Solid state fermentation of a waste rapeseed meal leading to production of surfactin, biopolymers and feed enzymes

Ida Szmigiel[1], Marta Domżał-Kędzia[1], Robert Pluskota[2], Anna Krasowska[1], Marcin Łukaszewicz[1]

[1]Department of Biotransformation, Faculty of Biotechnology, University of Wroclaw,50-383, Wroclaw, Poland
[2]InventionBio Sp. Z.o.o., Wojska Polskiego 65, 85-825, Bydgoszcz, Poland
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Corresponding author: ida.szmigiel@uwr.edu.pl

Introduction

Solid state fermentation (SSF) has been succesfully used for a production of secondary metabolites, enzymes or food. The fermentation process is based on a non-soluble material that acts as a source of a nutrients for microroganisms. This kind of process mainly uses agricultural and industrial waste as a solid base, which are cheap and easily accessible.

In our reasearch we are using rapeseed meal (RSM) which is formed as a waste product during process of oil pressing from rapeseed. RSM contain a high fraction of cell wall polysaccharides and are a major source of proteins, amino acids, polysaccharides, and carbohydrates in animal feed. To increase the bioavailability of animal feed contents, supplementary feedstock mixes (SFM) are produced that have various enzymatic, prebiotic, and probiotic properties. Enzymes that deconstruct the cell wall of plants, such as xylanases and cellulases, are among the SFM components. Our studies are also focused on a production of high-value added products like biosurfactants and biopolymers.

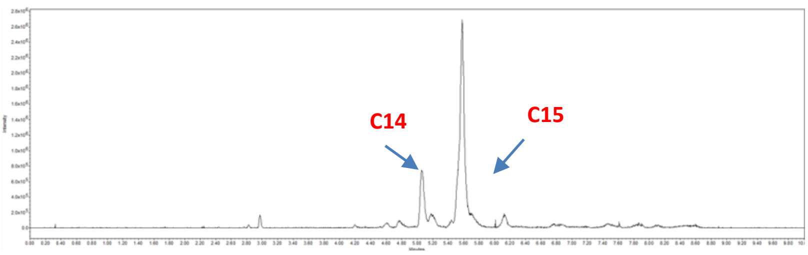
Biosurfactants are surfactants of natural origin produced by microorganisms. *Bacillus subtilis* strains used in our research are capable of producing a range of lipopeptide biosurfactants such as surfactin (SU) (Jajor et al. 2016). SU is a well-known cyclic lipopeptide and exhibits various biological activities, e.g. antimicrobial, antiviral, and antitumor, hence SU has potential applications for the pharmaceutical, cosmetic, and food industries.

Bacterial strains used in our studies are also capable of producing biopolymers like levan and γ-polyglutamic acid (γ-PGA). Levan is a fructose polymer synthetized from sucrose by various microorganisms and few plant species. This exopolysaccharide is a component of some biofilms and acts as a shield to microorganisms. Fructans like levan (Domżał-Kędzia et al. 2019), due to resistance to changing environmental conditions, are not degradated in our digestive system and acts as a prebiotic for human gut microflora. γ-PGA in turn is composed by monomers of glutamic acids. Same as levan, polyglutamic acid is produced by various microorganisms, ingredient of a biofilms and acts as a natural moisturizer.

Methods

Quantitative analyses of surfactin were performed using Ultra-High Performance Chromatography – Mass Spectrometry (UHPLC - MS) methods. Quantitative analyses of xylanolytic and cellulolytic enzymes were performed using dinitrosalicylic acid (DNS). Qualitative analyses of levan and γ-PGA were performed using Gel Permeation Chtromatography (GPC/SEC) methods and Nuclear Magnetic Resonance Spectroscopy (NMR) methods.

Results



Surfactin analog	Molecular mass	Pseudo-molecular ions [*m/z*]
C14	1021	1022, 1044
C15	1035	1036, 1058

Fig.1. Separation of crude extract from rapeseed meal after 24 h of fermentation using UHPLC – MS. During fermentation process we obtain mainly C14 and C15 analogs of surfactin.

B. subtilis 87 Y						
time [h]	4	8	12	16	20	24
xylanases [%]	0	1	45	95	75	75
celulases [%]	0	0	25	95	70	70

Fig.2. Percentage share of xylanases and cellulases produced on rapeseed meal after 24 h fermentation.

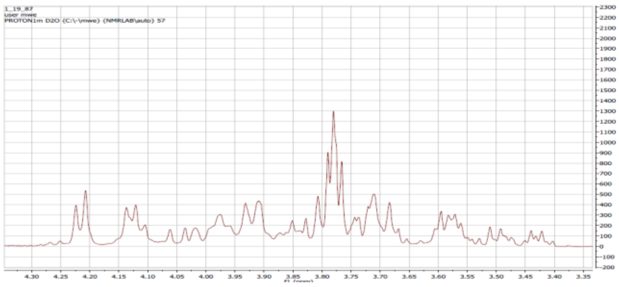


Fig.3 Identification of levan on 24 h fermented rapeseed meal by H^1NMR.

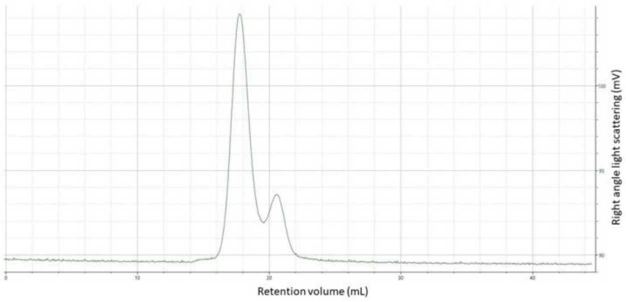


Fig.4 Identification of two fractions of levan on 24 h fermented rapeseed meal by GPC/SEC.

Conclusions

Rapeseed meal can be very useful source of nutrients for microorganisms. In our study we use environmental bacterial *B. subtilis* strains with special metabolic abilities. During fermentation process of RSM we obtain necessary feed stock enzymes, surfactin, biosurfactant with a wide range of properties as well as biopolymers like levan. Among five structural analogs of surfactin (C12-C16), we obtain mainly two of them: C14 and C15 (Fig.1). The highest production of xylanases and celulases we observe around sixteenth hour of growth (Fig.2). We also showed that we are able to produce two fractions of biopolymer levan (Fig 2&3).

References

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