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A search for nature's robust proteases with zein as a substrate

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Zein is produced in large quantities as a byproduct of corn starch manufacturing since it constitutes a majority of the total protein of maize seed (44–70%). Enzymatic treatment of zein significantly improves its aqueous solubility and provides peptides that are used as animal feed, functional food, or biologically active carriers for other bioactive molecules. Moreover, zein-derived peptides exhibit antioxidant, anti-inflammatory, antihypertensive, anticancer, and antimicrobial activities in human organisms¹. Few attempts up to this day have been made to screen for microorganisms that are capable of zein degradation. Available protocols for proteases identification almost exclusively rely on screening on casein, skim milk, and gelatin agar in limited experimental conditions. We have screened different *Bacillus* sp strains isolated from across Serbia for zein-degrading proteases. To do so we developed an inexpensive, simple, and reproducible way of high throughput functional screening of zein-degrading proteases on zein-containing gels. Besides detecting proteases with specificity towards zein, a developed diffusion assay was designed to support screening for naturally occurring robust proteases with high potential for industrial application. By using classical methods of protein purification, we isolated an alkaline thermostable protease from *Bacillus amyloliquefaciens* strain 12B that is resistant to the presence of detergents, organic solvents, and high salt concentrations.

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