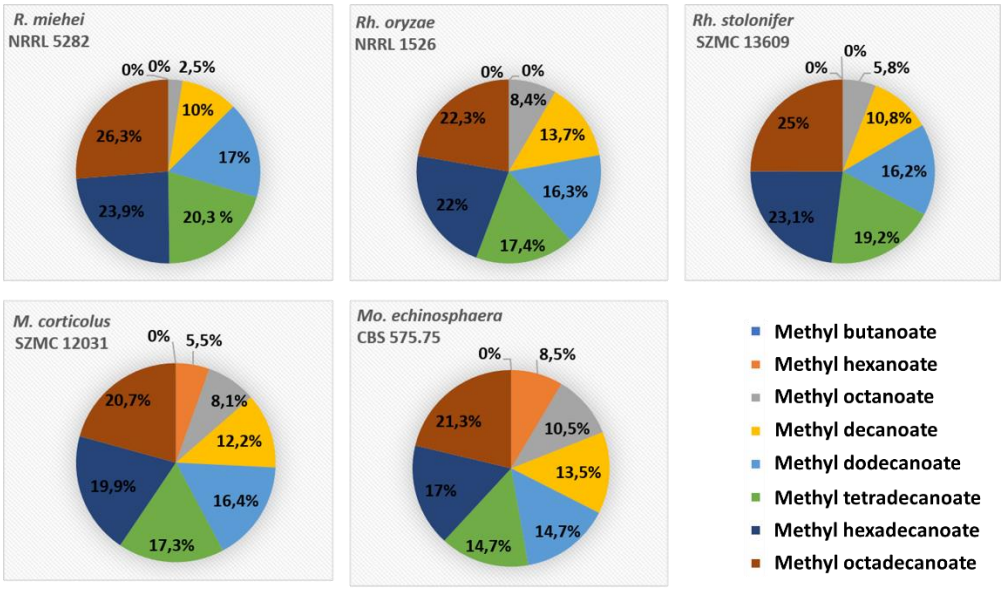

Fig. 1. Percent distribution of methyl esters formed during lipase-catalyzed esterification reactions. Reactions were performed at 40 °C for 72 h in *n*-heptane containing 1 M methanol and various fatty acids each in 10 mM concentration. The total methyl ester yield was taken as 100%.

Table 2. Concentration of methyl esters (µmol/l) detected during lipase-catalyzed esterification reactions. Reaction mixtures were incubated at 40 ºC for 72 hours.

Methyl ester yield (µmol/l)	**Source of crude lipase**				
	***R. miehei* NRRL 5282**	***Rh. oryzae* NRRL 1526**	***Rh. stolonifer* SZMC 13609**	***M. corticolus* SZMC 12031**	***Mo. echinosphaera* CBS 575.75**
Methyl butanoate	n.d.	n.d.	n.d.	n.d.	n.d.
Methyl hexanoate	n.d.	n.d.	n.d.	12.85 ± 3.56	21.09 ± 0.27
Methyl octanoate	6.76 ± 2.02	10.85 ± 7.24	12.11 ± 3.10	18.39 ± 1.20	25.83 ± 0.51
Methyl decanoate	24.98 ± 6.51	15.89 ± 0.71	21.98 ± 5.92	27.62 ± 0.70	33.42 ± 2.44
Methyl dodecanoate	41.90 ± 2.29	19.02 ± 1.71	32.63 ± 3.30	37.08 ± 0.70	36.49 ± 2.10
Methyl tetradecanoate	49.69 ± 0.65	20.37 ± 2.88	38.62 ± 1.62	39.01 ± 0.77	36.24 ± 1.89
Methyl hexadecanoate	58.76 ± 1.16	25.83 ± 3.40	46.46 ± 1.77	44.95 ± 1.00	41.79 ± 1.49
Methyl octadecanoate	64.33 ± 1.12	26.53 ± 5.57	50.21 ± 2.37	46.96 ± 2.12	52.84 ± 6.20

n.d. – not detected

5) Enzymological and biological conditions have been studied to improve the efficiency of bioactive phenolic enrichment from plant residues.

Several types of residues were selected as substrates for the *in vivo* and *in vitro* tests planned in this objective. These fruit and vegetable byproducts are derived from plants having high antioxidative capacity, and rich in phenolic glycosides. The β-glucosidase enzymes can release the phenolic aglycone moiety from these glycosides, thereby, they can mobilize antioxidative compounds from the substrate. In our tests, the high-yield β-glucosidase producer *R. miehei* NRRL 5282 isolate and its cellulolytic cocktail produced under wheat bran-based solid-state fermentation were selected for the *in vivo* fermentation and *in vitro* enzyme treatment experiments, respectively. In the *in vivo* tests, the solid plant-waste medium was supplemented with soy flour as nitrogen source, and moisturized with distilled water. Then, this was inoculated with 5 x 10^6 spores of *R. miehei*, and incubated at 37 °C for predetermined time periods (about 9-18 days). During the incubation, samples were taken periodically and extracted with water or ethanol:water (50:50) solution. The phenolic content and antioxidant activity of the extracts were then determined, including radical scavenging and ferric reducing capacity tests. The β-glucosidase activity was also measured to analyze the correlation between the deglycosylation and the antioxidant capacity. In the *in vitro* analyses, combined carbohydrase treatments using *R. miehei* cellulase and *Aspergillus niger* pectinase were also carried out.

At first, we have investigated the antioxidative effect of hemp, flax, poppy and pumpkin seed residues after solid-state fermentation. These substrates are byproducts remained after extraction of the corresponding plant-seed oil. Overall results showed increase in the amount of soluble free phenolic components during the fermentation process. The antioxidant capacities (both ferric reducing and free radical scavenging activities) of the crude extracts were also enhanced, especially when flax- and hemp seed were used as substrates. Fermentation on poppy-, flax- and pumpkin seed residues showed correlation between β-glucosidase activity and radical scavenging/ferric reducing capacities; however, despite the high antioxidant power determined, low β-glucosidase activity was found on hempen seed. Furthermore, we studied the antimicrobial property of these antioxidative extracts against some foodborne pathogens and spoilage bacteria such as *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, *Pseudomonas putida, Escherichia coli*, *Salmonella enterica* and *Listeria monocytogenes*. For sample preparation, extracts were lyophilized overnight and then the final concentration of each sample was set to 100 mg/ml using 50% ethanol solution. Ten µl of concentrated extract was added to 5-mm paper discs placed on the Petri plates smeared with 10^8 bacterial cells. After 24-h incubation, hemp and flax seeds showed the highest activity against most of the bacteria tested. Both *Bacillus* strains were sensitive to the seed extracts while the resistant bacteria were *Sa. enterica* and *E. coli* (Table 3). In cases when inhibition was observed, positive correlation between total phenolic content and antimicrobial activity was generally found. Our results indicate the promising applicability of *R. miehei* fermentation to enhance and release health-protective extractable phenolics from oilseed byproducts. A BSc diploma work (G. Babos) was defended from the fermentation studies in 2015. Presently, a manuscript is under preparation from these results. Data obtained so far were presented at two conferences:

Kotogán A, Kerekes EB, Babos G, Krisch J, Papp T, Chandrasekaran M, Kadaikunnan S, Alharbi NS, Vágvölgyi C, Takó M (2015) **Bioconversion of oilseed residues by *Rhizomucor miehei* for production of phenolic antioxidants.** ACTA MICROBIOLOGICA ET IMMUNOLOGICA HUNGARICA 62:(S2) pp. 167-168. (17th International Congress of the Hungarian Society for Microbiology. Budapest, Magyarország: 2015.07.08 -2015.07.10.)

Takó M, Zambrano C, Kotogán A, Kerekes EB, Krisch J, Papp T, Vágvölgyi Cs (2016) **Antimicrobial effect of antioxidative extracts obtained after solid-state bioprocessing of oilseed residues using the zygomycete *Rhizomucor miehei*.** Power of Microbes in Industry and Environment 2016. Krk, Croatia, 2016.09.28-2016.10.01. Abstracts p. 66. (ISBN:978-953-7778-14-9)

Table 3. Antimicrobial activity of crude extracts from *R. miehei* NRRL 5282 fermented oilseed residues against foodborne pathogens and spoilage bacteria.

Bacteria	Pumpkin seed			Flax seed			Poppy seed			Hemp seed		
	t/(fermentation)/day											
	0	4	9	0	4	9	0	4	9	0	4	9
Bacillus subtilis	7.7± 0.4	6.3±0.4	9.0±0.1	5.3±0.4	5.3±0.4	7.7±0.4	8.0±0.1	7.7±0.4	6.3±0.4	6.0±0.7	6.0±0.4	7.7±0.4
B. cereus	n.d.*	n.d.	7.3±0.9	10.0±2.0	7.0±0.1	6.7±0.4	8.0±0.7	8.7±0.4	n.d.	8.3±0.4	8.0±0.7	9.7±0.4
Staphylococcus aureus	7.3±0.4	6.0±0.1	n.d.	5.7±0.4	5.3±0.4	n.d.	6.0±0.1	7.7±0.4	n.d.	7.0±0.1	5.7±0.4	6.7±0.9
MRSA	n.d.	n.d.	6.7±0.4	n.d.	n.d.	6.0±0.1	n.d.	n.d.	n.d.	n.d.	7.7±0.4	7.7±0.4
Pseudomonas aeruginosa	6.0±0.1	6.7±1.1	5.7±0.4	6.3±0.4	6.0±0.1	n.d.	7.0±0.1	8.0±0.1	n.d.	n.d.	6.7±0.4	7.3±0.4
P. putida	6.3±0.4	6.0±0.1	5.3±0.4	n.d.	n.d.	7.7±0.4	n.d.	n.d.	5.3±0.4	6.3±0.4	8.3±0.4	8.3±0.4
Escherichia coli	n.d.	n.d.	6.7±0.4	n.d.	5.3±0.4	n.d.	6.0±0.1	n.d.	n.d.	5.3±0.4	7.7±0.4	n.d.
Salmonella enterica	n.d.	n.d.	n.d.	n.d.	n.d.	6.0±0.1	6.0±0.1	6.0±0.0	6.0±0.1	n.d.	n.d.	5.3±0.4
Listeria monocytogenes	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	8.0±0.7	7.3±0.9	7.3±0.4	5.3±0.4	5.3±0.4	5.3±0.4

*not detectable

The presented values are averages calculated from the data of three independent experiments. Antimicrobial activities are represented by the width of growth inhibition zone (mm) ± standard deviation.

Our additional researches in the topic had focused on plant-wastes, i.e. jostaberry, black and white grape, apple, pitahaya (also known as dragon fruit) and mango residues, remained after fruit processing:

The *R. miehei* NRRL 5282 bioconversion of lyophilized jostaberry pomace leads to enhanced total phenolic content, and positive correlation between total phenolic content and β-glucosidase activity was also found. Both ferric reducing and free radical scavenging capacities were significantly improved, which correlated to the elevated concentration of free phenolics. However, a decrease in total monomeric anthocyanin concentration could be observed after three days fermentation, suggesting that these compounds were degraded and/or oxidized by the fermenting fungi. Data were compared to those obtained during *M. corticolus* SZMC 12031 bioconversion, and presented at a conference. Our results indicate that both *R. miehei* and *M. corticolus* fermentations are applicable methods for liberation and enrichment of health-relevant extractable phenolics from jostaberry pomace:

Takó M, Kotogán A, Babos G, Sója A, Papp T, Vágvölgyi C, Krisch J (2015) **Enhancement of the bioavailability of extractable berry phenolics by solid state fermentation.** 6th Congress of European Microbiologists (FEMS 2015). Maastricht, The Netherlands, 2015.06.07-2015.06.11. Paper FEMS-2831.

Bioactive phenolic enrichment from lyophilized and oven-dried Jonagold apple, Othello black grape and yellow pitahaya residues was studied in detail. Grape pomace remained after juice pressing, and the mixture of peels, cores, peduncles and seeds of apple and pitahaya samples were used as substrates. The experiments covered not only the phenolic liberation assays, but also included antimicrobial, anti-biofilm forming, and anti-*quorum sensing* activity measurements of the extracts obtained after *R. miehei* NRRL 5282 bioconversion and carbohydrase treatment. In general, the β-glucosidase activity and the extractable polyphenolic content increased during the fermentation. Moreover, our studies demonstrated positive correlation between the β-glucosidase and the total phenolic content and antioxidant activity during fungal growth on lyophilized pitahaya samples, indicating that the *R. miehei* can produce enhanced level of antioxidative phenolics from the substrate, and the β-glucosidase of the fungus has important role in this process. The carbohydrase treatment with *R. miehei* cellulase cocktail generally increased the total phenolic content and improved the antioxidant activity of the extracts. In case of the apple and pitahaya samples, the cellulase and *A. niger* pectinase combined treatment also enhanced the total phenolic yield. Liquid chromatography analysis of individual phenolic compounds was also performed. Depending on the substrate used, the gallic acid, vanillic acid, catechin and/or epicatechin content increased significantly during the fermentations and enzymatic treatments. Three conference presentations and a journal paper have been published from these results:

Zambrano C, Kotogán A, Komáromi L, Takó M, Krisch J, Vágvölgyi Cs (2016) **Production of phenolic antioxidants from apple residues.** International Conference on Science and Technique Based on Applied and Fundamental Research (ICoSTAF'16). Szeged, Hungary, 2016.06.02. Abstracts p. 48. (ISBN:978-963-306-482-5)

Takó M, Zambrano C, Kotogán A, Krisch J, Papp T, Mondal KC, Vágvölgyi Cs (2016) **Solid-state fermentation of dragon fruit residues to produce phenolic antioxidants using *Rhizomucor miehei*.** 18th Danube-Kris-Mures-Tisa (DKMT) Euroregional Conference on Environment and Health. Novi Sad, Serbia, 2016.06.02-2016.06.04., Book of abstracts. pp. 86-87. (ISBN:978-86-6253-059-2)

Zambrano C, Kovács F, Kotogán A, Papp T, Mondal KC, Hargitai F, Vágvölgyi C, Krisch J, Takó M (2017) **Phenolic antioxidant mobilization in black grape, apple and dragon fruit residues by enzymatic treatment using a cellulolytic cocktail from *Rhizomucor miehei*.** 7th Congress of European Microbiologists (FEMS 2017). Valencia, Spain, 2017.07.09-2017.07.13. Paper FEMS7-2768.

Zambrano C, Kotogán A, Bencsik O, Papp T, Vágvölgyi Cs, Mondal KC, Krisch J, Takó M (2018) **Mobilization of phenolic antioxidants from grape, apple and pitahaya residues via solid state fungal fermentation and carbohydrase treatment.** LWT-FOOD SCIENCE AND TECHNOLOGY 89: pp. 457-465. *IF: 2.329*

For antimicrobial activity testing, at first, *B. subtilis*, *B. cereus*, *S. aureus*, MRSA, *P. aeruginosa*, *P. putida*, *E. coli*, *Sa. enterica* and *L. monocytogenes* bacteria were subjected to

paper-disc agar-plate assay, in which a volume of 10 µl extract (100 mg/ml concentration set after lyophilization) was loaded to the disc. There were no significant differences in the overall antimicrobial activity of the oven-dried and lyophilized samples (Table 4); however, the carbohydrase treatment enhanced the antimicrobial activity of certain fruit residue extracts. The sensitive bacteria towards the extracts were the *Bacillus*, *S. aureus*, MRSA and *Sa. enterica* isolates, while low effect has been detected in case of the *Pseudomonas*, *L. monocytogenes* and *E. coli* strains.

Table 4. Antimicrobial activity of oven-dried (OD) and lyophilized (LYO) black grape, apple and pitahaya extracts (100 mg/ml) pre- and post enzymatic treatment against foodborne pathogens and spoilage bacteria. Activities are represented by the width of growth inhibition zone around the paper-disc (see the key below).

Bacteria/fruit extract treatments			*B. subtilis*	*B. cereus*	*L. monocytogenes*	*S. aureus*	MRSA	*E. coli*	*Sa. enterica*	*P. putida*	*P. aeruginosa*
Black grape	OD	Control [1]	++	+	+	++	+	+	++	+	+
		Cellulase (T1)	++	++	++	++	++	++	+++	+	+
		Control [2]	++	++	+	++	+	+	++	+	+
		Cellulase/ Pectinase (T2)	++	++	+	+++	++	++	++	+	+
	LYO	Control [1]	++	+	+	++	++	+	++	+	+
		Cellulase (T1)	++	+++	+	++	++	++	++	+	+
		Control [2]	+	++	+	++	++	++	++	+	+
		Cellulase/ Pectinase (T2)	++	++	+	++	++	++	++	+	+
Apple	OD	Control [1]	++	+	+	+	+	+	++	+	+
		Cellulase (T1)	++	++	+	++	+	+	++	++	+
		Control [2]	++	++	+	+	+	+	++	+	NE
		Cellulase/ Pectinase (T2)	NE	++	++	++	++	++	++	+	+
	LYO	Control [1]	+	++	+	+	+	+	++	+	+
		Cellulase (T1)	++	++	+	+	++	+	++	+	+
		Control [2]	+	++	+	++	++	++	++	+	+
		Cellulase/ Pectinase (T2)	++	+	+	++	++	++	++	+	+
Pitahaya	OD	Control [1]	+++	+	+	++	+	+	++	+	NE
		Cellulase (T1)	++	++	+	++	+	+	++	+	+
		Control [2]	+++	++	+	++	++	++	+++	+	+
		Cellulase/ Pectinase (T2)	+	++	+	+++	+	++	+	+	+
	LYO	Control [1]	+++	++	+	++	+	+	++	+	+
		Cellulase (T1)	++	++	+	+	+	+	+	+	NE
		Control [2]	+	++	+	++	++	++	+++	+	+
		Cellulase/ Pectinase (T2)	+++	++	+	+	+	+	+	+	+

Inhibition zone effect
NE No effect
\+ 5.00-7.49 mm
++ 7.50-9.99 mm
+++ >10 mm
[1] without enzymatic treatment
[2] *A. niger* pectinase treatment

To continuing these investigations, minimum inhibitory concentration (MIC) of the extracts was determined by using microdilution assay. After the carbohydrase treatments, reduced MIC was detected in case of many extracts compared to enzyme-free control. This can be attributed to the elevated concentration of individual phenolic compounds found in the extracts after the enzyme treatments (see Zambrano et al. 2018: LWT-Food. Sci. Technol. 89, 457-465). To prove this suggestion, antimicrobial activity of the major small phenolics detected in the extracts has also been determined. In these tests, we could also gain valuable information about the antimicrobial capacity of some pure phenolic compounds; it is worth mentioning that many of them have not yet been studied from this aspect. Presently, these data are under evaluation by our research group.

Anti-biofilm forming and anti-*quorum sensing* properties of selected extracts were also investigated. Because many of the tested foodborne pathogens and spoilage bacteria can form biofilms causing serious problems in the food industry, we also consider the investigation of these activities to be important. Majority of the extracts involved in this study inhibited the biofilm formation of *E. coli*, *S. aureus* and *L. monocytogenes* (Table 5). These preliminary results led us to complete the examinations with the other enzyme-treated extracts; evaluation of the data is in progress.

Anti-*quorum sensing* activity of the extracts was studied using the violacein pigment producer *Chromobacterium violaceum* 6269 model strain. The synthesis of violacein in *C. violaceum* is under *quorum sensing* regulation, which property makes this bacterium suitable

for screening of compounds with anti-*quorum sensing* effect. Based on the liquid-culture assays performed, cellulase (S1) and cellulase/pectinase (S2) treatments significantly stimulated the anti-*quorum sensing* activity of the lyophilized and oven-dried grape samples compared to enzyme-free control (C1) (Fig. 2). For the lyophilized apple and oven-dried pitahaya samples, carbohydrase treatment caused a moderate increase in the inhibition.

Table 5. Effect of enzyme-treated oven-dried (OD) and lyophilized (LYO) black grape, apple and pitahaya extracts (in 50 mg/ml concentration) on the biofilm formation of foodborne pathogens and spoilage bacteria.

			E. coli	*S. aureus*	*L. monocytogenes*	MRSA
Black grape	*OD*	Cellulase	72.9	55.9	77.9	8.9
		Cellulase + *Pectinase*	99.9	45.5	41.3	10.8
	LYO	Cellulase	71.1	39.3	NT	17.4
		Cellulase + *Pectinase*	80.0	24.5	NT	7.5
Apple	*LYO*	Cellulase	NT	30.9	NT	NT
		Cellulase + *Pectinase*	NT	32.8	NT	NT
Pitahaya	*OD*	Cellulase	NT	NT	NT	NT
		Cellulase + *Pectinase*	100.0	1.1	NT	NT
	LYO	Cellulase	NT	NT	NT	NT
		Cellulase + *Pectinase*	96.0	NT	NT	NT

NT: not tested;
Data were expressed in percent inhibition; full inhibition was considered to be 100%.

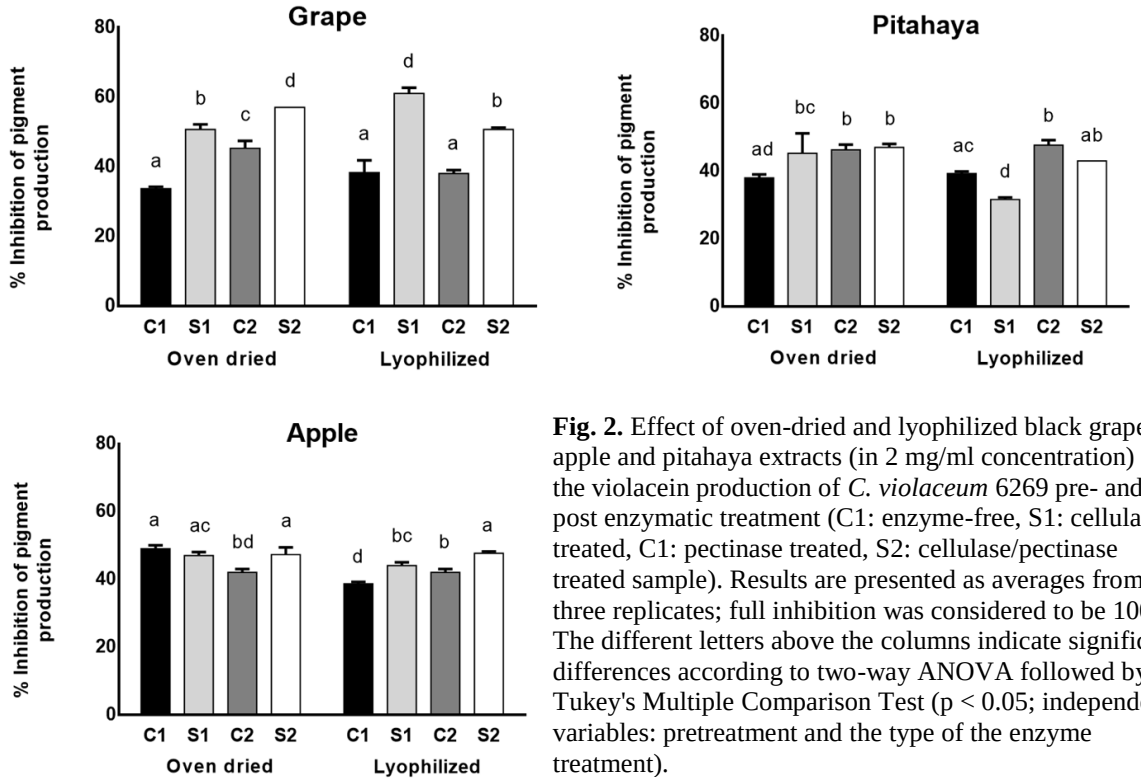

Fig. 2. Effect of oven-dried and lyophilized black grape, apple and pitahaya extracts (in 2 mg/ml concentration) on the violacein production of *C. violaceum* 6269 pre- and post enzymatic treatment (C1: enzyme-free, S1: cellulase treated, C1: pectinase treated, S2: cellulase/pectinase treated sample). Results are presented as averages from three replicates; full inhibition was considered to be 100%. The different letters above the columns indicate significant differences according to two-way ANOVA followed by Tukey's Multiple Comparison Test ($p < 0.05$; independent variables: pretreatment and the type of the enzyme treatment).

Fermentation and enzyme treatment studies regarding to white grape, mango, tomato and naranjilla samples were also performed within this topic. At present, evaluation of the datasets of these experiments is in progress. Partial results of the antimicrobial studies have been presented at two conferences:

Zambrano C, Kotogán A, Kerekes E, Vágvölgyi Cs, Krisch J, Takó M (2017) **Effect of carbohydrate hydrolyzing enzyme treatment on the antioxidant and antimicrobial activity of fruit pomace extracts.** 19th Danube-Kris-Mures-Tisa (DKMT) Euroregional Conference on Environment and Health. Szeged, Hungary, 2017.06.09-2017.06.10. Program and abstracts. p. 34. (ISBN:978-963-306-535-8)

Zambrano C, Kerekes EB, Kotogán A, Bencsik O, Mondal KC, Vágvölgyi Cs, Papp T, Takó M, Krisch J (2017) **Production of bioactive phenolic compounds from fruit residues by carbohydrase enzymes.** MIKOLÓGIAI KÖZLEMÉNYEK-CLUSIANA 56:(1) pp. 16-18. (6th Hungarian Mycological Conference, Szeged, Hungary: 2017.07.03 -2017.07.05.)

Furthermore, our research group conducts intensive researches concerning to the potential application of lipid-based materials, especially essential oils, as preservatives in the food industry. Knowledge and some results obtained during the antimicrobial assays have been utilized in that researches as well. In conjunction with these studies, a book chapter and a conference abstract were published:

Kerekes EB, Vidács A, Török Jenei J, Gömöri C, Takó M, Chandrasekaran M, Kadaikunnan S, Alharbi NS, Krisch J, Vágvölgyi C (2015) **Essential oils against bacterial biofilm formation and quorum sensing of food-borne pathogens and spoilage microorganisms.** In: Méndez-Vilas A (Ed.) The Battle Against Microbial Pathogens: Basic Science, Technological Advances and Educational Programs. Formatex Research Center, pp. 429-437. (ISBN:978-84-942134-6-5)

Kerekes EB, Vidács A, Gömöri Cs, Nacsa-Farkas E, Takó M, Vágvölgyi Cs, Krisch J (2017) **Essential oils as food preservatives: from lab experiments to use in real foods.** NATURAL VOLATILES & ESSENTIAL OILS 4:(3) p. 51. (48th International Symposium on Essential Oils. Pécs, Hungary: 2017.09.10 -2017.09.13.)

Other achievements during the project period:

This postdoctoral program allowed the PI to establish the research group of "Microbial Fermentation and Exoenzyme Technology" at the Department of Microbiology, University of Szeged, and contributed to his successful application for an assistant professor position to the Department at the end of 2016. Results achieved during the progression of the project have contributed to the PI's successful application for the János Bolyai Research Scholarship of the Hungarian Academy of Sciences in 2017.

Under the supervision of the PI, a PhD thesis containing enzymological results on lipases was successfully defended in 2017 (A. Kotogán: *Analysis of extracellular lipase enzymes from zygomycetes fungi: enzyme production, characterization of synthetic and hydrolytic reactions*; University of Szeged) by a researcher participating in the project. A further dissertation program is in progress concerning to the *in vivo* and *in vitro* analysis of bioactive phenolic production from plant residues (C. Zambrano: *Enrichment of bioactive phenolics in plant byproducts using fermentative and enzymatic processes*; University of Szeged; Supervisor: M. Takó; expected PhD defense is at 2018). Four BSc and three MSc diploma work were successfully defended from researches conducted within the frame of the project.

The topic of the project has contributed to establish fruitful cooperative works with the research group of Dr. K.C. Mondal at the Department of Microbiology, Vidyasagar University, India. This collaboration resulted in several joint publications. To strengthen this fruitful collaboration with the Indian partners, a joint research project proposal has been submitted under the Indo-Hungarian Intergovernmental S&T Cooperation Programme in 2017. We have also maintained our strong collaboration with Dr. J. Krisch from the Institute of Food Engineering, Faculty of Engineering, University of Szeged.

Enzymological results, methods and knowledges obtained during the project have been incorporated to the courses "Microbial Physiology" and "Soil Microbiology" at the University of Szeged.