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Summary of doctoral thesis „Biotransformation of rapeseed meal using *Bacillus subtilis*"

Agricultural and agro-industrial activities generate large quantities of lignocellulosic by-products such as fruit pomace, straw, stalks, cobs, peels and skins. The primary forms of organic waste include household food waste, agricultural waste, industrial waste, as well as human and animal waste.

Organic waste may be recycled and reused by means of biotransformation through fermentation with microorganisms. Solid-state fermentation (SSF) is the cultivation of microorganisms on a solid, insoluble substrate with a small proportion of water. This mode of microorganism cultivation delivers many benefits, including economic, energetic and ecological. SSF processes are used to generate a wide range of bio-based products, including a variety of enzymes, biosurfactants and biofuels. In addition, SSF is used to produce fermented feeds or additives for animal feed. Their nutritional properties are significantly improved by the SSF process, enriching them with beneficial microorganisms, while at the same time getting rid of any anti-nutritional compounds that limit the use of these substrates in the raw state.

Rapeseed meal, formed after the extraction of oil from rapeseed, has great potential for use in animal feed. It is a rich source of protein with a well-balanced amino acid composition, however, it also contains a large quantities of fiber, which monogastric animals are not able to digest. It is, however, possible to carry out solid fermentation with selected microorganisms, which may eliminate substances limiting the consumption of meal by animals. Rapeseed meal is also a very good substrate for the production of high-value microbiological products, such as feed enzymes or biosurfactants. Relatively cheap substrates and suitable microorganisms which produce valuable substances for profitable production are highly sought after.

During this study, the biotransformation of rapeseed meal using the *Bacillus subtilis* strain 87Y, originally isolated from the earthworm *Eisenia fetida*, was investigated. Initially, the metabolic abilities of *B. subtilis* strain 87Y were characterized in terms of enzymatic activity and surfactin production.

Highly specific enzymatic activity, including xylanolytic and cellulolytic activity, was determined during the SSF of rapeseed meal using *B. subtilis* 87Y. In addition, cultivation conditions were evaluated in terms of surfactin production by *B. subtilis* strain 87Y, in

comparison to liquid cultivation in a rich medium previously developed at the Biotransformation Department (Faculty of Biotechnology, University of Wroclaw) for the purpose of surfactin production. The solid cultivation method obtained similar biosurfactant yields to cultivation using a rich and expensive liquid medium.

Next, the enhancement of the nutritional value of rapeseed meal by SSF was evaluated via analysis of chemical and elemental composition. The loss of crude fiber, normally indigestible for monogastric animals, through SFF was confirmed.

An important aspect of rapeseed meal fermentation is the ability of *B. subtilis* strain 87Y to reduce mycotoxins from the meal to improve its potential as feed material. Infections with mycotoxins are a common problem that occurs during the cultivation of crops or during their storage. Consumption by animals of feed contaminated with mycotoxin-producing molds has tremendous adverse health effects. Hence, the ability to eliminate these toxic compounds through fermentation with microorganisms is of utmost importance. Rapeseed meal super-contaminated with deoxynivalenol, zearalenone, as well as aflatoxins B1 and B2, exhibited significant reduction in these compounds through *B. subtilis* strain 87Y fermentation.

The next stage of the work was to investigate potential probiotic properties of *B. subtilis* strain 87Y using *in vitro* methods. Strain 87Y was observed to be capable of survival in an acidic environment high in bile salts. Moreover, by using and modifying the insert method, *B. subtilis* strain 87Y was co-cultured with *Lactococcus lactis*, *Staphylococcus aureus*, *Salmonella enteritidis* and *Salmonella typhimurium* subspecies and were shown to be able to promote the growth of probiotic *L. Lactis*, while inhibiting *S. aureus* and both *Salmonella* spp.

Further, it was decided to expand the investigation to *in vivo* studies. Broiler chickens were given feed with containing 3% either unfermented or *B. subtilis* 87Y fermented rapeseed meal and the cecal microbiome was analyzed. Analysis of the content of the cecum, by means of the plate method using chromogenic media, demonstrated a beneficial effect of both the addition of unfermented and fermented rapeseed meal to the chicken fodder. Beneficial effects included the inhibition of pathogenic strains such as *Klebsiella pneumoniae* and *Enterobacter cloacae*, as well as an increase in the amount of *Lactobacillus* probiotics. Further analysis of the metagenome obtained from the content of the cecum revealed *Blastocystis hominis* parasite in the ceca of chickens fed with no additives. Moreover, a

histological analysis of the cecal tissue was performed, which revealed better morphology of the cecal mucosa in chickens fed with fermented *B. subtilis* 87Y fermented rapeseed meal.

As part of this study, it was also decided to study the components of the plant cell wall, specifically oats, on which *B. subtilis* 87Y was found to produce the highest yield of surfactin. Enzymatic activity, expression of genes encoding specific enzymes that break down cell wall elements and microscopic analysis of oat cell walls all suggested that xylan and its derivatives probably directly increase the production of biosurfactant.

Taken together, these results presented by this thesis demonstrate the metabolic benefits of fermentation with *B. subtilis* strain 87Y in terms of enzyme and surfactin production, as well as mycotoxin reduction. Furthermore, strain 87Y exhibited both *in vitro* and *in vivo* probiotic properties. It was concluded that rapeseed meal has great potential as an easily available and cheap fermentation substrate for the production of high-value biological products.