Paenibacillus Polymyxa MVY-024: Plant Growth Promoting Bacteria Which is Easy to Apply on an Industrial Scale

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Abstract

In this study, thirteen isolates which were possibly expected to fix nitrogen, were isolated from the soil and pea root nodules and identified by gene analysis of 16S rDNA sequences. Two of these isolates which were able to form endospores and growth on nitrogen free media were selected for spring wheat development research. The isolate *Paenibacillus* sp. S7 identified as *Paenibacillus polymyxa* was found to significantly increased amount of ammonium and mineral N amounts in the soil. Furthermore, increased nitrogen accumulation in grain and a chlorophyll index were obtained after wheat treatment. *Paenibacillus* sp. S7 isolate was selected for further studies and accession number MT900581 and strain name MVY-024 in NCBI nucleotide bank for this isolate was assigned. During cultivation of *Paenibacillus* sp. MVY-024, sugarcane molasses and yeast extract were determined as the most suitable carbon and nitrogen sources, optimal concentrations were 100 gL^{-1} and 10 gL^{-1}, respectively. The optimal pH range for cells culturing was between 6.5 and 7.0, optimal air flow rate was 0.4 vvm. It was found that air flow has effect for biomass production and cells endospores formation. After *Paenibacillus* sp. MVY-024 biomass cultivation optimization, cultured cells number was on average 2.2×10^9 cfu mL^{-1}.

Introduction

Is a huge assortment of microbial fertilizers in agricultural industry, but new species of bacteria which are able to fix nitrogen from atmosphere are constantly being sought. For superior microbial fertilizers it is important to select effective, competitive and resistant to environmental factors nitrogen fixing bacteria strains. *Paenibacillus polymyxa* is Gram-positive endospore forming, plant growing promoting, and biological nitrogen fixing rhizobacteria which has high potential as a bacterial fertilizer in agriculture [1,2]. This bacteria is found in rhizosphere and also as root and stem endophyte of various crop plants especially wheat [34]. Nitrogen fixation, production of auxin and other indolic and phenolic compounds, siderophores, phosphate solubilisation, directly promote plant growth and development [567]. Biofilm formation on plant root and producing antibiotics by *P. polymyxa*, such fusaricidins, polymyxins and many others [8,9,10] ensure strong effect against variety of pathogenic microorganisms and indirectly promote plant growth also [11,12]. *P. polymyxa* microbial product is easily compatible with mineral fertilizers and chemical plant protection products, *P. polymyxa* cells survive in the environment for a long period of time, because of the spores formation under adverse conditions [13].

There a lot of scientific researches about production optimization of secondary metabolites during *P. polymyxa* fermentation, however, biomass cultivation of these bacteria is poorly described and complicated process. Cell endospore formation is important factor to ensure microbial product stability and efficiency on soil and plant [14]. The main problem in biomass cultivation process of *P. polymyxa* is that after exponential fermentation phase lot of cells are not able to form endospores and die. Fermentation conditions and nutrition media optimization helps to improve vegetative cells endospores formation, increases viable cells number in the final microbial product, provides an opportunity to ensure production efficiency and reduce product price, making the product more attractive for smallholder

farmers [15], [16]. In this study we will present results of *P. polymyxa* MVY-024 strain effect on spring wheat development and review the best fermentation conditions, the most suitable nitrogen and carbon sources for *P. polymyxa* MVY-024 biomass cultivation.

Materials And Methods

Samples collection

Soil samples and pea seedlings were collected in Panevezys region, Lithuania. Soil samples were collected from field where wheat grew. The pea seedlings were uprooted from the pea field, seedlings were picked randomly. Isolation of microorganisms was performed the same day when the samples were collected. The experimental research on plants, including the collection of plant material, complied with relevant institutional, national, and international guidelines and legislation. The appropriate permissions and/or licenses for collection of plant were obtained for the study.

Isolation of diazotrophic microorganisms

During isolation of diazotrophic soil microorganisms 1 g of soil sample was added to 10 mL of sterile deionized water and suspended. The soil suspension was incubated for 20 minutes in a shaking incubator at 30°C temperature. After incubation suspension was diluted in 10^{-3}; 10^{-4}; 10^{-5}; 10^{-6} series according to the serial dilution method and 100 µL of each dilution was plated onto solid NF media using spread plate method. Plates were incubated in bacteriological incubator at 30°C for 48 hours [17]. Each isolate was tested for the growth on Ashby's Mannitol [18], Winogradsky's [19], NF [20], NFB [21] nitrogen-free agar media.

During isolation of diazotrophic microorganisms from pea root nodules, roots were washed under running tap water for 10 minutes, then using tweezers pink colour nodules were carefully taken from the roots. The nodules surface disinfected for 30 s in 70% ethanol solution and 5 times washed with sterile deionized water. Then nodules were treated for 1 min. in 3 % sodium hypochlorite solution and 10 times washed with sterile deionized water [22]. Disinfected nodules were transferred to a tube with 5 mL sterile deionized water and homogenized using a sterile glass rod. Prepared bacterial suspension was diluted in 10^{-3}; 10^{-4}; 10^{-5}; 10^{-6} series according to the serial dilution method and 100 µL of each dilution was plated onto solid NF media using spread plate method. Plates were incubated in bacteriological incubator at 30°C for 48 hours. Each isolate was tested for the growth on Ashbys Mannitol, Winogradsky's, NF and NFB nitrogen-free agar media.

Endospore formation test

The ability of bacteria to form endospores was evaluated microscopically and by standard plating procedure. Suspensions of all strains were incubated in laboratory water bath at 75 ° C for 15 min and 1 mL of the heat-treated bacterial suspension was plated onto MPA agar medium and incubated in

bacteriological incubator for 48 hours at 30 ° C. After incubation bacterial colonies were counted and phenotype traits were tested [23].

Molecular identification of isolated microorganisms

Isolates identification were carried out using partial 16S rDNA sequence analysis. All partial 16S rDNA sequences were determined by PCR with primers 8 F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') [24] and compared to the GenBank database in the National Center for Biotechnology Information (NCBI) using BLAST program. To determine the phylogenetic relationships phylogenetic tree was constructed using MEGA 5.0 software.

Vegetative growth promotion in wheat plants by *Paenibacillus* spp.

The vegetative pot experiment was conducted in 2019-2020, under regulated greenhouse conditions. Soil was used from organically managed field from the top 0–20 cm. The soil was a loamy Endocalcaric Epigleyic Cambisol (Drainic, Loamic) CM-can.glp-dr.lo [25] and was characterised as high fertility level with 3.9 % humus, 151 mg N kg^{-1}, 93 mg P kg^{-1} and 156 mg K kg^{-1}. The soil (5 kg dry weight pot^{-1}) was filled in 8.5 l PVC pots. Two microbiological products of *Paenibacillus* sp. S1 and *Paenibacillus* sp. S7 were spread on the top soil in the form of water solution containing 400 mL H_2O and 38 µL suspension of bacteria. It was 1.5×10^8 cfu per pot. Solution was applied two times: in the beginning and 3 weeks later. Only water applied on the control treatment. The spring wheat "Collada" was sown in pots, in five replications, 10 plants per each pot. Growth conditions in the greenhouse during the experiment were controlled: 16/8 h light/dark; photosynthetically active radiation at canopy level: 600 mol m^2 s^{-1}; temperature 20°C day and 16°C night time; irrigation of 200-400 mL per pot, 2 times a week.

Soil and plant analysis

The ammonium ($N\text{-}NH_4$) in the soil was measured spectrophotometrically (with Cary 60 UV-Vis, USA) 4 days, 2 weeks and 2 months after first *Paenibacillus* spp. application. Mineral nitrogen (Nmin) content measured after 2 months as the sum of $N–NO_3$ and NH_4. Spring wheat in the pots were analysed during BBCH 37 (SPAD) and BBCH 87 growing stages for the grain yield per plant, thousand kernels weight (TKW), proteins in grain and kernels per spike. The concentration of protein and N yield in grain was measured using Kjeldahl method [26].

According to results of soil nitrogen changes and spring wheat experiments, accession number MT900581 and strain name MVY-024 for *Paenibacillus* sp. S7 in NCBI nucleotide bank were assigned.

Optimization of carbon and nitrogen sources for *Paenibacillus* sp. MVY-024 cultivation

Optimization of carbon and nitrogen sources is performed in shake-flask experiment using AF medium which was also used for inoculum preparation. Glucose, sucrose, glycerol, mannitol, molasses and starch were selected as a carbon sources and soybean, peptone, urea, ammonium sulphate, casein peptone,

agropeptone, yeast extract and meat extract were used as a nitrogen source (Table 1). Taking into account that 100 g of cane molasses contains around 70 g of sugars, the molasses concentration was recalculated. During optimization of carbon source, the nitrogen source was selected yeast extract.

Table 1
Nitrogen and carbon sources optimization.

Carbon source optimization				**Nitrogen source optimization**			
Carbon source, g L^{-1}		**Nitrogen source, g L^{-1}**		**Nitrogen source, g L^{-1}**		**Carbon source, g L^{-1}**	
Control	0	Yeast extract	0	Control	0	Molasses	0
Glucose	40	Yeast extract	10	Soybean peptone	10	Molasses	57
Sucrose	40	Yeast extract	10	Carbamide	10	Molasses	57
Glycerol	40	Yeast extract	10	Ammonium suphate	10	Molasses	57
Mannitol	40	Yeast extract	10	Casein peptone	10	Molasses	57
Molasses	57	Yeast extract	10	Agropeptone	10	Molasses	57
Starch	40	Yeast extract	10	Yeast extract	10	Molasses	57
				Meat extract	10	Molasses	57
Molasses concentration optimization				**Yeast extract concentration optimization**			
Control	0	Yeast extract	0	Control	0	Molasses	0
Molasses	25	Yeast extract	10	Yeast extract	5	Molasses	0
Molasses	50	Yeast extract	10	Yeast extract	10	Molasses	0
Molasses	100	Yeast extract	10	Yeast extract	15	Molasses	0
Molasses	200	Yeast extract	10	Yeast extract	20	Molasses	0

Under sterile conditions 200 mL of sterile medium was poured to a 1 L Erlenmeyer flask and 2 mL of fresh *Paenibacillus* sp. MVY-024 inoculum was inoculated to the flask. The concentration of *Paenibacillus* sp. MVY-024 cells in the initial inoculum was 5.0×10^8 cfu mL^{-1}. All samples using different carbon or nitrogen sources were incubated for 24 hours at 30 ºC, 130 rpm in a shaking incubator. After incubation, the number of bacterial cells in suspensions were determined on solid MPA (20 g meat extract, 5 g glucose, 10 g agropeptone, 20 g agar in 1000 mL deionized water, pH 6,6-7,0) medium according to the serial dilution-spread plate method. Plates were incubated in bacteriological incubator at 30°C for 48 hours.

After determination that sugarcane molasses and yeast extract are the best carbon and nitrogen sources for *Paenibacillus* sp. MVY-024 biomass production, the same shake-flask experiment was performed using different concentrations (Table 1).

pH and temperature optimization

To determine the optimum initial pH for *Paenibacillus* sp. MVY-024 biomass production, the pH of the medium was adjusted to the desired pH by adding 1 M HCl and 1 M NaOH before sterilization. pH values of AF medium: 6.0, 6.5, 7.0, 7.5, 8.0. 200 mL of sterile medium was poured to a 1 L Erlenmeyer flask and 2 mL of fresh *Paenibacillus* sp. MVY-024 inoculum was inoculated. All samples using different pH values were incubated for 24 hours at 30 ºC, 130 rpm in a shaking incubator. After incubation, the number of bacteria cells in suspensions was obtained on solid MPA medium according to the serial dilution-spread plate method. Plates were incubated in bacteriological incubator at 30°C for 48 hours.

After determination of optimal pH for biomass production, the same shake-flask experiment was repeated in the same conditions but using different temperature values: 28°C, 30°C, 32°C, 34°C, 36°C. Samples were diluted in serial dilutions, plated on MPA solid medium and were incubated in bacteriological incubator at 30°C for 48 hours. The number of bacterial colonies was counted.

Optimization of air flow

Paenibacillus sp. MVY-024 air flow optimization was performed in a fermenter (EDF 5.4_1). Cells culturing was performed in AF medium, using molasses and yeast extract as carbon and nitrogen sources in determined optimal concentrations. Based on the results of temperature and pH optimization, biomass culturing was performed at 32°C when the pH value was 7.0 ± 0.5. During fermentation process pH value of medium was adjusted by using automatic titration with 2 M NaOH and 2 M H_2SO_4 and antifoam was used to reduce foaming. Feeding was started after 8 hours of fermentation and was fed in to the bioreactor for 5 hours. Every hour 60 mL of feeding was used. During air flow optimization, the same partial pressure of oxygen and different air flow rates were selected. The partial pressure of oxygen in the medium 20±2, air flow rates: 0.1 vvm, 0.2 vvm, 0.4 vvm, 0.8 vvm, 1.2 vvm, 1.6 vvm and 2.0 vvm. The air to the bioreactor was supplied through a 0.2 µm pore size filter. The stirrer was set to automatic mode, from 45 to 800 rpm. 200 mL of inoculum and 3 L of NF medium were used for fermentation. During the process, parameters such as temperature, pH, agitation rate and partial pressure of oxygen were monitored and recorded. The optimal cell culturing time in bioreactors was about 70 hours, during this time all fermentable bacterial cells had to form endospores.

Statistical analysis

All statistical analyses were performed using SAS software version 9.4 (SAS Institute Inc., Copyright © 2002–2010). Graphical representation of data was performed using Microsoft Office 2013 software package. Homogeneity and normality were verified using Bartlett's test. Experimental data were analysed by one-way analysis of variance (ANOVA) and mean comparisons between treatments were performed using Duncan's mean separation test. The smallest significant difference R_{05} was calculated using a probability level of $p<0.05$.

Results

Isolation and description of physical properties of isolates

Thirteen isolates were isolated from soil and pea roots nodules on NF agar media. Each isolate was tested for the growth on Ashby's, Winogradsky's, NF nitrogen-free agar media (Table 2).

Table 2
Growth of isolates on semi-selective media, no growth (-), weak growth (+), medium growth (++), intensive growth (+++)

Isolates	Ashby's	Winogradsky's	NF	NFB	Gram Reaction	Endospore formation
Isolates from soil						
Isolate S1	**+++**	**+**	**+++**	**+**	+	+
Isolate S2	**++**	**++**	**+**	**+**	-	-
Isolate S3	**++**	**++**	**+++**	**+**	-	-
Isolate S4	**-**	**-**	**+**	**-**	+	+
Isolate S5	**++**	**+++**	**++**	**-**	+	+
Isolate S6	**+**	**++**	**+**	**-**	+	+
Isolate S7	**++**	**++**	**+++**	**+**	+	+
Isolate S8	**+**	**+**	**+**	**-**	+	+
Isolate S9	**+**	**++**	**++**	**-**	+	+
Isolates from pea root nodules						
Isolate R1	+	+	++	-	+	+
Isolate R2	-	-	++	-	+	+
Isolate R3	+++	+	++	+	-	-
Isolate R4	+++	-	+++	+	-	-

Three isolates S1, S3, S7 showed quite strong growth on all media. Isolates S3 and S7 demonstrated the most intensive growth on NF agar media and at an average on Ashby's and Winogradsky's nitrogen free media (Table 2). Isolate S1 growth on Ashby's medium was better compared to S3 and S7 but growth on Winogradsky's media was weaker. Isolates S4, S6 and R2 showed the weakest growth on all semi-selective media. NFB media showed that only half of isolated bacteria formed pellicle and possibly are capable to fix nitrogen from atmosphere, positive nitrogen fixation reaction was demonstrated culturing isolates S1, S2, S3, R3 and R4. The growth of isolates on each media was different because of individual demand for salts and carbon source.

Gram staining and endospores determination method revealed that nine isolates are Gram positive and are capable to form endospores, the other four isolates are Gram negative and does not produce endospores (Table 2). Given the results of isolate growth on semi-selective media, nitrogen fixation reaction and the fact that microorganisms which are capable to form endospores are much more resistant to adverse environmental factors [27,28] isolates S1 and S7 were selected for spring wheat growth promotion investigation.

Phylogenetic analysis

After processing the primary sequence data the DNA sequences of the isolates encoding 16S rRNA were collected and compared with the 16S rRNA gene sequences of typical bacterial cultures. Based on the identification results of the obtained 16S rRNA gene sequences phylogenetic tree was constructed (Fig. 1). Phylogenetic analysis based on 16S rDNA sequences showed that S4, S5, S6, S8, S9, R1 and R2 isolates belongs to *Bacillus* spp., S2 and S3 isolates are members of *Ensifer* spp., R3 isolate belongs to *Lelliottia* spp., R4 is member of *Rhizobium* spp. and the target S1 and S7 isolates belongs to *Paenibacillus* spp.

The comparing of 16S rDNA sequence obtained from S1 and S7 isolates showed that sequences are homological 99 % and belongs to different *Paenibacillus* species. Isolate S1 is closely related to *P. peoriae* FO 15541, with percent identity 99 % and to *P. kribbensis* PB172 with a high percent identity 98 %. Isolate S7 strain is homologous to *P. polymyxa* DSMZ 36 strain, with percent identity 99 %.

Soil nitrogen changes as affected by *Paenibacillus* sp. S1 and *Paenibacillus* sp. S7

Four days after bacteria application, the amount of $N\text{-}NH_4$ in the soil did not vary significantly among the treatments (Table 3). However, significantly higher amounts ($p<0.05$) of $N\text{-}NH_4$ were observed after 2 weeks for both *Paenibacillus* spp. strains comparing with the control treatment. After 2 months wheat growing period (BBCH 87), the amount of $N\text{-}NH_4$ was lower, but *Paenibacillus* sp. S7 held the highest ammonium and mineral N amounts in the soil.

Table 3

The alteration of soil ammonium and total mineral N (mg kg^{-1}) as affected by *Paenibacillus* spp. strains in different time lags after microorganism application.

No.	Treatment	Soil N-NH_4, after 4 days	Soil N-NH_4, after 2 weeks	Soil N-NH_4, after 2 months	Soil N_{min}, after 2 months
1.	Control	36.5 ab	36.5 a	1.12 a	3.13 ab
2.	*Paenibacillus* sp. S7	42.3 b	47.1 b	2.87 b	4.48 b
3.	*Paenibacillus* sp. S1	41.6 ab	48.1 c	0.29 a	1.48 a
Probability Pr>F		0.1415	0.001**	0.026*	0.042*
The values marked with the same letter have no significant differences at ($P \leq 0.05$).					
* $p < 0.05$.					
** $p < 0.001$.					

The nitrogen accumulation in grain was significantly higher ($p<0.05$) for the treatment with *Paenibacillus* sp. S7, comparing with *Paenibacillus* sp. S1 (Table 4). Chlorophyll, in the leaf tissues indicating N was also significantly higher for control and *Paenibacillus* sp. S7. All yield components, including, grain yield per plant, thousand kernels weight (TKW), proteins in grain and kernels per spike, differed significantly from the control, when the *Paenibacillus* spp. S7 strain was applied on the soil. After *Paenibacillus* sp. S7 application grain yield was 9 %, TKW was 5 % and protein content was 11 % higher comparing to the control. The effect of *Paenibacillus* sp. S1 was very similar to the control.

Table 4

Spring wheat productivity and yield quality under effect of *Paenibacillus* spp. strains.

No.	Treatment	N yield in grain, g^{-1} per plant	Leaf chlorophyll, SPAD	Grain yield per plant, g	TKW, g	Protein in grain, %	Kernels per spike, unit
1.	Control	0.0355 ab	45.8 a	1.42 a	40.9 a	11.5 a	34.7 a
2.	*Paenibacillus* sp. S7	0.0361 b	46.8 b	1.54 b	43.1 b	12.8 b	36.0 b
3.	*Paenibacillus* sp. S1	0.0297 a	45.1 a	1.46 ab	40.4 a	11.1 a	36.2 b
Probability Pr>F		0.041*	0.025*	0.361	0.196	0.118	0.831
The values marked with the same letter have no significant differences at ($P \leq 0.05$).							
* $p < 0.05$.							

According to results of soil nitrogen changes and spring wheat experiments, accession number MT900581 and strain name MVY-024 for *Paenibacillus* sp. S7 in NCBI nucleotide bank were assigned. This strain *Paenibacillus* sp. MVY-024 was selected for biomass production.

Effect of carbon and nitrogen sources on *Paenibacillus* sp. MVY-024 biomass production

The results obtained from shake-flask experiments revealed that the different carbon and nitrogen sources have effect on the amount of biomass production (Fig. 2). The lowest biomass production was obtained using glucose, sucrose and glycerol. Differences between glucose and sucrose variants were insignificant. The highest biomass production was in the nutrition medium with molasses, here concentration of cells was 48 times higher comparing to control variant. Determined number of cells 2.5×10^8 cfu mL^{-1}. Biomass production in media using mannitol and starch as carbon sources showed approximately 2-fold less number of cells compared to molasses.

The results obtained from shake-flask experiments revealed that the weakest biomass growth was found in samples using urea and ammonium sulphate as nitrogen sources (Fig. 3). The highest biomass production was when yeast extract was used. The number of cells using yeast extract as nitrogen source 2.5×10^8 cfu mL^{-1}, it was 48 times higher compared to control variant. Agropeptone and meat extract showed a similar result which is not significantly different. Based on the results obtained, the most suitable nitrogen source for *Paenibacillus* sp. MVY-024 biomass cultivation is yeast extract.

Studies have shown that the most suitable carbon and nitrogen sources for *Paenibacillus* sp. MVY-024 biomass production are sugar cane molasses and yeast extract, so these substrates were chosen for further studies. To determinate the optimal concentrations of molasses and yeast extract for *Paenibacillus* sp. MVY-024 biomass cultivation research was continued in the shake-flask experiments. The results indicated that the lowest cell biomass production was obtained when concentration of yeast extract was 5 g L^{-1}, increasing the yeast extract concentration from 10 g L^{-1} to 20 g L^{-1} showed higher biomass production, but differences between these variants were insignificant (Fig. 4). However, optimal yeast extract concentration for *Paenibacillus* sp. MVY-024 biomass production is 10 g L^{-1}, the number of cells on average in this concentration was 2.4×10^8 cfu mL^{-1}.

Experiment with different molasses concentrations showed that the lowest number of cells were when molasses concentration was 25 g L^{-1} (Fig. 5). However, increasing the molasses concentration to 100 g L^{-1} the number of cells increased more than 250-fold compared to control sample and the cultured bacterial biomass of the cultured cells was on average 1.3×10^9 cfu mL^{-1}. When the molasses concentration was increased to 200 g L^{-1}, no significant difference was observed, the number of cells obtained were the same as the number of cells in the sample, where molasses concentration was 100 g L^{-1}. In conclusion, the optimal concentration of molasses for *Paenibacillus* sp. MVY-024 biomass cultivation is 100 g L^{-1}.

Optimization of nutrition broth pH and temperature for *Paenibacillus* sp. MVY-024 biomass production

In this experimental study adjusting pH values of nutrition broth was found that the most suitable pH values for *Paenibacillus* sp. MVY-024 biomass growth are 6.5-7.0 (Fig. 6). The number of cells in these pH values was 1,1 × 10^9 cfu mL^{-1} and 9,7 × 10^8 cfu mL^{-1}, the difference was insignificant. At higher or lower pH values, *Paenibacillus* sp. MVY-024 biomass production was decreased.

Experiment using different temperatures for MAY-024 biomass production showed that the highest cell number was determined when temperature was around 32 °C and 34 °C (Fig. 7). The number of cells in these temperatures was 1.9 × 10^9 cfu mL^{-1} and 1.8 × 10^8 cfu mL^{-1}, the difference was insignificant. During biomass cultivation in higher or lower temperatures, number of *Paenibacillus* sp. MVY-024 cells decreased. The difference of number of cells, during biomass cultivation at 28 and 36 °C temperatures was insignificant.

Optimization of air flow for *Paenibacillus* sp. MVY-024 biomass production

Results of the experiment showed that the airflow feed rate is significant for biomass production and spore formation (Fig. 8). Biomass production was similar in all samples the first 20 hours of fermentation where different air flow rates were used. In several fermentations the number of bacteria cells reached a maximum value. According to the 30 hour results the number of cells started to decrease in the samples where air flow was 0.8 vvm and lower, and in the samples where air flow was 1.2 vvm and higher, the number of cells increased compared to the results of 20 hours. Samples where air flow was 0.1 vvm, 0.2 vvm, 0.4 vvm and 0.8 vvm at 30–40 h fermentation hours showed the first endospore-forming cells. All cells in fermentations where air flow was 0.8 vvm and lower were formed free endospores until 70 h of fermentation. Cells did not form any endospores in the samples where air flow was 1.2 vvm and higher, until 70 h of fermentation, fermentations were continiuos until 110 h, around 60-80 % of vegetative cells formed spores, but number of cells decreased, it was obtained less than 1 × 10^8 cfu mL^{-1} so fermentations were stopped.

The results showed that the highest number of cells in the final sample was obtained when the air flow was 1.6 vvm and 2.0 vvm. The average number of cells was 4.8 × 10^9 cfu mL^{-1} and 3.9 × 10^9 cfu mL^{-1} in these fermentations. However, during fermentations where airflow rates were 1.6 vvm and 2.0 vvm any endospores formation was not achieved in over 70 hours, so it could be that these airflows are not suitable for culturing *Paenibacillus* sp. MVY-024 cells. The optimal air flow for biomass production of *Paenibacillus* sp. MVY-024 is 0.4 vvm, during this fermentation process all cells formed endospores in 70 hours and the number of cells obtained was the highest compared to other successful fermentations. The number of spores was averaged 2.2 × 10^9 cfu mL^{-1} in final sample.

Discussion

It is a lot of studies which prove *P. polymyxa* positive effect on plants. Scientists from Russian demonstrated that *P. polymyxa* CCM 1465 and *P. polymyxa* 92 strains increase the mitotic index of the root cells 1.2- and 1.6-fold after inoculation on wheat seedlings. Also it was determined that these two

strains and their produced EPSs promoted wheat growth and development, increasing root and shoot length up to 22% and root and shoot dry weight up to 28% compared with the control [29]. Study with *P. polymyxa* SbCT4 strain also confirmed positive effect on wheat and maize growth promotion, root length and dry weight were significantly higher comparing to the control [30]. Canola and tomato growth parameters were significant better after *P. polymyxa* P2b-2R treatment. Tomato seedlings inoculated P2b-2R strain were assimilated nearly 90 % more biomass than controls, nearly 40 % longer than controls, and fixed nearly 17 % of nitrogen from the atmosphere [31].

During optimization of carbon and nitrogen sources it was determined that sugarcane molasses 100 gL^{-1} and yeast extract 10 gL^{-1} are the most suitable for *P. polymyxa* MVY-024 biomass production, the number of cells spores in the final product was 2.2×10^9 cfu mL^{-1}. In Gong and his colleagues studies, where carbon and nitrogen sources were optimized for *P. polymyxa* BY-28 cell growth, the best nitrogen sources were determined polypeptone and bean powder, given result 3.1×10^8 cfu mL^{-1} and 3.0×10^8 cfu mL^{-1}, cells number with yeast extract was 2.8×10^8 cfu mL^{-1}. The most suitable carbon sources were indicated maltose 3.3×10^8 cfu mL^{-1} and glucose 3.2×10^8 cfu mL^{-1} [32]. In previous studies glucose and sucrose also mentioned as the best carbon source for exopolysaccharides production by *P. polymyxa* strains [33],[34],[35]. However, in our study the weakest biomass production was determined when glucose and sucrose were used as a carbon sources for *Paenibacillus* sp. MVY-024 culturing.

According to the results of J. Liu and his colleagues, optimizing the medium of *P. polymyxa* EJS-3, the maximum dry cell mass was obtained when pH value was 6.0 [33]. The results of V. Raza the most suitable pH for *P. polymyxa* SQR-21 cell growth was 6.5 [36]. In our experimental study it was found that the most suitable pH values for *Paenibacillus polymyxa* sp. MVY-024 biomass growth are 6.5-7.0 and the temperature is 32°C. In previous studies for cell culturing of *P. polymyxa* BY-28 optimal temperature was 30°C [32], for 2,3-Butanediol production by and *P. polymyxa* ZJ-9 determined optimal temperature was 30°C, for *P. polymyxa* DSM 365 optimal temperature was 35°C [37], for *P. polymyxa* PM 3605 strain 37°C [38]. Based on the results obtained and reported in the literature, optimal media pH for *P. Polymyxa* growth and metabolites production is from 6.0 to 7.0, meanwhile, temperature is from 30°C to 37°C, so culturing temperature and pH tolerance are specific for each *P. polymyxa* strain.

Although there are no researches about effect of different air flow rates culturing *P. polymyxa* biomass a few scientific publications represents that intensive aeration during fermentation process increases the synthesis of acetoin and acetate, which can inhibit *Paenibacillus polymyxa* biomass growth and synthesis of other secondary metabolites [39],[40],[41], it is possible that that acetoin and acetate production in higher air flow rates affected endospores formation. In this research it was observed that when the air flow was 1.2 vvm or higher, the volume of condensate increased proportionally with increasing air flow rate. Thus, as the air flow rate increases a part of the fermentation product evaporates.

Conclusions

In this study, nitrogen fixing *Paenibacillus* sp. S7 strain was isolated and identified as *Paenibacillus polymyxa*. Accession number MT900581 for *Paenibacillus* sp. MVY-024 isolate was assigned. Results of this study showed that using this strain significantly higher $N\text{-}NH_4$ in the soil during all the spring wheat growing period was observed. Also, higher nitrogen accumulation in grain, leaf chlorophyll levels of spring wheat, grain yield per plant was 9 %, TKW was 5 % and protein content was 11 % were higher comparing to the control. After optimization of fermentation parameters and culturing media, significant higher yield of *Paenibacillus* sp. MVY-024 biomass was determined. Finally, experiments in this study show that *Paenibacillus* sp. MVY-024 has a positive effect for nitrogen changes in soil and spring wheat development and growth, and it is easily apply on an industrial scale.

Declarations

Availability of data and materials

Not applicable.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests that could have influenced the information reported in this paper.

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Figures

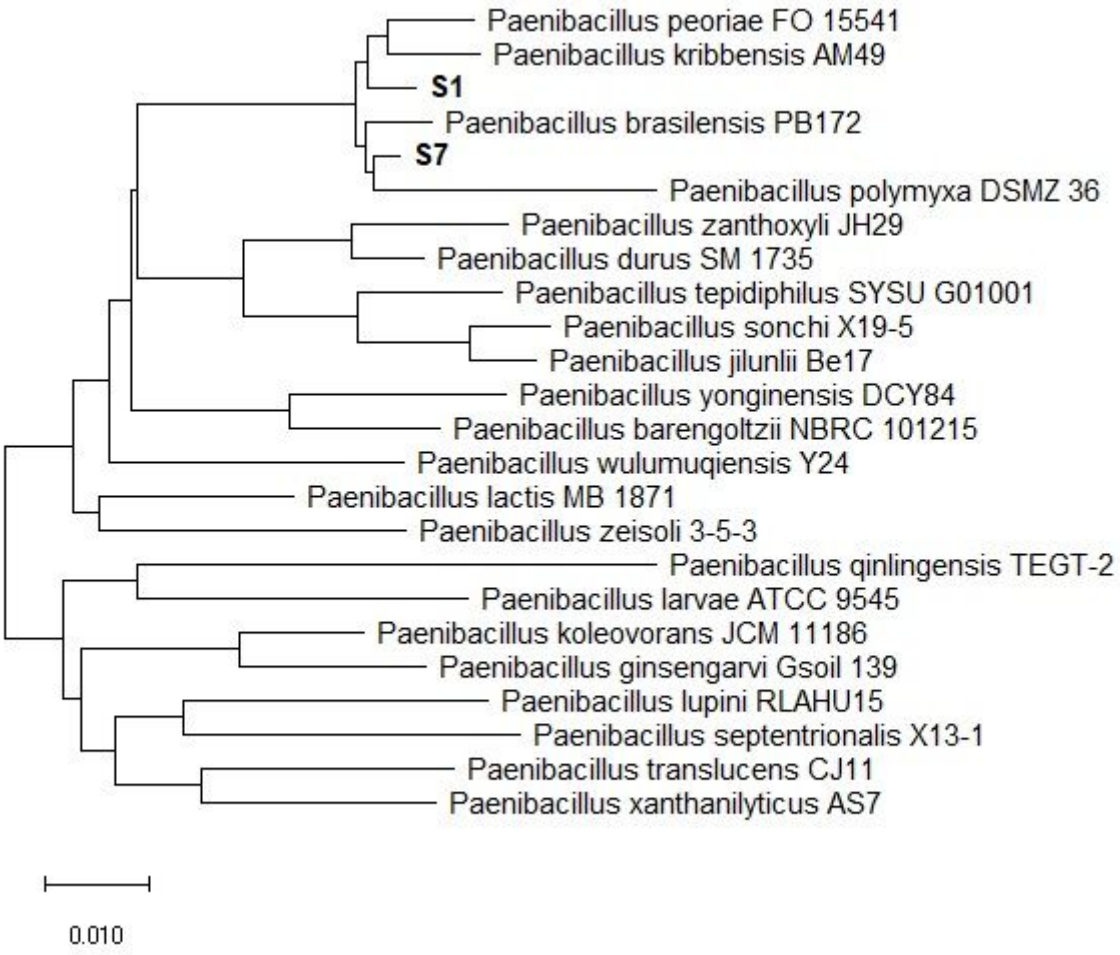

Figure 1

The phylogenetic relationships of Paenibacillus sp. S1 and Paenibacillus sp. S7 within the genus of Paenibacillus spp. investigated using 16S rRNA gene sequence analysis. The phylogenetic tree was constructed by using MEGA 5.0 software package, neighbor-joining statistical method with 1000 replicates of Bootstrap. The scale bar illustrate 0.01 substitutions per nucleotide position.

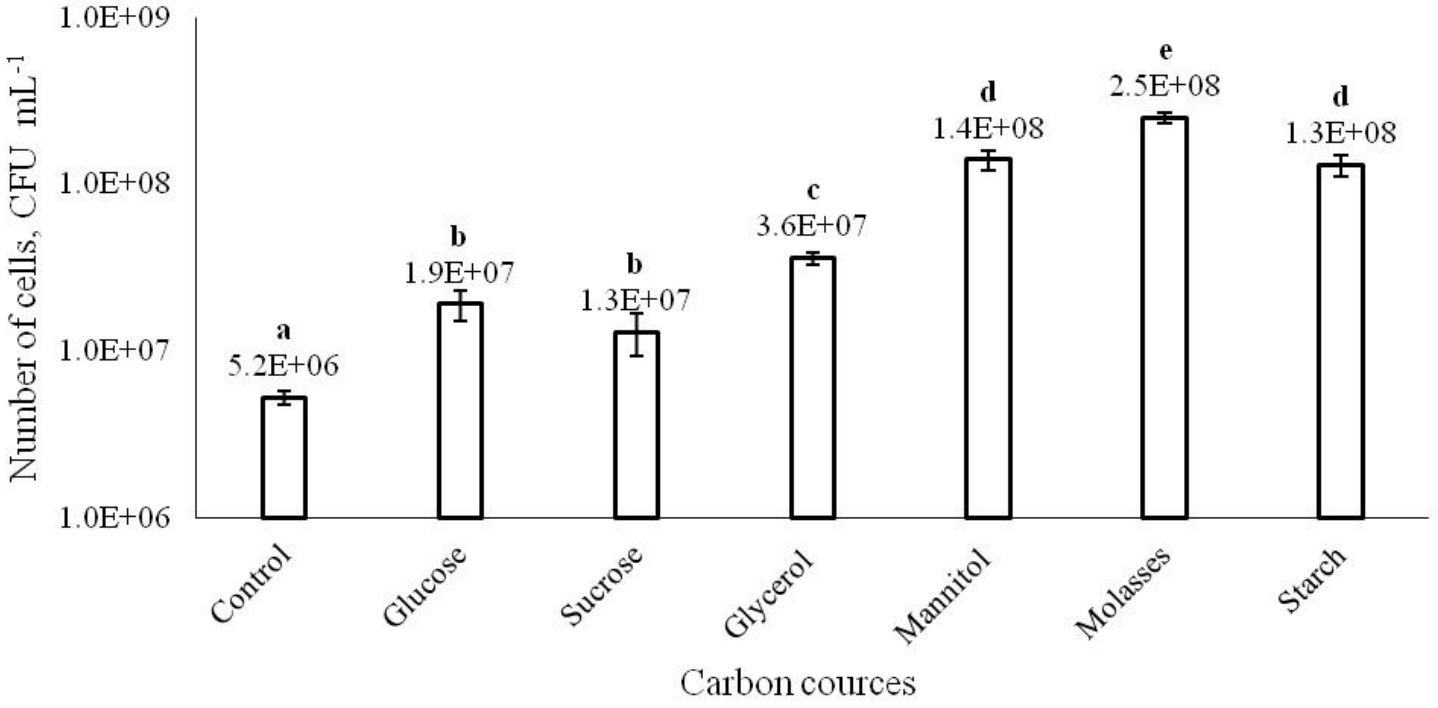

Figure 2

Effect of different carbon sources on Paenibacillus sp. MVY-024 biomass production. The values marked with the same letter have no significant different at ($P \leq 0.05$).

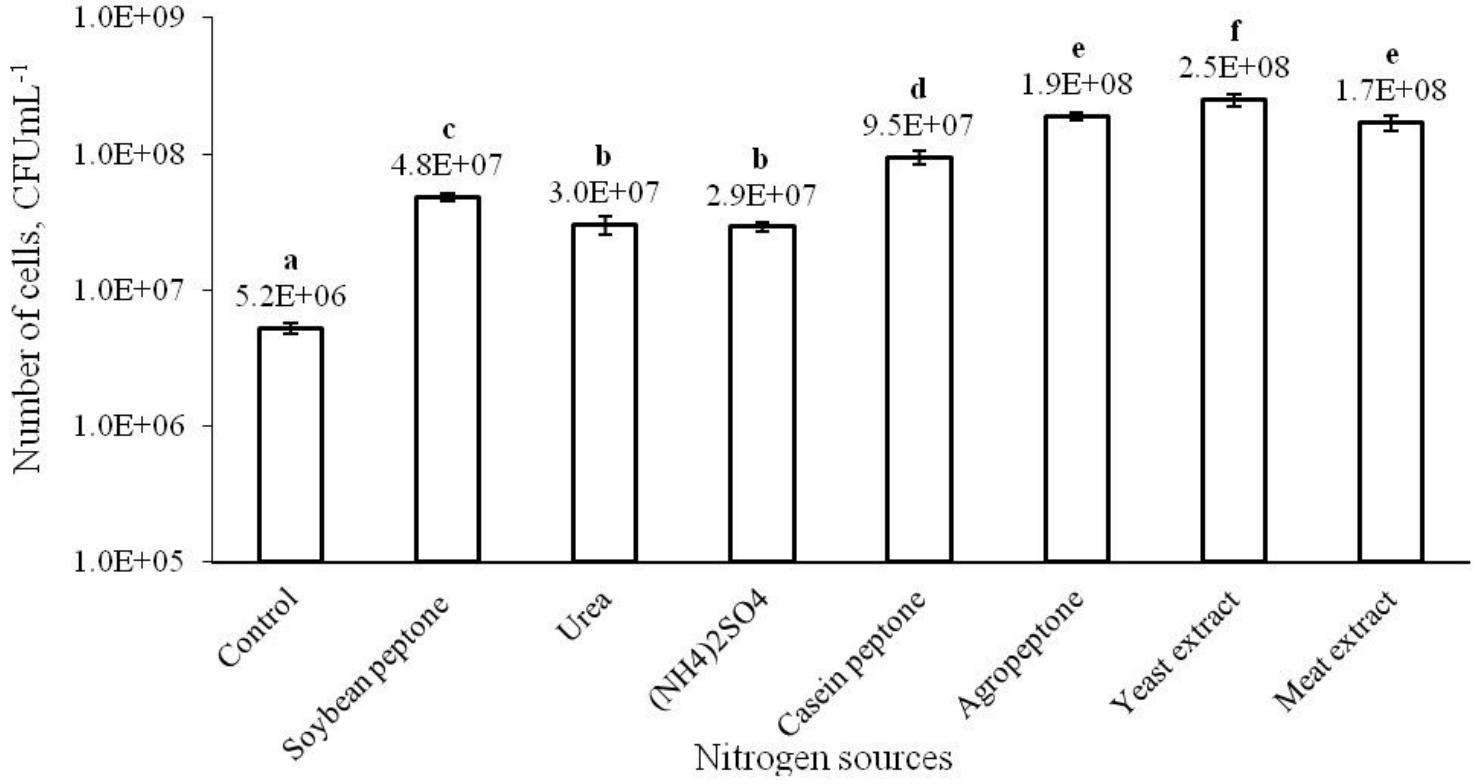

Figure 3

Effect of different nitrogen sources on Paenibacillus sp. MVY-024 biomass production. The values marked with the same letter have no significant different at ($P \leq 0.05$).

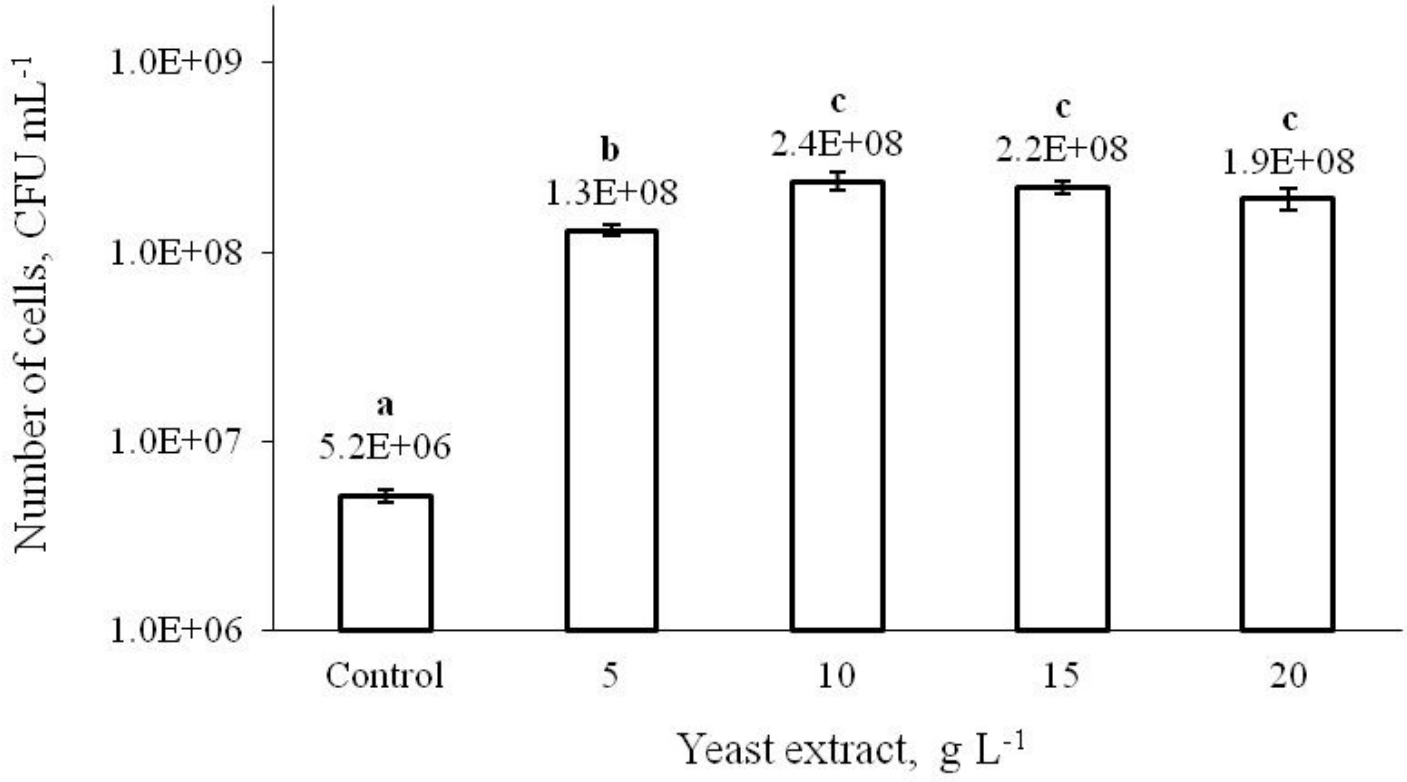

Figure 4

Effect of different concentrations of yeast extract on Paenibacillus sp. MVY-024 biomass production. The values marked with the same letter have no significant different at ($P \leq 0.05$).

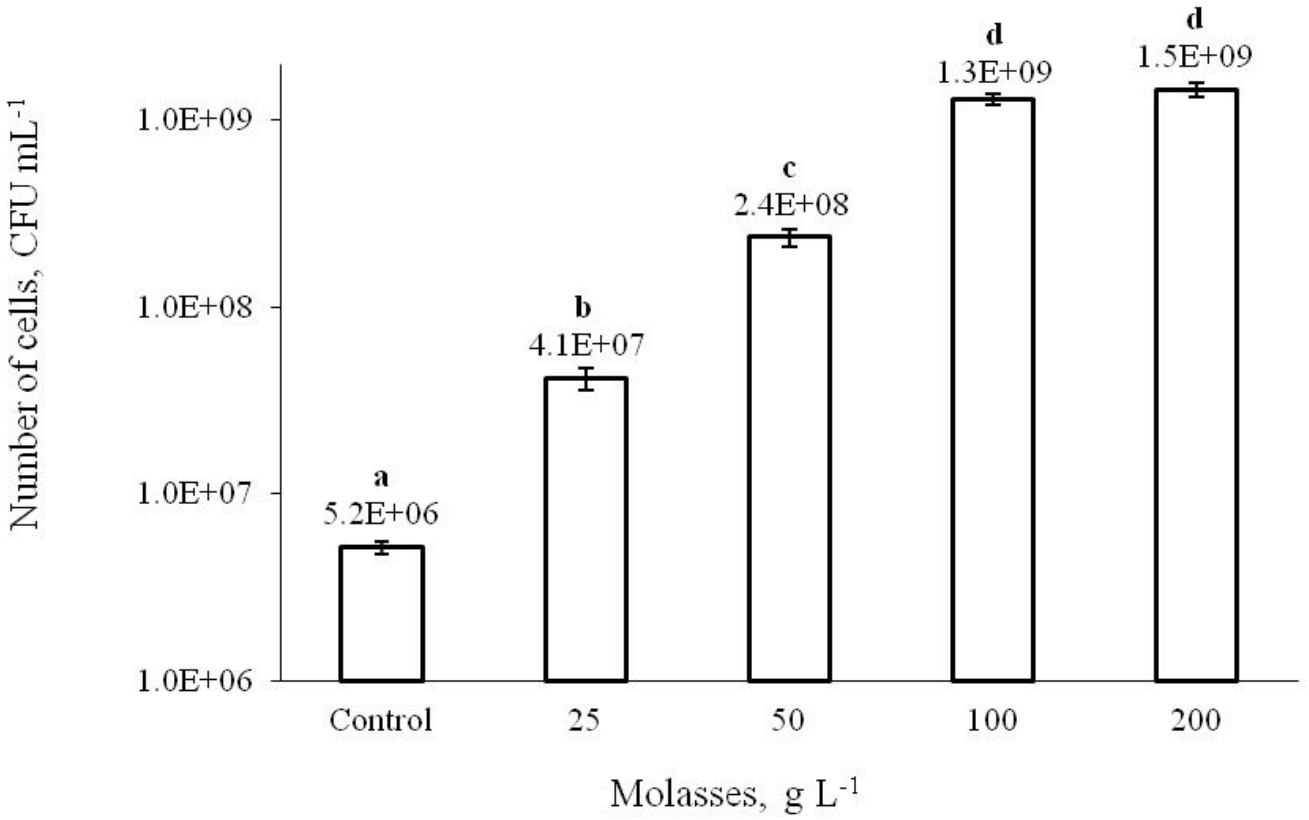

Figure 5

Effect of different concentrations of molasses on biomass production of Paenibacillus sp. MVY-024. The values marked with the same letter have no significant different at ($P \leq 0.05$).

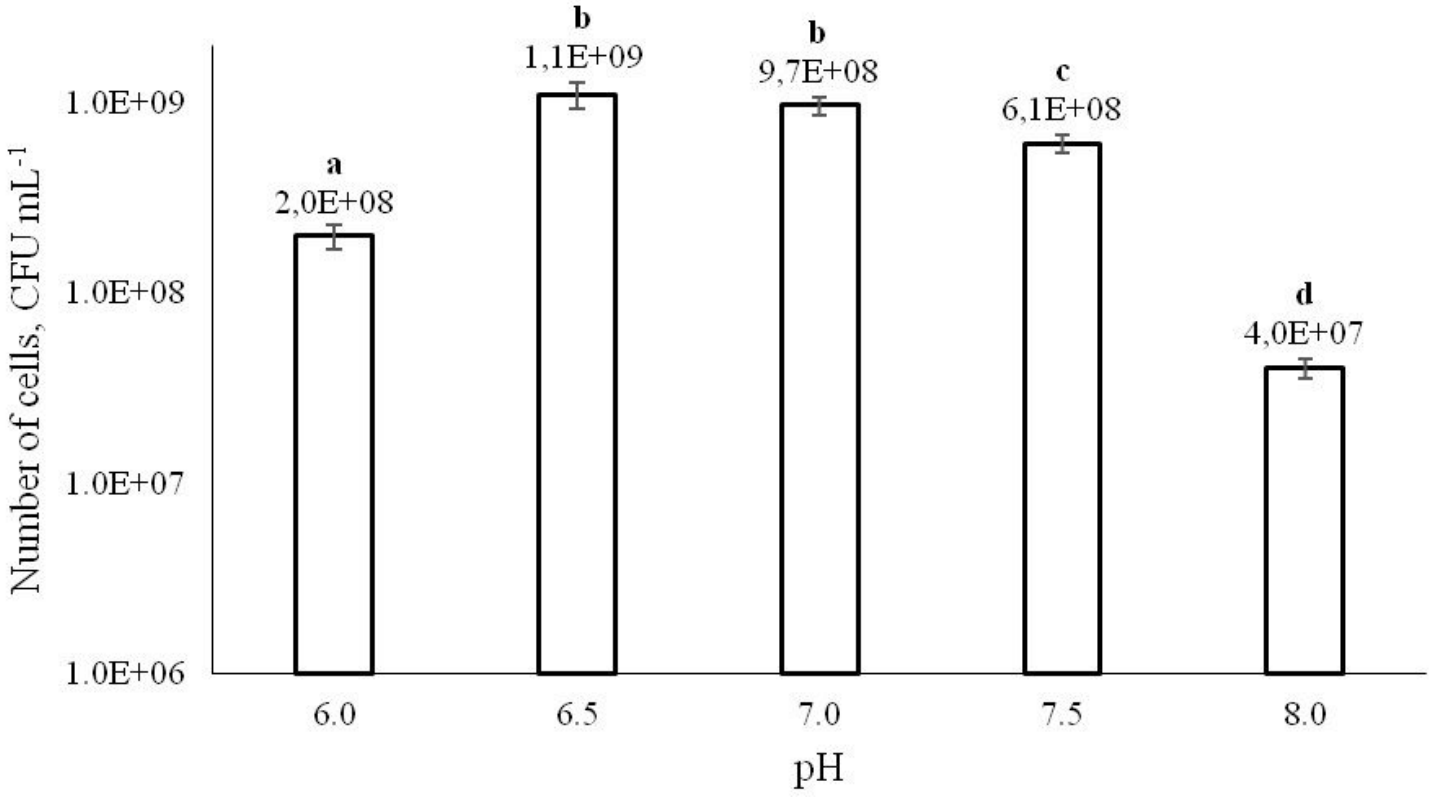

Figure 6

Effect of pH values on biomass production of Paenibacillus sp. MVY-024. The values marked with the same letter have no significant different at ($P \leq 0.05$).

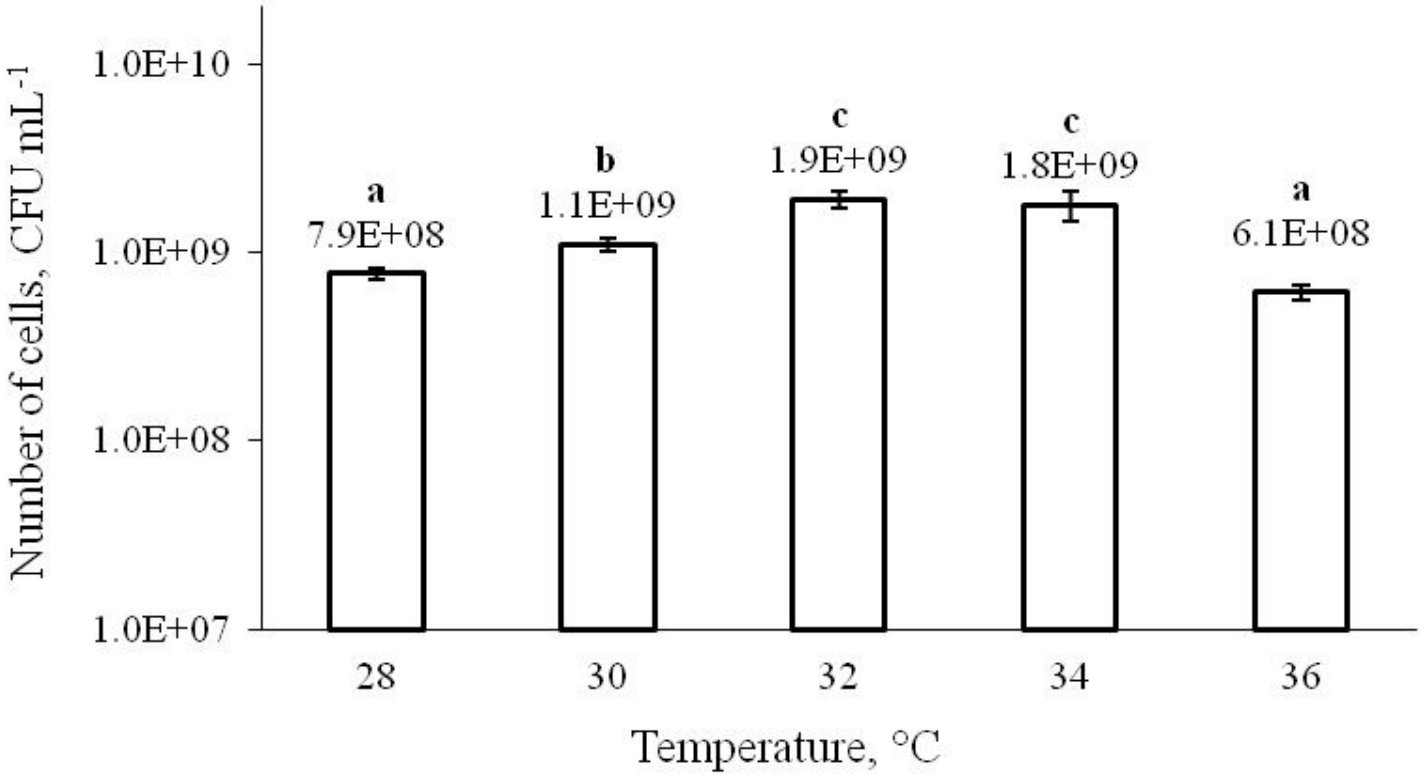

Figure 7

Effect of temperature on biomass production of Paenibacillus sp. MVY-024. The values marked with the same letter have no significant different at ($P \leq 0.05$).

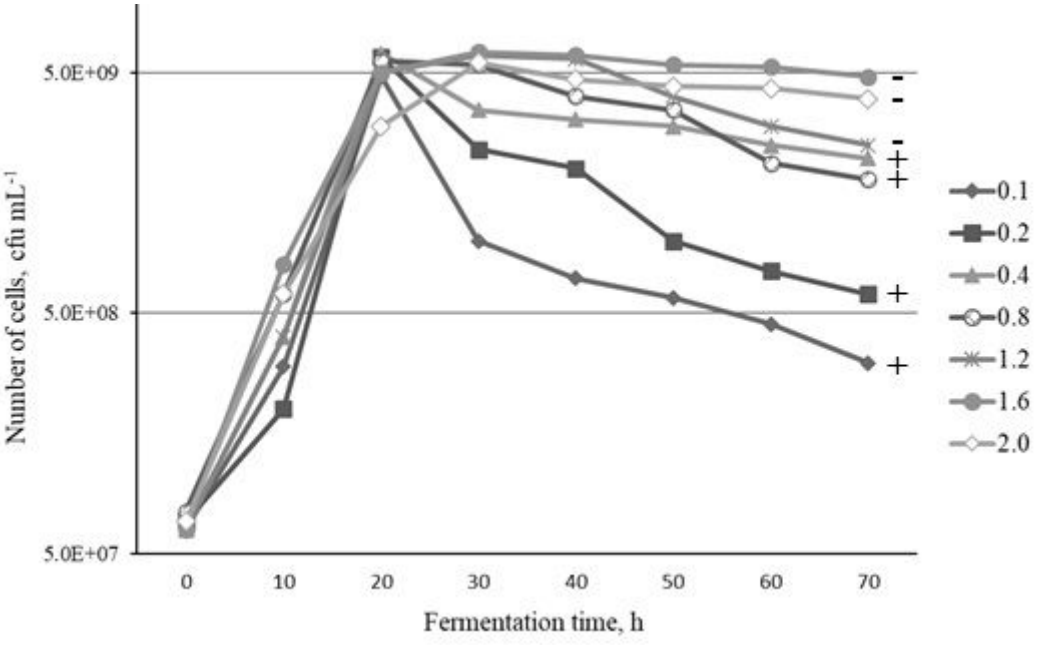

Figure 8

Effect of air flow on biomass production of Paenibacillus sp. MVY-024. Differences between averages are significant at ($P \leq 0.05$).