# Influence of the composition of the culture medium on the growth, sporulation and biological activities of probiotic strain *Heyndrickxia coagulans* BJ

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**Abstract.** The influence of the composition of the nutrient medium on the growth and sporulation of *Heyndrickxia coagulans* BJ during submerged cultivation was investigated. The logistic curve and exponential mathematical models were employed to study the kinetics of the growth and sporulation processes. The strain demonstrated maximal value of the maximum specific growth rate of 0.174 $h^{-1}$ and maximum specific sporulation rate of 0.198 $h^{-1}$ in nutrient medium B. The obtained culture liquids were investigated for biological activities. Three ninhydrin-positive compounds with antifungal activity and different Rf values were detected through thin-layer chromatography ($Rf_1$ = 0.55; $Rf_2$ = 0.07; and $Rf_3$ = 0.81). The strain exhibited proteolytic, amylolytic and lipolytic activities.

## 1 Introduction

Microorganisms represent an extraordinarily diverse and ubiquitous component of the biosphere. Many possess considerable biotechnological potential and are exploited as industrial producers of bioactive compounds [1]. Their high reproductive capacity, metabolic versatility, and amenability to controlled cultivation in bioreactors make them valuable resources across biotechnology, medicine, agriculture, and environmental management [2,3]. They contribute fundamentally to soil fertility, biological water purification, and the production of foods, pharmaceuticals, and energy resources [4-7].

Escalating antibiotic resistance and growing public concern regarding synthetic pesticides have intensified the search for new, environmentally sustainable biological alternatives. Within this context, members of genus *Heyndrickxia* have attracted increasing scientific and applied interest. Strains belonging to this group synthesize a broad spectrum of secondary metabolites with antimicrobial activity, with demonstrated relevance in both medical [8,9] and agricultural [10-12] applications.

*Heyndrickxia coagulans* has emerged as a highly promising probiotic due to its robust spore-forming ability, which facilitates exceptional resistance to harsh conditions in food processing and the gastrointestinal tract (e.g., heat, acidity, bile salts) [13-15]. Once ingested, the spores can survive stomach acidity and reach the intestine, where they germinate and transiently colonize the gut [16-18]. This resilience not only ensures viability during storage and administration but also supports consistent delivery of beneficial effects. Importantly, safety assessments of several strains have found no evidence of mutagenicity, genotoxicity, or harmful gene content – making *H. coagulans* a safe candidate for human and animal probiotic applications [15].

*In vivo* and clinical studies further highlight the functional benefits of *H. coagulans*. Supplementation with different strains was associated with improved digestion (improved protein utilization and nutrient absorption), modulation of gut microbiota toward potentially beneficial species, and enhancement of host immune responses, including elevated mucosal immunoglobulins and natural-killer cell activity [16,19,20]. Moreover, in broilers and human trials, *H. coagulans* improved gut barrier integrity, exerted anti-inflammatory effects, and increased antioxidant capacity [17,21,22]. These two properties make *H. coagulans* a versatile probiotic, which combines survival under stress, digestive support, microbiota modulation, and immunomodulation, and justify its increasing use in functional foods, supplements, and potentially even non-traditional applications (e.g., animal feed, stress-resistant preparations).

*Heyndrickxia coagulans* and related bacilli have attracted the interest of researchers as sustainable biocontrol agents because they produce a range of antimicrobial metabolites (including lipopeptides and bacteriocin-like compounds), hydrolytic enzymes, and organic acids that can suppress phytopathogenic fungi and bacteria and modulate the rhizosphere microbiome [23,24]. Several studies report direct antagonism of *H.*

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*coagulans* strains against important plant pathogens. *B. coagulans* isolates inhibited species of *Fusarium, Rhizoctonia* and other phytopathogenic fungi and reduced disease symptoms in controlled assays [25,26]. These antimicrobial effects may be combined with activities, such as phosphate solubilisation, production of siderophores, ACC-deaminase activity, and induction of plant systemic resistance to formulate preparations for crop protection and growth promotion [27].

The remarkable resistance of *Heyndrickxia coagulans* spores to heat, acidity, and bile further positions this species as an appealing candidate for industrial probiotic formulations [28] and for robust, field-stable microbial products used in crop protection [24,29]. Harnessing this potential in commercial formulations depends critically on two interconnected requirements: (i) the capacity to generate high-quality biomass and stress-resistant spores at scale, and (ii) the consistent expression or preservation of functional biological activities – such as enzyme secretion, antimicrobial compound production, and biofilm- or adhesion-associated traits – following production and formulation.

Culture medium composition exerts a profound influence on both factors. In *Bacillus* species, the nature of carbon and nitrogen sources, C:N ratio, mineral content, and growth phase cues can substantially affect growth kinetics, metabolic allocation toward secondary metabolite pathways, and the efficiency of sporulation. These parameters ultimately shape the performance, stability, and efficacy of the resulting microbial products [30,31].

The aim of this study was to examine how nutrient medium composition affects the growth, sporulation, and selected biological activities of *Heyndrickxia coagulans* M relevant to its prospective applications.

## 2 Materials and Methods

### 2.1 Microorganisms

*Heyndrickxia coagulans* BJ isolated from spontaneously coagulated pasteurized milk and identified by physiological-biochemical and molecular-genetic tests, was used in the present study. The strain was maintained on a nutrient medium with the following composition (g/dm$^3$): peptone form meat – 10, NaCl – 5, glucose – 10, pH 7.0÷7.5, incubated at 37°C for 48 h and stored at 4±2°C.

*Aspergillus flavus* ATCC 16883 from the collection of the Department of Microbiology and Biotechnology at the University of Food Technologies was used as a test-microorganism for the study of antimicrobial activity.

### 2.2 Nutrient media

***LBG-agar***

Composition (g/dm$^3$): tryptone - 10; yeast extract - 5; NaCl - 10; glucose - 10; agar-agar - 15. The pH was adjusted to 7.0-7.5. Sterilization – 20 min at 121ºC.

***Medium A***

Composition (g/dm$^3$): molasses - 20; peptone - 10; corn extract - 3; $CaCl_2$ - 0.22; $MgSO_4$ - 0.11; $K_2HPO_4$ - 0.24. The pH was adjusted to 7.0-7.5. Pasteurization – 30 min at 100ºC.

***Medium B***

Composition (g/dm$^3$): malt - 20; corn extract - 3; molasses - 20; $CaCl_2$ - 0.22; $MgSO_4$ - 0.11; $K_2HPO_4$ - 0.24. The pH was adjusted to 7.0-7.5. Pasteurization – 30 min at 100ºC.

***Medium C***

Composition (g/dm$^3$): peptone - 10; NaCl - 5; meat extract - 5; glucose – 10; $CuCl_2$ – 0.001; $MgSO_4$ – 0.5. The pH was adjusted to 7.0-7.5. Sterilization – 20 min at 121ºC.

***LBG-agar supplemented with skimmed milk for the determination of proteolytic activity***: 100 cm$^3$/dm$^3$ of skimmed milk was added to the molten nutrient medium.

***LBG-agar supplemented with fat and indicator for the determination of lipolytic activity*:** 50 cm$^3$/dm$^3$ of fat containing 0.75 g/kg fat Victoria Blue indicator was added to the molten nutrient medium.

***LBG-agar supplemented with starch (1%) instead of glucose for the determination of amylolytic activity***

### 2.3 Methods of analysis

#### *2.3.1 Cultivation of Heyndrickxia coagulans BJ*

Cultivation of the strain was carried out in 500 cm$^3$ Erlenmeyer flasks with 100 cm$^3$ of nutrient medium at a temperature of 37°C for 24÷48 h on a rotary shaker (220 min$^{-1}$). Inoculation was carried out with 1% (v/v) 18 h vegetative inoculation material.

#### *2.3.2 Determination of the number of viable cells and spores of Heyndrickxia coagulans BJ*

The number of viable cells was determined by preparation of serial tenfold dilutions and spread plating on LBG-agar medium. The Petri dishes were incubated for 24÷48 h at 37±1°C until the appearance of single colonies. The same procedure was followed for the determination of the number of spores, but prior to dilution the samples were treated at 80°C for 20 min to destroy the vegetative cells.

#### *2.3.3 Modelling process kinetics and identification of model parameters of Heyndrickxia coagulans BJ*

The modelling of the growth kinetics of *Heyndrickxia coagulans* M cultivated in the different fermentation media was done using the classical logistic curve equation (1), and the sporulation kinetics of the strain was modelled with the exponential growth model (2):

$$\frac{dX_b}{d\tau} = \mu_m X_b - \beta {X_b}^2 \quad \mu_m X_b - \frac{\mu_m}{X_{bk}} {X_b}^2 \quad \mu_m \left(1 - \frac{X_b}{X_{bk}}\right) X_b \tag{1}$$

$$\frac{dX_{bs}}{d\tau} = kX_{bs} \Rightarrow X_{bs} = X_{0_{bs}} e^{k\tau} \quad (2)$$

where: $\mu_m$ - maximum specific growth rate of vegetative cells, $h^{-1}$ ; $X_b$ and $X_{bk}$ – current and final concentration of vegetative cells in dimensionless form; $k$ – maximum rate constant of sporulation; $X_{bs}$ and $X_{0_{bs}}$ - current and initial spore concentration in dimensionless form; $\beta$ – coefficient of intra-population competition, $X_b/m^3$.h.

The differential equations were solved by the 4th order Ringe-Kutta method in MS Office Excel 2013. The general and discretized forms of the equation were:

$$\frac{dy}{dx} = F(x, y)$$

$$\frac{y_{i+1} - y_i}{x_{i+1} - x_i} = \frac{y_{i+1} - y_i}{h} = F(x.y)$$

where $h$ is a step in the solution.

For a system of ordinary differential equations ($k$=1,...,$k$ equations):

$$\frac{dy_1}{dx_1} = F_1(x, y_1, y_2, \dots\dots\dots, y_k)$$

$$\frac{dy_2}{dx_2} = F_2(x, y_1, y_2, \dots\dots\dots, y_k)$$

$$\frac{dy_k}{dx_k} = F_k(x, y_1, y_2, \dots\dots\dots, y_k)$$

The algorithm for solving ordinary differential equations by the Runge-Kutta method of the 4th order is as follows:

$$y_{k,i+1} = y_{k.i} + \frac{1}{6}(k_{k,1} + 2.k_{k,2} + 2.k_{k,3} + k_{k,4})$$

$$k_{k,1} = hF_k(x_i, y_{1,i}, y_{2,i}, \dots\dots\dots y_{k,i})$$

$$k_{k,2} = hF_k(x_i + \frac{h}{2}, y_{k,i} + \frac{k_{k,1}}{2})$$

$$k_{k,3} = hF_k(x_i + \frac{h}{2}, y_{k,i} + \frac{k_{k,2}}{2})$$

$$k_{k,4} = hF_k(x_i + h, y_{k,i} + k_{k,3})$$

The Solver function in MS Excel 2013 was used to identify the parameters of the models. They were determined by the minimization of the square of the difference between the experimental data and the data obtained from the corresponding model.

### *2.3.4 Preparation of SDS-PAGE samples*

The sterile filtrate was prepared according to a previously published method [32]. In brief, the culture medium was centrifuged at 3500 $min^{-1}$ for 15 min to separate the biomass, followed by filtration of the supernatant through a membrane filter (0.45 μm) (sample 1). The proteinaceous substances from the supernatant were isolated by precipitation with HCl (pH 2.0) overnight at 4°C, followed by centrifugation at 12000 r/min, 4°C, for 20 min. The obtained supernatant was sample 2 and the pellet was treated with 1.5 $cm^3$ methanol and incubated at 4°C for 8 h. The supernatant after centrifugation at 10000 r/min, 4°C, for 20 min was labelled sample 3 and the pellet was dissolved in 0.5 $cm^3$ 0.5 N NaOH for 15 min and after that was diluted with 1.0 $cm^3$ $H_2O$ (sample 4).

### *2.3.5 SDS-PAGE Electrophoresis*

Gel electrophoresis under denaturing conditions (SDS-PAGE) of the obtained samples was performed using an omniPAGE mini Cleaver system (model CVS10DSYS, Cleaver Scientific Ltd., Rugby, UK) according to the method of Laemmli (1970), with a 15% resolving gel (pH 8.8) [33]. All samples subjected to SDS-PAGE were adjusted to equal protein concentrations. Each protein sample was mixed with Simple buffer (containing 4% SDS, 25% glycerol, 4% 2-mercaptoethanol, and 0.02% bromophenol blue, dissolved in 0.25 M tris(hydroxymethyl)aminomethane, and proteins were denatured for 2 minutes in a boiling water bath. The cooled samples (30 μl) were loaded into the wells of a 6% stacking gel. The gels were stained with 0.2% Coomassie Brilliant Blue R-250 (Serva Electrophoresis GmbH, Heidelberg, Germany) for 20 minutes, followed by destaining in a solution containing 10% ethanol and 7% acetic acid for 12 hours.

Molecular-weight determination (Mw) of the obtained fractions was carried out using the "BIO-RAD Precision Plus Protein Standards (10,000-250,000 Da)". Gel data were processed and visualized using the specialized software TotalLab1D v. 14.1 (BioStep GmbH, Burkhardtsdorf, Germany). To facilitate the interpretation and discussion of the results, the protein fractions were categorized into three groups: low-molecular-weight fractions (LMF, <50 kDa), medium-molecular-weight fractions (MMF, 50-150 kDa), and high-molecular-weight fractions (HMF, >150 kDa).

### *2.3.6 Thin-layer chromatography (TLC)*

Thin-layer chromatography was performed on silica gel TLC plates ($COF_{254}$, 20×20 cm; Merck, Germany) and mobile phase of butanol : acetic acid : water (3:1:1 v/v/v), placed in a chromatography chamber to a depth of 0.5 cm. At the original line of each plate, 50 μl of the prepared culture media was applied using a micropipette. The chromatogram, after development, was dried at 60°C for approximately 15 min and sprayed with ninhydrin solution to detect peptide antibiotics according to the method of Arx et al. (1967). After spraying, pink spots appeared on the chromatograms. The Rf-values of the spots were determined.

### *2.3.7. Biochromatography*

A copy of the TLC chromatograms, which was not sprayed with ninhydrin solution, was subjected to bioautography according to the method of Leifert et al. (1995). The chromatogram was dried for 5 hours at 70°C, after which it was placed onto an agar nutrient medium previously inoculated with *Aspergillus flavus* ATCC 16883. The setup was left at low temperature for 30 minutes to allow diffusion of the antibiotic substances into the agar plates. Subsequently, the TLC plates were removed, and the bioautograms were incubated at 28°C. The active spots were identified, and their Rf-values were determined [34].

### *2.3.8 Determination of enzyme activity*

Melted nutrient medium was poured into Petri dishes (15 $cm^3$ each), and after the medium solidified, six wells (6 mm in diameter) were made in each plate using a cork borer. Fresh 24 h culture of the strain was used. A spore suspension (60 µL) with a spore concentration of $10^{12}$ cfu/$cm^3$ was added to the wells. The diameter of the hydrolysis zones (in mm) was determined after 48 hours.

### *2.3.9 Processing of the results*

Data from triplicate experiments were processed using MS Office Excel 2013 software, using statistical functions to determine the standard deviation and maximum error of estimate at $\alpha<0.05$ significance levels.

## 3 Results and discussion

In microbial populations the biomass accumulation, sporulation and metabolite production are highly dependent on nutrient availability and environmental conditions. The effect of the composition of the nutrient medium on the development of *Heyndrickxia coagulans* BJ was investigated through submerged cultivation in shaken flasks, and the dynamics of biomass accumulation, the sporulation process, and the percentage of sporulation during cultivation were monitored. The results of these studies are presented in Fig. 1 to 3.

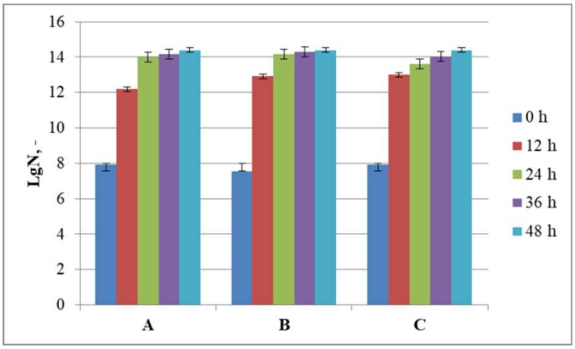


**Fig. 1.** Total viable cell count of *Heyndrickxia coagulans* BJ during submerged cultivation in shake flasks on the different nutrient media.

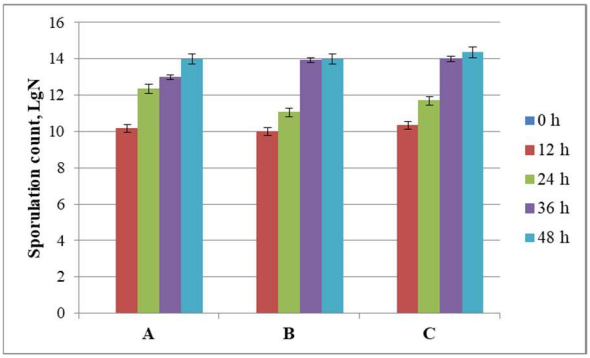


**Fig. 2.** Spore count of *Heyndrickxia coagulans* BJ during submerged cultivation in shake flasks on the different nutrient media.

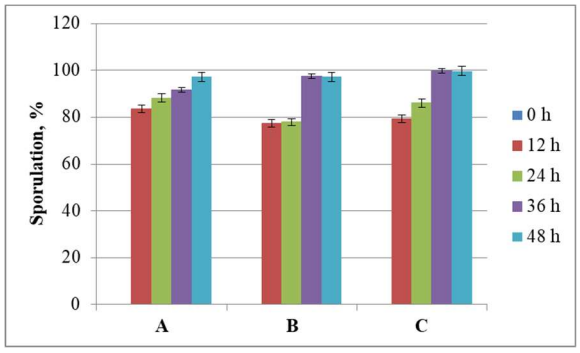


**Fig. 3.** Sporulation percentage of *Heyndrickxia coagulans* BJ during submerged cultivation in shake flasks on the different nutrient media.

The results presented in Fig. 1 show that the total viable cell count of the *Heyndrickxia coagulans* BJ strain increased continuously up to 24 h when cultivated in media A and B, reaching values of approximately $10^{13}$-$10^{14}$ cfu/$cm^3$. After this point, the total viable cell concentration remained relatively constant. In medium C the maximum viable cell count – also in the range of $10^{13}$-$10^{14}$ cfu/$cm^3$ – was reached at 36 h and remained stable until the end of the process.

No spores were detected during the first 12 h of cultivation. Sporulation of *Heyndrickxia coagulans* BJ began 12 h after the start of cultivation in all three fermentation media (Fig. 2). The experimental data in Fig. 2 also indicate that the spore concentrations in the three media were comparable (approximately $10^{10}$ cfu/$cm^3$). In medium B and C the number of spores continued to increase until 36 h, reaching concentrations of about $10^{14}$ cfu/$cm^3$, which then remained relatively constant until the end of the process. In the molasses-based medium, spore concentration increased continuously throughout the cultivation process, also reaching approximately $10^{14}$ cfu/$cm^3$.

The sporulation percentage was also monitored through the cultivation process. These results are shown in Fig. 3. At 12 h, the sporulation percentage was 83.57%, 77.40%, and 79.40% for medium A, B and C, respectively. During the process, the sporulation percentage in medium B and C increased to maximum values of 97.24% and 99.71% at 36 h, remaining stable until 48 h. In medium A, sporulation increased continuously, reaching 97.24% by the end of the process.

Understanding the kinetics of the process is essential for scaling up and operating fermentation in bioreactors of different volumes. For this reason, key kinetic parameters of growth and sporulation for *Heyndrickxia coagulans* BJ were calculated for the three selected nutrient media. To enable kinetic modelling, the total viable cell count and spore count were converted into dimensionless values so that the modelled kinetic curves would start from the same reference point. The results of the parametric identification of the kinetic models are presented in Table 1.

**Table 1**. Kinetic parameters of growth in the logistic curve model for the *Heyndrickxia coagulans* BJ strain

| | Medium A | Medium B | Medium C |
|---|---|---|---|
| $\mu_m$, $h^{-1}$ | 0.128 | 0.174 | 0.155 |
| $\beta$, $X_b/cm^3.h$ | 0.0700 | 0.0914 | 0.0847 |
| $x_{\kappa b}$ | 1.82 | 1.91 | 1.83 |
| $R^2$ | 0.9999 | 0.9999 | 0.9987 |
| E | 0.15 | 0.17 | 0.16 |

The data presented in Table 1 indicate that the logistic curve model used to describe the growth kinetics exhibited high correlation coefficients between the experimental data and the model predictions. The correlation coefficient ranged from 0.9987 to 0.9999. Notably, the identification errors were very low, well below 1, with values of 0.15, 0.17, and 0.16 for the different media. These low identification errors, combined with the high correlation coefficients, demonstrate that the model accurately and adequately describes the process kinetics and can be reliably used to predict the process within the studied time frame.

The data in Table 1 show that *Heyndrickxia coagulans* BJ achieved a high maximum specific growth rate ($\mu_{max}$) of 0.174 $h^{-1}$ in medium B, compared to 0.128 $h^{-1}$ in medium A and 0.155 $h^{-1}$ in medium C. Moreover, the predicted theoretical maximum concentrations of the total viable cell count in dimensionless form were 1.82, 1.91, and 1.83 for molasses, malt, and supplemented meat-peptone broth, respectively. These values closely match the experimentally determined maxima: 1.82 and 1.91 for media A and B, respectively, and 1.93 for medium C, confirming the adequacy of the model.

The mathematical model also provides comparable values for the intra-population competition coefficient: 0.0700, 0.0914, and 0.0847 $X_b/cm^3 \cdot h$ for medium A, B and C, respectively. These coefficients are significantly lower than one, indicating that the composition of the fermentation media, the agitation rate, and the cultivation temperature were appropriately selected. Under these conditions, the strain developed without inhibitory effects that could lead to cell death due to competition for nutrients and oxygen (Table 1). This, in turn, demonstrates that this model accurately fits the experimental data and can be used to predict the sporulation process within the selected cultivation time interval.

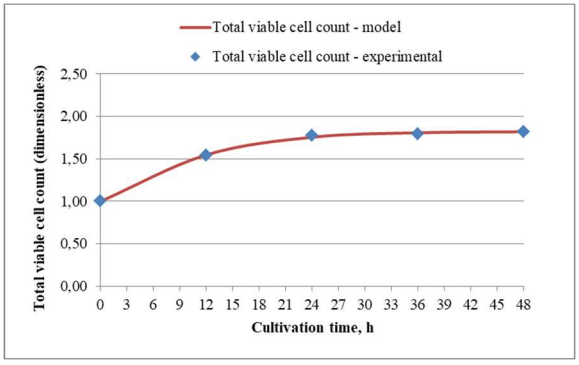


**Fig. 4.** Comparison of the experimental data for the total number of cells in dimensionless form with those from the logistic curve model for *Heyndrickxia coagulans* BJ cultivated in medium A.

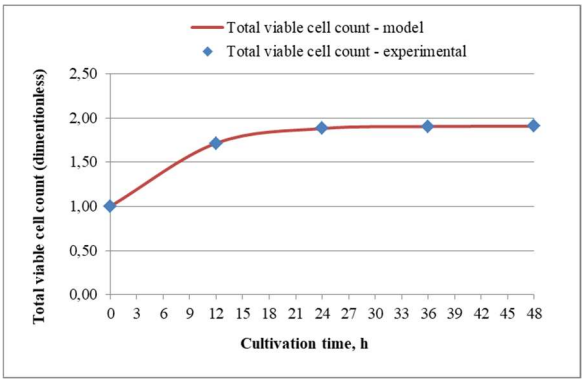


**Fig. 5.** Comparison of the experimental data for the total number of cells in dimensionless form with those from the logistic curve model for *Heyndrickxia coagulans* BJ cultivated in medium B.

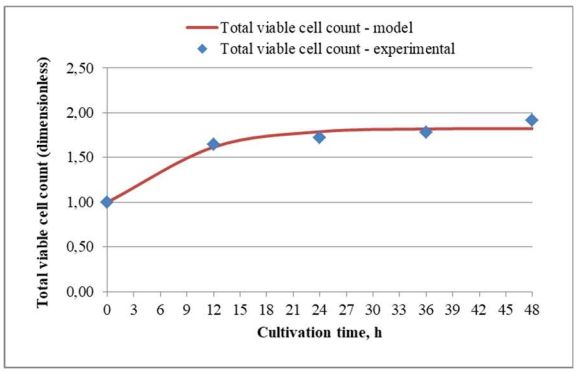


**Fig. 6.** Comparison of the experimental data for the total number of cells in dimensionless form with those from the logistic curve model for *Heyndrickxia coagulans* BJ cultivated in medium C.

The data in Table 2 show that the exponential model describing the sporulation kinetics of *Heyndrickxia coagulans* BJ, cultivated in the three fermentation media, was characterized by high correlation values for all three media – 0.9998, 0.9934, and 0.9970 for medium A, B and C, respectively, as well as low identification error values of 0.33 and 0.34. This indicates that this model also aligns very closely with the experimental data and can be used to predict the sporulation process within the selected cultivation time frame.

**Table 2**. Maximum specific sporulation rate of *Heyndrickxia coagulans* BJ cultivated in the three fermentation media.

| | Medium A | Medium B | Medium C |
|---|---|---|---|
| $k$, $h^{-1}$ | 0.183 | 0.198 | 0.197 |
| $R^2$ | 0.9998 | 0.9934 | 0.9970 |
| E | 0.33 | 0.34 | 0.34 |

The data also show that the strain exhibited high sporulation rate constants, which are nearly identical for the medium B and C at 198 $h^{-1}$ and 197 $h^{-1}$, respectively. The sporulation rate in medium A was slightly lower but still relatively high. The higher kinetic parameters observed in media B and C make them more suitable for conducting the cultivation process aimed at producing a spore concentrate.

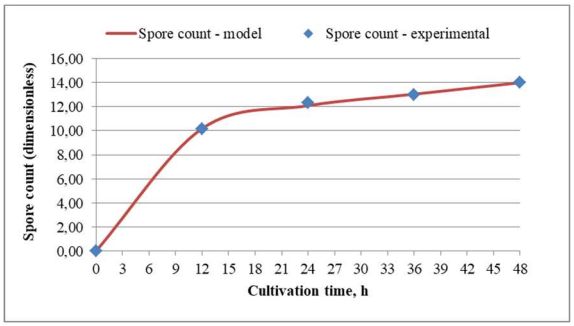


**Fig. 7.** Comparison of the experimental data for the number of spores in dimensionless form with those from the exponential model for *Heyndrickxia coagulans* BJ cultivated in medium A

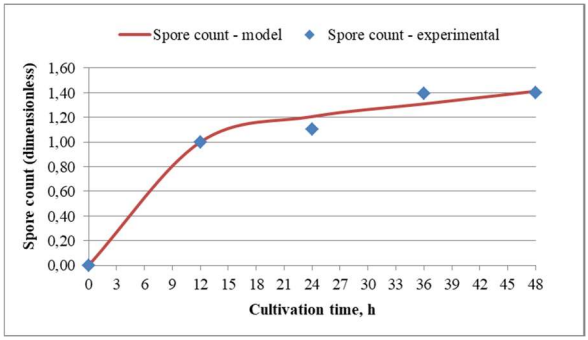


**Fig. 8.** Comparison of the experimental data for the number of spores in dimensionless form with those from the exponential model for *Heyndrickxia coagulans* BJ cultivated in medium B

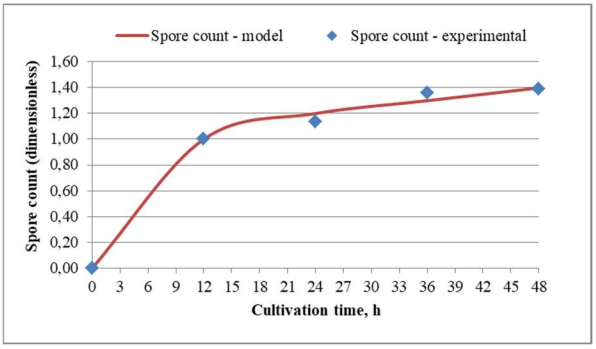


**Fig. 9.** Comparison of the experimental data for the number of spores in dimensionless form with those from the exponential model for *Heyndrickxia coagulans* BJ cultivated in medium C

Assessing the capacity of the isolated strain to degrade substrates found in food and soil, such as starch, protein and lipids is essential to future applications. These enzyme activities can contribute to the strain survival in the gastrointestinal tracts and its probiotic properties [35-37]. In plant protection applications such enzymes can facilitate root colonization and pathogens displacement [38-40]. Three enzyme activities of *Heyndrickxia coagulans* were evaluated using the agar well diffusion method (Table 3).

**Table 3.** Enzyme activity of *Heyndrickxia coagulans* BJ.

| | Hydrolysis zone, mm |
|---|---|
| Amylolytic activity | 17.66±0.47 |
| Proteolytic activity | 15.25±0.43 |
| Lipolytic activity | 8.17±0.24 |

*$d_{well}$ = 6mm*

The strain displayed moderate to high proteolytic and amylolytic activities and low lipolytic activity. According to reference literature the detected proteolysis zone diameters correspond to specific proteolytic activity between 10–65 U/mg protein [41].
Another key feature of both biocontrol agents and probiotics is their capacity to inhibit microbial growth. Members of the *Bacillus* group synthesize a range of antimicrobial compounds, including protein-like bacteriocins [42,43]. The four samples obtained from the culture medium of *Heyndrickxia coagulans* BJ were analysed by SDS-PAGE (Fig. 10). A fraction was detected in sample 1,that cannot enter the concentrating gel and in Fig. 7 it is marked with a molecular weight (Mw) >250 kDa. After the biologically active proteins and peptides were extracted to obtain sample 3, sample 2 still contained proteins that were not extracted under the described conditions. SDS-PAGE analysis revealed that sample 2 contained only a single fraction (100%) with a molecular weight of 11 or 12 kDa. The result from sample 3 of the *H. coagulans* BJ strain shows that only one fraction (100%) with a molecular weight below 10 kDa was detected for this strain. The results obtained for sample 3 confirm the literature data indicating that precipitation of bioactive proteins and peptides, followed by their extraction with methanol, is an effective method for their isolation [41].

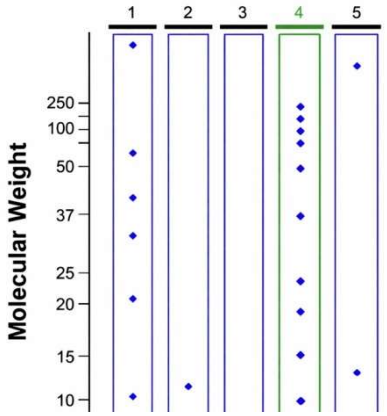


**Fig. 10.** SDS-PAGE of samples 1 to 4: 1 – Sample 1; 2 – Sample 2; 3 – Sample 3; 4 – Protein ladder 10-250 kDa; 5 – Sample 4

After the bioactive proteins and peptides were extracted (sample 3), the remaining precipitate was dissolved in NaOH (sample 4) to ensure complete utilization of the proteins obtained by acid precipitation and to assess their antimicrobial activity. The SDS-PAGE results show that in sample 4, similar to sample 1, a high-molecular-weight fraction (>250 kDa, 1.38-3.63% TTP) is present, which does not enter the concentrating gel.

**Table 4**. Percentage distribution of protein fractions (in samples 1-4 of the studied strain

| Sample | Protein fractions, % | | |
|---|---|---|---|
| | Low molecular wight | Medium molecular weight | High molecular weight |
| 1 | 90.81 | 6.86 | 2.33 |
| 2 | 100 | - | - |
| 3 | 100 | - | - |
| 4 | 1.38 | - | 98.62 |

In the performed thin-layer chromatography (TLC), all spots obtained from the tested strain were ninhydrin-positive, confirming their proteinaceous nature. The TLC analysis revealed spots with Rf-values corresponding to the three growth media, as presented in Table 5.

**Table 5.** Rf-values of the obtained protein spots of *H. coagulans* BJ after staining with ninhydrin.

| | |
|---|---|
| Medium A | 0.628; 0.445; 0.323; 0.213; 0.122; 0.03 |
| Medium B | 0.609; 0.518; 0.335; 0.122 |
| Medium C | 0.609; 0.356; 0.122 |

In order to prove the antimicrobial activity of the identified proteinaceous fractions a biochromatogram was conducted (Table 6).

**Table 6.** Rf-values of the inhibition zones produced by *Heyndrickxia coagulans* BJ on the three fermentation media against *A. flavus*.

| Medium A | Medium B | Medium C |
|---|---|---|
| 0.51 ; 0.07 | - | 0.81 |

In medium A strain *Heyndrickxia coagulans* BJ exhibited inhibition of *Aspergillus flavus* growth, observed as a spots with an Rf-value of 0.51 and 0.07, and in medium C – with Rf-value of 0.81. The spot with an Rf-value of 0.51 is close to the Rf-value of bacillomycin L methyl ester [44] but it cannot be conclusively confirmed that the compounds are identical. The remaining calculated Rf-values showed similarity with the data reported in the available literature. Medium B did not produce spots with antimicrobial activity.

## 4 Conclusion

The total viable cell count of *Heyndrickxia coagulans* BJ increased continuously during cultivation in all investigated nutrient media, reaching approximately $10^{13}$-$10^{14}$ CFU/cm$^3$. No spores were detected during the first 12 h. Sporulation of *H. coagulans* BJ began at 12 h in all three media and similar spore concentrations were reached across the three media (~$10^{10}$ CFU/cm$^3$). The maximal sporulation percentage 99.71% was detected in medium C. The strain exhibited both the highest growth rate and sporulation rate in medium B – 0.174 h$^{-1}$ and 0.198 h$^{-1}$, respectively. Two models were identified, which accurately describe the growth and sporulation processes – the logistic curve model for the growth process and the exponential model for the sporulation process. *Heyndrickxia coagulans* BJ displayed valuable enzyme activities and SDS-PAGE electrophoresis and biochromatograms confirmed the production of bacteriocin-like substances with antimicrobial activity against *Aspergillus niger*.

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