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EFFLUENTS FROM INDUSTRIAL PROCESSING OF THE FOOD OF ANIMAL ORIGIN AS MEDIA FOR BIOCONTROL AGENTS PRODUCTION

EFLUENTI IZ INDUSTRIJSKE PRERADE HRANE ŽIVOTINJSKOG POREKLA KAO MEDIJUMI ZA PROIZVODNJU BIOKONTROLNIH AGENASA

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ABSTRACT

Intensive food production to feed the growing human population requires significant material and energy resources, simultaneously generating large amounts of waste, whose treatment adds an additional cost to the overall production process. Wastewaters arising from meat and dairy processing industries contain large amounts of organic and inorganic nutrients, which present a heavy environmental burden, but, on the other hand, could serve as nutrient sources for microbial growth. The aim of this study was to investigate the suitability of meat processing wastewater and whey from the dairy industry as media for the growth of biocontrol strain Bacillus velezensis IP22. Both waste-based media have contributed to antibacterial and antifungal activity to a similar degree, with highly comparable inhibition zone diameters against Xanthomonas campestris, Xanthomonas euvesicatoria and Aspergillus flavus. Furthermore, biocontrol activity of the cultivation broths obtained using the waste-based media was slightly lower compared to the nutrient broth medium, suggesting the possibility to replace the expensive chemically defined medium with a complex medium alternative. This approach based on circular economy principles promotes responsible resource utilization through the reuse of food industry effluents for the production of value value-added products, opening a chapter of possibilities to establish an industrial symbiosis network between companies in different industry branches.

***Keywords**: industrial processing; wastewater; Bacillus velezensis; Xanthomonas campestris; Xanthomonas euvesicatoria.*

REZIME

Intenzivna proizvodnja hrane za ishranu rastuće ljudske populacije zahteva značajne materijalne i energetske resurse, istovremeno generišući velike količine otpada čiji tretman predstavlja dodatni trošak celokupnom proizvodnom procesu. Otpadne vode iz industrijske prerade mesa i mleka sadrže velike količine organskih i neorganskih nutrijenata, koji predstavljaju opterećenje za životnu sredinu, ali takođe mogu poslužiti kao izvor hranljivih materija za rast mikroorganizama. Cilj ovog rada bio je ispitivanje mogućnost primene otpadnih voda iz prerade mesa i surutke iz mlečne industrije kao medijuma za rast biokontrolnog soja Bacillus velezensis IP22. Praćenje rasta bakterija u odabranim podlogama na bazi efluenata prehrambene industrije pokazalo je pogodnost otpadnih voda iz prerade mesa i surutke kao odličnih supstrata za umnožavanje Bacillus velezensis IP22 i povećanje broja živih ćelija. Oba medijuma na bazi industrijskog otpada su u sličnom stepenu doprinela antibakterijskoj i antifungalnoj aktivnosti, sa uporedivim prečnicima zona inhibicije protiv Xanthomonas campestris, Xanthomonas euvesicatoria i Aspergillus flavus, pri čemu je uočen veći nivo otpornosti fungalnog patogena prema ispitivanim biokontrolnim agensima. Biokontrolna aktivnost postignuta primenom kultivacionih tečnosti dobijenih korišćenjem medijuma na bazi otpada bila je nešto niža u poređenju sa hranljivim bujonom, što sugeriše mogućnost da se skupa hemijski definisana podloga zameni kompleksnim alternativnim medijuma, koji nisu pokazali inhibitorne efekte na rast i metaboličku aktivnost Bacillus velezensis IP22. Ovaj pristup zasnovan na principima cirkularne ekonomije promoviše odgovorno i maksimalno korišćenje resursa kroz ponovnu upotrebu otpadnih voda prehrambene industrije za dobijanje proizvoda sa dodatom vrednošću, otvarajući novo poglavlje mogućnosti uspostavljanja mreže industrijske simbioze između kompanija u različitim industrijskim granama.

***Ključne reči:** otpadna voda iz prerade mesa, surutka, Bacillus velezensis, Xanthomonas campestris, Xanthomonas euvesicatoria.*

INTRODUCTION

The increasing human population demands an increased supply of primary resources such as food and agricultural products at regional and global levels to meet societal needs (*Giller et al., 2021*). With increasing industrialization comes increased waste generation, which is considered a major environmental challenge (*Gaur et al., 2020*). Food industry effluents present one of the biggest concerns when it comes to environmental impact because of their large content of organic and inorganic nutrients, which are a heavy environmental burden (*Dmitrović et al., 2022*).

Dairy is the part of the food industry that serves the global population with nutritionally rich products included in the everyday diet, such as milk, cheese, milk powder, ghee, butter, etc. At the same time, the dairy industry generates millions of tons of byproducts. In Europe, nearly 29 million tons of dairy products end up as waste every year (*Usmani et al., 2022*). The main byproduct of the dairy industry is cheese whey, which corresponds to the net fraction remaining after milk coagulation. The production of 1 kg of cheese produces around 9–10 liters of whey, which creates a significant problem for the environment if discarded without treatment (*Asgharnejad et al., 2021*). Dairy effluents are characterized by high chemical oxygen demand (COD) and biological oxygen demand (BOD) levels due to the milk proteins and lactose they contain. Concentrations of COD and BOD in cheese whey are usually in the range of 50-102 g/L and 27-60 g/L respectively (*Dmitrović et al., 2022*). For the comparison, the average BOD and COD values for urban wastewaters are 0.20 and 0.41 g/L respectively (*Pires et al., 2021*). Furthermore, whey usually contains large amounts of nitrogen and phosphorus, as well as residual lactose and fat from milk processing into cheese products (*Asgharnejad et al., 2021*).

Meat has been the main source of protein in the human diet for thousands of years and an important part of the food pyramid (*de Castro Cardoso Pereira and dos Reis Baltazar Vicente, 2013*). The meat processing industries are one of the biggest industries, with an annual production of 355.5 million tons in 2021 (*FAO, 2022*). The amount of wastewater generated in meat production depends on the product and factory specialization. Every step in the meat processing industry, including animal slaughtering, washing, and cutting, deboning and packaging meat, and processing meat into various products such as dry- and heat-treated meat products, generates wastewater (*Skripsts et al., 2022*). In poultry production, the water required per animal is about 11.5 L, while the water requirement for beef production units is around 1325 L per animal (*Asgharnejad et al., 2021*), considering all stages of meat processing until obtaining fresh meat products. The concentration of COD and BOD in meat processing wastewater is mostly caused by the presence of blood, fat, and mucosa and is in the range of 2–6 g/L and 1.3–2.3 g/L, respectively, caused by a high content of suspended solids arising mostly from the aforementioned debris and resulting in high nitrogen and fat content in meat processing wastewater (*Latiffi et al., 2019*).

Taking into account the high amount of organic and inorganic nutrients suitable for microbial growth present in wastewater from the meat and dairy industries, one of the possible routes for wastewater treatment could be microbial bioconversion of available nutrients to value-added products, such as microbial biocontrol agents. In this way the biocontrol agents' production cost would be significantly reduced, resulting in higher market competitiveness of the final biocontrol product. Biological control is a relatively novel chapter in agricultural production that aims to employ biological agents for plant protection against pathogens instead of conventionally used agrochemicals. Microbial biocontrol agents are promising alternatives for plant disease management due to their high efficacy, multiple mechanisms of action, lower chance of antimicrobial resistance development, and selective biocontrol activity. Bacteria from the *Bacillus* genus are the most widely used as active components of microbial biocontrol agents due to their multiple beneficial biocontrol traits, including the production of a wide spectrum of volatile and diffusible antimicrobial agents, supreme competitive traits in terms of growth space occupation and nutrients acquisition, plant growth promotion capabilities and plant immunity boosting against plant pathogens (*Pajčin et al., 2020*).

Plant diseases caused by bacterial and fungal pathogens lead to large economic losses worldwide (*Nazarov et al., 2020*). Bacterial spot is one of the most common pepper and tomato diseases in terms of infection persistence and economic losses caused by *Xanthomonas euvesicatoria*. Crop losses caused by this pathogen are usually very severe if the infection occurs at an early growth stage (*Potnis et al., 2015*). The infection is characterized by lesions on leaves, stems, and fruits. The pathogen spreads mostly through the seed and infected crop remains, but also by the rain and overhead irrigation. Disease severity implies an infection ratio as high as 50–95% with significant economic losses due to the necessity to remove infected plants and fruits in order to prevent the repeated occurrence of the infection in the same field. Usually used disease suppression methods include chemical treatments using copper-based bactericides in combination with ethylene bis-dithiocarbamates, but frequent application of these preparations has led to the emergence of resistant *Xanthomonas euvesicatoria* strains (*Pajčin et al., 2020*).

Another bacteria from the genus *Xanthomonas* that causes great damage to crops is *Xanthomonas campestris*. The black rot of crucifers caused by this bacterial pathogen is considered the most destructive disease of crucifers worldwide (*Schaad and Dianese, 1981*). The typical leaf symptoms of black rot are V-shaped lesions manifested at the leaf margin as a result of pathogen entrance through hydathodes, causing vein blackening. Preventing black rot infection is difficult and relies on the use of pathogen-free planting material and the elimination of other potential inoculum sources. On the other hand, when infections occur, the usual treatments are sprays of antibiotics, copper fungicides, crop rotation, and control of cruciferous weeds (*Singh et al., 2010; Vicente and Holub, 2013*).

When it comes to fungal pathogens, *Aspergilus flavus* represents one of the major pests for crops of maize, peanuts, and cotton. The estimation of the European Commission (EC) confirms the influence of mycotoxin contamination, resulting in annual global crop losses of 5 to 10%. *Aspergilus flavus* is characterized by a high potential for aflatoxin production. There are four major types of aflatoxin: B1, B2, G1, and G2, and from the food safety point of view, the most relevant is aflatoxin B1 (AFB1). The International Agency for Research on Cancer (IARC) characterized AFB1 as a carcinogen (Group 1a) (*Vlajkov et al., 2021*).

The suppression of the aforementioned bacterial and fungal plant pathogens becomes a major problem in agricultural and food production. Hence, there is a necessity to consider other eco-friendly and bio-based alternatives for plant disease management, where the application of *Bacillus*-based biocontrol agents represents one of the promising directions. The aim of this study was to investigate the suitability of meat processing wastewater and whey from the dairy industry as media for the production of biocontrol agents based on *Bacillus velezensis* IP22, and to examine antifungal and antibacterial activity of the produced biocontrol agents against a range of plant pathogens, including *Xanthomonas campestris* Mn 7-2, *Xanthomonas euvesicatoria* PL1 and *Aspergillus flavus* SA2B SS.

MATERIAL AND METHOD

Producing microorganisms and phytopathogens

Bacillus velezensis IP22, used as producing microorganism in this study, was isolated from fresh cheese and previously identified to the species level (*Pajčin et al., 2020*). Phytopathogens used in this study were bacteria *Xanthomonas campestris* Mn 7-2, the black rot pathogen of cruciferous crops (*Grahovac et al., 2020*), *Xanthomonas euvesicatoria* PL1, pepper bacterial spot pathogen (*Pajčin et al., 2020*), and *Aspergillus flavus* SA2B SS, corn fungal pathogen producing aflatoxins (*Vlajkov et al., 2021*). All microorganisms were stored at the Laboratory for biochemical engineering, Faculty of Technology Novi Sad, and refreshed under the following conditions (medium, temperature): nutrient agar (Himedia Laboratories, India), 28 ℃ for producing microorganism, YMA (yeast maltose agar, *Grahovac et al., 2021*), 26 ℃ for *Xanthomonas* spp. and SMA (Sabouraud maltose agar, Himedia Laboratories, India), 26 ℃ for *Aspergillus flavus* SA2B SS.

Media and cultivation conditions

The inoculum of the producing microorganism *Bacillus velezensis* IP22 was prepared using nutrient broth (28 ℃, 170 rpm, 48 h). Ten percent (v/v) of the inoculum was used for inoculation of the cultivation media. The following cultivation media were examined: nutrient broth (Himedia Laboratories,

India), meat processing wastewater (cumulative wastewater from the production process obtained from a meat processing plant based in Serbia) and whey (obtained after cheese production from a dairy plant based in Serbia). Media based on food industry effluents were used as obtained, without any treatment or dissolution, except sterilization (121 °C, 2.1 bar, 20 min). Cultivations of the producing microorganism were performed on a laboratory shaker at 28 °C with an external agitation rate of 170 rpm and spontaneous aeration for 96 h, with everyday sampling to determine biomass content and antimicrobial activity against target bacterial and fungal phytopathogens.

Biomass content determination

The biomass content of *Bacillus velezensis* IP22 in cultivation broth samples was determined using the plate count method. Briefly, serial dilutions of cultivation broth were prepared (up to 10^{-8}), and the dilutions designated as 10^{-6}, 10^{-7} and 10^{-8} were placed in a Petri dish (1 mL) and mixed with melted and tempered (50±1 °C) nutrient agar (15 mL). Incubation was performed at 28 °C for 48 h, followed by an enumeration of emerged colonies to determine the biomass content in CFU/mL (colony forming unit/mL).

Antimicrobial activity assay

Suspensions of phytopathogens were prepared in sterile saline (10^6 CFU/mL for *Xanthomonas* spp., 10^5 spores/mL for *Aspergillus flavus* SA2B SS). Test plates were prepared by mixing the suspensions (1 mL) with melted and tempered (50±1 °C) test media (YMA for *Xanthomonas* spp., SMA for *Aspergillus flavus* SA2B SS). After media solidification in the Petri dish, three paper discs were placed at YMA and three wells were made in SMA. Cultivation broth samples collected at different cultivation time points were tested for their antibacterial (3x10 µL) and antifungal activity (3x100 µL) in triplicate tests. Incubation was carried out at 26 °C for 72 h and 120 h for antibacterial and antifungal assays, respectively, followed by diameter measurement of the emerged inhibition zones.

Experimental data analysis

LabPlot v. 2.9 (github.com/KDE/labplot) software was employed for generating cultivation course monitoring plots. Statistica 13.2 software (Dell, USA) was used for statistical analyses, with all tests performed at the significance level ≤0.05 (95%).

RESULTS AND DISCUSSION

Biomass content of *Bacillus velezensis* IP22 during cultivation on commercial and waste-based media

During the cultivation of *Bacillus velezensis* IP22 using the nutrient broth, meat processing wastewater and whey, periodic sampling of cultivation broth was performed to determine the viability and number of bacterial cells. Another goal was to investigate the possible inhibitory effects of waste-based media on the growth and metabolic activity of the producing microorganism. The results of the cultivation course monitoring in terms of bacterial growth are presented in Fig. 1. The data presented in this figure depict the conventional bacterial growth curve independently of the medium used for cultivation. The highest biomass content was achieved after 72 h of cultivation in each of the investigated cases. Furthermore, this fact also points out the possibility to reduce bioprocess duration from 96 h to 72 h, suggesting a possible route to cut down the overall production cost and achieve significant savings with simultaneous optimal exploitation of the available production resources. Furthermore, the examined waste-based media arising from the processing of animal food products didn't show any inhibitory effect on the growth of the producing microorganism *Bacillus velezensis* IP22, confirming that these industrial effluents could be applied as media basis without any further pretreatment, except sterilization. Thus, mutual benefit could be achieved on both sides - effluent generators and effluent adopters, with savings achieved in terms of waste treatment and waste utilization instead of less cost-effective commercial media, respectively. Another important point is that effluent-based media preparation hasn't required consumption of additional process water, pointing out the saving of this valuable resource and also reducing the cost of process water preparation, also resulting in less generated wastewater at the end of the production cycle.

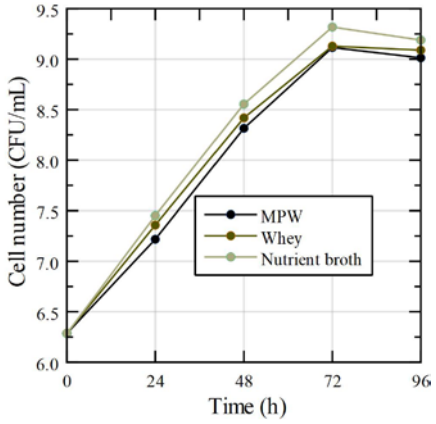

Fig. 1. Biomass content of Bacillus velezensis IP22 during cultivation on commercial and waste-based media (MPW – meat processing wastewater)

Antimicrobial activity of biocontrol agents produced using commercial and waste-based media

In order to investigate the suitability of meat processing wastewater and whey from the dairy industry as potential media for the production of biocontrol agents based on *Bacillus velezensis* IP22, antimicrobial activity of the cultivation broth samples collected at predefined time points was tested against bacterial and fungal pathogens. To investigate differences between waste-based media and commercial chemical defined medium and their effects on biocontrol activity of *Bacillus velezensis* IP22, nutrient broth as the common medium for bacterial cultivation was also included in the screening, besides MPW and whey from the dairy industry. The effects of the aforementioned cultivation media on antimicrobial activity against *Xanthomonas campestris* Mn 7-2, *Xanthomonas euvesicatoria* PL1 and *Aspergillus flavus* SA2BSS were analyzed using the inhibition zone diameters as the main indicators of antimicrobial activity. The results of the analysis of variance (ANOVA) are given in Table 1. As could be seen, the effect of the applied medium (nutrient broth, MPW, or whey) was statistically significant at a confidence level of 95% only in the case of antimicrobial activity against *Xanthomonas campestris* Mn 7-2. This could be explained by higher sensitivity of the aforementioned strain towards *Bacillus*-based biocontrol agents in comparison to other tested plant pathogens, which was presented as follows, but also in other studies (*Pajčin et al., 2022a*), thus implying that small changes in the medium applied for biocontrol agents production could affect the level of achieved antibacterial activity against this pathogenic strain. In

the other two cases, there wasn't a significant effect of the cultivation medium to inhibition zone diameters obtained during antimicrobial assays against *Xanthomonas euvesicatoria* PL1 and *Aspergillus flavus* SA2B SS, indicating the smaller extent of the medium contribution to the overall antimicrobial activity of *Bacillus velezensis* IP22 against the aforementioned plant pathogens.

Table 1. ANOVA (analysis of variance) for media effects on antimicrobial activity of the produced biocontrol against Xanthomonas campestris Mn 7-2 (XC), Xanthomonas euvesicatoria PL1 (XE) and Aspergillus flavus SA2BSS (AF)

Effect	Pathogen	SS	MS	DF	F	*p*-value
Intercept	XC	8341.78	8341.78	1	15015.20	**<0.0001**
	XE	6642.25	6642.25	1	4511.72	**<0.0001**
	AF	3025.00	3025.00	1	6405.88	**<0.0001**
Medium	XC	6.89	3.44	2	6.20	**0.0347**
	XE	12.17	6.08	2	4.13	0.0744
	AF	2.17	1.08	2	2.29	0.1820
Error	XC	3.33	0.56	6	-	-
	XE	8.83	1.47	6		
	AF	2.83	0.47	6		

SS - sum of squares, MS - mean squares, DF - degree of freedom

Figures 2, 3 and 4 show the comparison between synthetic medium (nutrient broth) and waste-based media (meat processing wastewater - MPW and whey from the dairy industry) in terms of their antibacterial or antifungal activity against the selected plant pathogens: *Xanthomonas campestris* Mn 7-2, *Xanthomonas euvesicatoria* PL1 and *Aspergillus flavus* SA2BSS, respectively. In the case of the antibacterial activity against *Xanthomonas campestris* Mn 7-2 it could be observed that meat processing wastewater and whey were at the same level of statistical significance in terms of their effect on antibacterial activity (*p*-value 0.6038), while the commercial medium nutrient broth was at a separate homogeneous group in terms of statistically significant difference compared to waste-based media (*p*-values 0.0340 compared to whey and 0.0194 compared to MPW). However, differences in inhibition zone diameters less than 3 mm could be observed when comparing synthetic and complex media, suggesting that less than 10% of antimicrobial could be lost when applying the cost-effective and environment-friendly alternative medium in the production process.

Similar results could be observed in the case of biocontrol activity against *Xanthomonas euvesicatoria* PL1 (Fig. 3). As mentioned earlier, larger inhibition zones could be observed in the case of *Xanthomonas campestris* Mn 7-2, suggesting higher sensitivity of the black rot pathogen towards the *Bacillus*-based biocontrol agents. As in the previous case, a similar level of statistical significance could be observed when it comes to the effect of meat processing wastewater and whey on antibacterial activity against *Xanthomonas euvesicatoria* PL1 (*p*-value 0.2837), but in this case, the nutrient broth was grouped into the same homogenous group as meat processing wastewater (*p*-value 0.1437). These results point out slightly higher suitability of meat processing wastewater as a substrate for the production of biocontrol agents effective against the bacterial spot pathogen, as well as the possibility of successfully replacing the synthetic medium with meat industry wastewater in the cultivation of *Bacillus velezensis* IP22 as the biocontrol active component.

In the case of antifungal assays against *Aspergillus flavus* SA2B SS, a slightly higher biocontrol effect could be observed when applying whey as a substrate compared to meat processing wastewater, although each of the three tested media was at the same level of statistical significance.

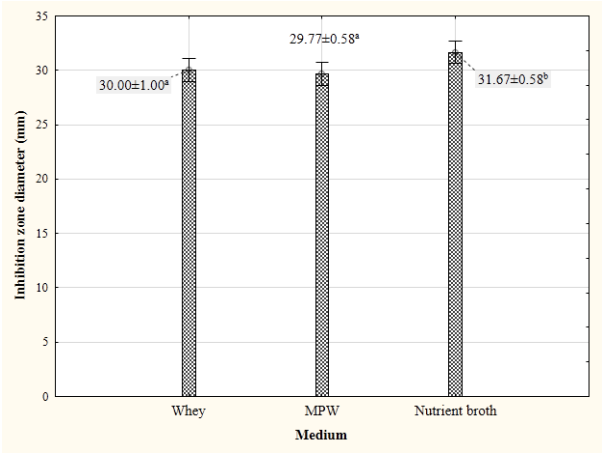

Fig. 2. Antimicrobial activity of Bacillus velezensis IP22 biocontrol agents produced on commercial and waste-based media against Xanthomonas campestris Mn 7-2 (MPW – meat processing wastewater)

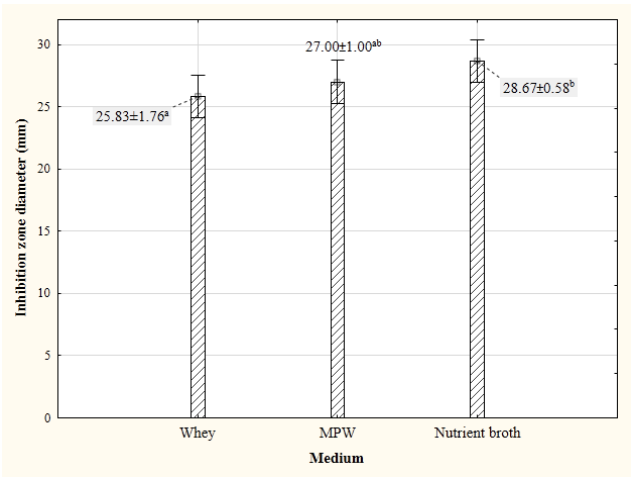

Fig. 3. Antimicrobial activity of Bacillus velezensis IP22 biocontrol agents produced on commercial and waste-based media against Xanthomonas euvesicatoria PL1 (MPW – meat processing wastewater)

Therefore, in the development of the production process for biocontrol agents effective against *Aspergillus flavus* SA2B SS meat processing wastewater and whey could be successfully applied as replacements for nutrient broth, with less than a 5% difference in biocontrol activity. It could also be concluded that fungal pathogen is more resistant to the investigated biocontrol agents compared to bacterial pathogens, although in field conditions bacterial infections are found to be significantly harder to suppress and control due to a previously mentioned problem of fast development of antimicrobial resistance towards agrochemicals (*Miller et al., 2022*). Hence biocontrol agents based on *Bacillus* spp. offer an additional advantage in the biological control of fungal and more importantly bacterial phytopathogen in terms of exhibiting more than one mechanism of action, therefore making pathogens harder to develop resistance (*Shafi et al., 2017; Fira et al., 2018*). Cultivation broth as the overall cultivation product, containing both viable bacterial cells and produced metabolites, was found to be highly efficient in terms of biocontrol activity in comparison with the separate potential active components, and one of the reasons could be a possibility to exhibit several mechanisms of biocontrol action (*Pajčin et al., 2022b*). However, a detailed investigation of the mechanisms of action involved in biocontrol activity of *Bacillus velezensis* IP22 against the investigated plant pathogens is required to determine eventual further downstream

steps to formulate the biocontrol product with maximized antibacterial and antifungal activity.

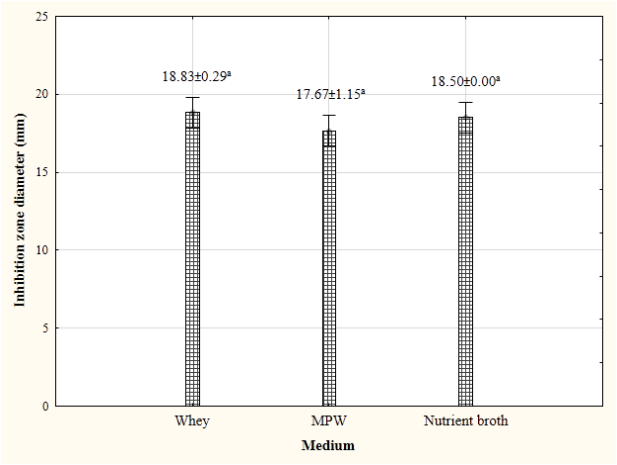

Fig. 4. Antimicrobial activity of Bacillus velezensis IP22 biocontrol agents produced on commercial and waste-based media against Aspergillus flavus SA2B SS (MPW – meat processing wastewater)

The possibility to apply industrial waste streams as a possible medium basis for biocontrol agent production was not thoroughly investigated in the literature. Dmitrović et al. (2022) have shown a successful utilization of whey and winery flotation effluent for the production of biocontrol agents based on *Bacillus amyloliqefaciens*. Another winery effluent, the barrel washing wastewater, could also be used as a substrate for *Bacillus*-based biocontrol agents production (*Pajčin et al., 2022c*). Fruit processing wastewater arising from alcoholic drinks production could be another potential medium for microbial growth to produce biocontrol agents (*Pajčin et al., 2022b*). The most investigated species of the *Bacillus* genus in terms of industrial waste utilization in the cultivation process is *Bacillus thuringiensis*, used for bioinsecticide production, which was shown to be able to grow in starch industry wastewater (*Brar et al., 2005; Kumar et al., 2019*), olive mill wastewater (*Jallouli et al., 2014*) and brewery sludge (*Wu et al., 2014*). The small number of studies in this field calls for an action to define sustainable and scalable bioprocess solutions for the utilization of food industry effluents as raw materials in biocontrol agents production to provide suitable and efficient alternatives to chemical pesticides and establish a basis for a more sustainable agricultural production system, as well as transition to greener technologies by removing the effluents from the industrial production processes and performing their valorization to obtain value-added products. Circular economy principles dictate the necessary shift from a linear disposal-oriented approach towards the networking of industrial subjects in order to achieve maximal exploitation of the available input and output resources, thus making a circular metabolism or industrial symbiosis network at larger perspective outside only one production facility (*Fraccascia and Giannoccaro, 2020; Neves et al., 2020*), hence contributing to the burning problem of industrial waste generation and disposal.

CONCLUSION

Production of food of animal origin is designated as one of the major environmental issues considering greenhouse gas emissions and a significant amount of biohazardous effluents which must be treated before their safe disposal to the environmental recipients. This study aimed to investigate effluents from the animal food industry (meat processing wastewater and whey from the dairy industry) as possible raw materials for the production of biocontrol agents based on *Bacillus velezensis* IP22, the biocontrol with proven antibacterial and antifungal activity against a wide range of plant pathogens. This approach resulted in obtaining valued-added biocontrol agents which could be applied in sustainable plant disease management. The investigated pathogens whose suppression was targeted were *Xanthomonas campestris*, the black rot pathogen of cruciferous crops, *Xanthomonas euvesicatoria*, pepper bacterial spot pathogen, and *Aspergillus flavus*, corn fungal pathogen producing aflatoxins. The monitoring of bacterial growth in the selected food industry effluents-based media has shown the suitability of meat processing wastewater and whey from the dairy industry as excellent substrates for the multiplication of *Bacillus velezensis* IP22 and increase in viable cell number, as observed by the regular bacterial growth curves, with the maximal cell number achieved after 72 h of cultivation at 28 °C in both of the tested media. Similarly, the peak of antibacterial and antifungal activity was also observed after three days of cultivation. In order to assess biocontrol efficacy obtained using the waste-based media as substrates, inhibition zone diameters against bacterial and fungal pathogens were compared between the meat processing wastewater medium, whey-based medium and nutrient broth as the commercial chemically defined medium. The results of this study have confirmed the possibility to utilize animal food industry effluents as substrates for biocontrol agents resulting in only a 5-10% loss in biocontrol activity compared to the commercial chemically defined medium as a less cost-effective option. In this way the biocontrol agents' production cost would be significantly reduced, resulting in higher market competitiveness of the final biocontrol product. Significant water savings would be achieved, especially at the larger production scale, considering that no process water was used for the preparation of the waste-based media, thus contributing to cost reduction due to savings on process water management. This study has therefore confirmed successful implementation of the circular economy principles in biotechnological production aiming the increased sustainability in the agricultural and food production sector, providing a basis for consideration of industrial symbiosis establishment. The suggested approach also requires defining the suitable downstream strategy to achieve maximal biocontrol efficiency of the formulated biocontrol agent in field testing experiments and further application in plant disease management.

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REFERENCES

Asgharnejad, H., Khorshidi Nazloo, E., Madani Larijani, M., Hajinajaf, N., & Rashidi, H.R. (2021). Comprehensive review of water management and wastewater treatment in food processing industries in the framework of water-food-environment nexus. Comprehensive reviews in food science and food safety, 20, 4779-4815. https://doi.org/10.1111/1541-4337.12782

Brar, S.K., Verma, M., Tyagi, R D., Valéro, J.R., & Surampalli, R.Y. (2005). Starch industry wastewater-based stable Bacillus thuringiensis liquid formulations. Journal of Economic Entomology, 98(6), 1890-1898. https://doi.org/10.1093/jee/98.6.1890

de Castro Cardoso Pereira, P.M., dos Reis Baltazar Vicente, A.F. (2013). Meat nutritional composition and nutritive role in the human diet. Meat Science, 93, 586-592. https://doi.org/10.1016/j.meatsci.2012.09.018

Dmitrović, S., Pajčin, I., Vlajkov, V., Grahovac, M., Jokić, A., & Grahovac, J. (2022). Dairy and wine industry effluents as alternative media for the production of Bacillus-based biocontrol agents. Bioengineering, 9(11), 663. https://doi.org/10.3390/bioengineering9110663

Fira, Đ., Dimkić, I., Berić, T., Lozo, J., & Stanković, S. (2018). Biological control of plant pathogens by Bacillus species. Journal of Biotechnology, 285, 44-55. https://doi.org/10.1016/j.jbiotec.2018.07.044

Fraccascia, L., & Giannoccaro, I. (2020). What, where, and how measuring industrial symbiosis: A reasoned taxonomy of relevant indicators. Resources, Conservation and Recycling, 157, 104799. https://doi.org/10.1016/j.resconrec.2020.104799

Gaur, V.K., Sharma, P., Sirohi, R., Awasthi, M.K., Dussap, C.G., & Pandey, A. (2020). Assessing the impact of industrial waste on environment and mitigation strategies: A comprehensive review. Journal of Hazardous Materials, 398, 123019. https://doi.org/10.1016/j.jhazmat.2020.123019

Giller, K.E., Delaune, T., Silva, J.V., Descheemaeker, K., van de Ven, G., Schut, A.G.T., van Wijk, M., Hammond, J., Hochman, Z., Taulya, G., Chikowo, R., Narayanan, S., Kishore, A., Bresciani, F., Teixeira, H.M., Andersson, J.A., van Ittersum, M.K. (2021). The future of farming: Who will produce our food? Food Security, 13, 1073-1099. https://doi.org/10.1007/s12571-021-01184-6

Grahovac, J., Pajčin, I., Vlajkov, V., Rončević, Z., Dodić, J., Cvetković, D., & Jokić, A. (2021). Xanthomonas campestris biocontrol agent: Selection, medium formulation and bioprocess kinetic analysis. Chemical Industry and Chemical Engineering Quarterly, 27(2), 131-142. https://doi.org/10.2298/CICEQ200508032G

FAO (2022). Food Outlook – Meat and Meat Products. https://www.fao.org/3/cb9427en/cb9427en_meat.pdf (accessed on 2nd March 2023)

Jallouli, W., Sellami, S., Sellami, M., & Tounsi, S. (2014). Efficacy of olive mill wastewater for protecting Bacillus thuringiensis formulation from UV radiations. Acta Tropica, 140, 19-25. https://doi.org/10.1016/j.actatropica.2014.07.016

Kumar, L.R., Ndao, A., Valéro, J., & Tyagi, R.D. (2019). Production of Bacillus thuringiensis based biopesticide formulation using starch industry wastewater (SIW) as substrate: A techno-economic evaluation. Bioresource Technology, 294, 122144. https://doi.org/10.1016/j.biortech.2019.122144

Latiffi, N.A., Mohamed, R., Shanmugan, V.A., Apandi, N.M., Tajuddin, R.M., & Kassim, A.H. (2019). Characteristics of water quality from meat processing wastewater. Journal of Advanced Research in Applied Sciences and Engineering Technology, 17(1), 78-84.

Miller, S.A., Ferreira, J.P., & LeJeune, J.T. (2022). Antimicrobial use and resistance in plant agriculture: A One health perspective. Agriculture, 12, 289. https://doi.org/10.3390/agriculture12020289

Nazarov, P.A., Baleev, D.N., Ivanova, M.I., Sokolova, L.M., Karakozova, M.V. (2020). Infectious plant diseases: etiology, current status, problems and prospects in plant protection. Acta Naturae, 12, 46-59. https://doi.org/10.32607/actanaturae.11026

Neves, A., Godin, R., Azevedo, S.G., & Matias, J.C.O. (2020). A comprehensive review of industrial symbiosis. Journal of Cleaner Production, 247, 119113. https://doi.org/10.1016/j.jclepro.2019.119113

Pajčin, I., Vlajkov, V., Dmitrović, S., Jokić, A., Grahovac, M., Dodić, J., & Grahovac, J. (2022b). Distillery fruit waste as a substrate for biocontrol agents production. Proceeding of the 27th Biotechnology Counseling, 25-26. March 2022. Čačak, Republic of Serbia, 315-320. https://doi.org/10.46793/SBT27.315P

Pajčin, I., Vlajkov, V., Dodić, J., Loc, M., Grahovac, M., & Grahovac, J. (2022a). Bacillus velezensis - biocontrol activity of cells and extracellular compounds against Xanthomonas spp. Journal on Processing and Energy in Agriculture, 25(1), 15-18. https://doi.org/10.5937/jpea26-36660

Pajčin, I., Vlajkov, V., Frohme, M., Grebinyk, S., Grahovac, M., Mojicevic, M., & Grahovac, J. (2020). Pepper bacterial spot control by Bacillus velezensis: Bioprocess solution. Microorganisms, 8(10), 1463. https://doi.org/10.3390/microorganisms8101463

Pajčin, I., Vlajkov, V., Loc, M., Dodić, J., Grahovac, M., & Grahovac, J. (2022c). Valorization of barrel washing winery wastewater through production of microbial biocontrol agents. Acta Periodica Technologica, 53(1), 223-230. https://doi.org/10.2298/APT2253223P

Pires, A.F., Marnotes, N.G., Rubio, O., García, A.C., & Pereira, C.D. (2021). Dairy by-products: A review on the valorization of whey and second cheese whey. Foods, 10(5), 1067. https://doi.org/10.3390/foods10051067

Potnis, N,; Timilsina, S., Strayer, A., Shantharaj, D., Barak, J.D., Paret, M.L., Vallad, G.E., & Jones, J.B. (2015). Bacterial spot of tomato and pepper: Diverse Xanthomonas species with a wide variety of virulence factors posing a worldwide challenge. Molecular Plant Pathology, 16(9), 907-920. https://doi.org/10.1111/mpp.12244

Schaad, N.W., & Dianese, J.C. (1981). Cruciferous weeds as sources of inoculum of Xanthomonas campestris in black rot of crucifers. Phytopathology, 71, 1215-1220. https://doi.org/10.1094/Phyto-71-1215

Shafi, J., Tian, H., & Ji, M. (2017). Bacillus species as versatile weapons for plant pathogens: a review. Biotechnology & Biotechnological Equipment, 31(3), 446-459. https://doi.org/10.1080/13102818.2017.1286950

Singh, D.P., Dhar, S., & Yadav, D.K. (2010). Effect of endophytic bacterial antagonists against black rot disease of cauliflower caused by Xanthomonas campestris pv. campestris. Indian Phytopathology, 63, 122-126.

Skripsts, E., Mezule, L., & Klaucans, E. (2022). Primary sludge from dairy and meat processing wastewater and waste from biomass enzymatic hydrolysis as resources in anaerobic digestion and co-digestion supplemented with biodegradable surfactants as process enhancers. Energies,15(12), 4333. https://doi.org/10.3390/en15124333

Usmani, Z., Sharma, M., Gaffey, J., Sharma, M., Dewhurst, R.J., Moreau, B., Newbold, J., Clark, W., Kumar Thakur, V., & Kumar Gupta, V. (2021). Valorization of dairy waste and by-products through microbial bioprocesses. Bioresource technology, 346, 126444. https://doi.org/10.1016/j.biortech.2021.126444

Vicente, J.G., & Holub, E.B. (2013). Xanthomonas campestris pv. campestris (cause of black rot of crucifers) in the genomic era is still a worldwide threat to brassica crops. Molecular Plant Pathology, 14(1), 2-18. https://doi.org/10.1111/j.1364-3703.2012.00833.x

Vlajkov, V., Grahovac, M., Budakov, D., Loc, M., Pajčin, I., Milić, D., Novaković, T., & Grahovac, J. (2021). Distribution, genetic diversity and biocontrol of aflatoxigenic Aspergillus flavus in Serbian maize fields. Toxins, 13(10), 687. https://doi.org/10.3390/toxins13100687

Wu, L.Y. (2012). Biochemical utilization of brewery sewage as a raw material for the production of Bacillus thuringiensis. Journal of Fujian Agriculture and Forestry University (Natural Science Edition), 41(4), 529-53.

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