Original Article

The study of Lovastatin production and modeling, and optimization of the Ethanol production process with the new strain of hanseniaspora opuntiae Mk-460485

Olga Yakovlevna Mezenova[1], Savelyeva Ekaterina Leonidovna[2], Kudaibergenova Aliya Kamalovna[3], Danshina Svetlana Dmitrievna[4], Kurbanova Madinat Nasrudinovna[5], Elena Andreevna Rogozina[6], Saeid Keshtkar[1]*

[1] Department of Food Biotechnology, Faculty of Mechanics and Technology, Kaliningrad State Technical University, Kaliningrad, Russia. [2] Department of Medical Genetics, Institute of Clinical Medicine of N.V.Sklifosovsky, Sechenov University, Moscow, Russia. [3] Department of Biophysics and Biomedicine, Faculty of Biology and Biotechnology, Al-Farabi Kazakh National University, Almaty, Kazakhstan. [4] Department of Propedeutics of Dental Diseases, Institute of Dentistry. E.V. Borovsky, Sechenov University, Moscow, Russia. [5] Russian Research Institute of Canning Technology – Branch of V.M.Gorbatov Federal Research Center for Food Systems of RAS, Moscow, Russia. [6] Faculty of Food Biotechnology and Engineering, ITMO University, St. Petersburg, Russia.

Correspondence: Saeid Keshtkar; Department of Food Biotechnology, Faculty of Mechanics and Technology, Kaliningrad State Technical University, Kaliningrad, Russia. E-mail: keshtkar.bio@gmail.com.

ABSTRACT

Lovastatin and ethanol are two important compounds greatly used in therapies and medicines. We have developed and patented a new type of yeast Hanseniaspora opuntiae MK-460485, which is characterized by increased resistance to stress factors and a high ability to produce bioethanol. In this study, we considered the ability of this strain to produce ethanol under optimal conditions as well as the possibility and amount of lovastatin production by it. The present study showed that the studied species have a very good ability to produce lovastatin, especially in the presence of mashed potatoes (up to 112 mg/l). Additionally, to determine the optimal growth conditions for new yeast with the production of the maximum amount of bioethanol, the method of mathematical modeling was used. The statistical method of Plackett-Burman with the Minitab 18.1© software determined the effective factors of ethanol production by the new strain and their ranges of variation: temperature - from 25 ° C to 42 ° C; molasses concentration (carbon source) in the nutrient medium - from 20 to 100%; the concentration of ammonium nitrate (source of nitrogen) is from 1 g/l to 5 g/l. The experiment plan for optimizing the ethanol production objective function was developed using the CCD method (Central Mix Design), software Design Expert 7.0.0©. Optimal conditions were obtained for ethanol production by this strain: molasses concentration - 56.5%, ammonium nitrate concentration - 3 g/l, and temperature – 30.41 ° C. Under optimized conditions, bioethanol production by the new strain increased from 16% to 19.6%.

Keywords: Lovastatin, Ethanol production, Yeast, Hanseniaspora opuntiae mk 460485, Optimization, Temperature, Statistical value, Ammonium chloride

Introduction

Lovastatin has a significant role in lowering blood cholesterol levels in both humans and animals, thus abating the risk of hypercholesterolemia. It works by interdict the 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, thereby limiting the rate of cholesterol synthesis. Ethanol could be obtained in various ways, but the most promising one is the biotechnological way of using yeasts. Lovastatin significantly decreases the blood cholesterol levels in humans as well as animals, thus eliminating the risk of hypercholesterolemia. It works by interdict the 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, thereby limiting the rate of cholesterol synthesis [1]. Statins are a group of cholesterol-lowering drugs that work by inhibiting the enzyme HMG-CoA reductase [2]. Statins are commonly used to prevent cardiovascular disease caused by high blood fats. Some types of statins: Atorvastatin,

Access this article online	
Website: www.japer.in	**E-ISSN:** 2249-3379

How to cite this article: Olga Yakovlevna Mezenova, Savelyeva Ekaterina Leonidovna, Kudaibergenova Aliya Kamalovna, Danshina Svetlana Dmitrievna, Kurbanova Madinat Nasrudinovna, Elena Andreevna Rogozina, Saeid Keshtkar. The study of Lovastatin production and modeling, and optimization of the Ethanol production process with the new strain of hanseniaspora opuntiae Mk-460485. J Adv Pharm Edu Res 2020;10(S4):164-170. Source of Support: Nil, Conflict of Interest: None declared.

Simvastatin, Fluvastatin, Lustatin, and HMG-CoA reductase enzyme.

The enzyme hydroxymethylglutaryl coenzyme A reductase is an enzyme that catalyzes the conversion of HMG-CoA to melatonin in the cell. This enzyme in the metabolism of the enzyme malonate determines the rate of reactions, i.e., controlling or accelerating it reduces or increases other reactions [1]. The malonate metabolic pathway is a very important metabolic pathway in eukaryotes and many bacteria that eventually leads to the production of many important compounds such as cholesterol [3]. In a comparison of benefits and harms, it is found that drugs have side effects in addition to therapeutic effects [4]. Statin, which is also used to reduce fat, increases women's aggression while reducing the rate of aggression in men. Besides, studies by researchers at the University of California, San Diego, show that while lowering blood lipids, it also reduces the risk of heart disease. Of course, various harms have been predicted for this drug; Among other things, it increases the risk of type 2 diabetes. [5] This compound is made as a fungal secondary metabolite. Simvastatin can be derived from fermented lovastatin by enzymatic deacylation of lovastatin. The compound can be produced by different Aspergillus species such as Aspergillus terreus, Aspergillus niger, Aspergillus parasiticus, Aspergillus flavus, Aspergillus flavipes, and some Monascus species including Monascus anka, Monascus rubber, Monascus paxi, and Monascus purpureus [5-9]. In the present research, we checked the possibility of the presence and extent of lovastatin in secondary metabolites of Hanseniaspora opuntiae MK-460485 by HPLC method as yeast-derived medicine.

In the present study, we also investigated the production of ethanol by yeast strain. Firstly, we designed and modeled the effective parameters on Hanseniaspora opuntiae MK-460485 and then implemented them in the real laboratory environment. Unlike what most people think, the dramatic increase in the use of fossil fuels affects the seas more than lands [10]. The marine environment is a very sensitive environment, on which the increase in global pollution is very distant [11]. Sea transport carries the largest volumes of cargo in world intercontinental traffic [12]. We should find a clean and profitable substitute to use instead of traditional fossil fuels. Bioethanol is greatly used in marine practice as an environmentally friendly biofuel, solvent, and for technical marine purposes [13]. The development of the production and use of bioethanol is relevant for solving environmental and economic problems [14]. Bioethanol is obtained in various ways, but the most promising one is a biotechnological method using yeast [15]. We have developed and patented a new type of yeast Hanseniaspora opuntiae MK-460485, which is characterized by increased resistance to stress factors and a high ability to produce bioethanol.

Subjects and Methods

Inoculum Preparation for Lovastatin

Our research group isolated the pure cultures of fungal strains and grew on dextrose agar (PDA) slants. The cultures were stored in a refrigerator at 4°C to avoid contamination. Inoculum preparation Vogel's medium (KH2PO4 0.5, peptone 0.1, yeast extract 0.2, NH4NO3 0.2, (NH4)2SO4 0.4, MgSO4 0.02, glucose 50 %, and trisodium citrate 0.5 g/100mL) were prepared in 500-mL flasks. The medium pH was maintained at 5.5 by using 0.1M NaOH/HCl solutions. It was sterilized in an autoclave at 121 °C for 15 min. Fungi spores were picked by loop and added into the flask. These flasks were placed in an orbital shaker at 120 rpm for 48 h (30 °C). Then we made 4 different media from the base medium by adding wheat bran, rice-based medium, mashed potatoes, and corn stover to them (by 20% of the volume of the culture).

Lovastatin Extraction

For SSF, distilled water was added to bring the volume up to 100 mL, and the flasks were placed on a shaker for 1h. These flasks were then acidified with 10 % 1 N HCl to pH 3.0 after 72 h for both SSF and LSF. An equal volume of ethyl acetate was added to the acidified broth, which was kept on a shaker for 2 h at 70 °C. This broth was filtered to separate the biomass and filtrate, followed by centrifugation at 4,000 g for 8 min before the upper phase was collected. Next, 10 mL 1 % trifluoroacetic acid was added to 1 mL. of the organic phase for the lactonization procedure, and the extract was vaporized at 80 °C. Lovastatin samples were analyzed using high-performance liquid chromatography (HPLC). The concentrated lovastatin was diluted at a 1:1 ratio with acetonitrile and passed through a filtration assembly (0.45 µm) The samples (20 µL) were injected into the HPLC apparatus (Shimadzu LC- 10AT) by syringe and analyzed with a UV detector at 238 nm and Shim pack CLS-ODS (C- 18) column at a flow rate of 1mL/min. The mobile phase consisted of 0.1 % phosphoric acid and acetonitrile with a volume ratio of 40:60.

Optimization of Ethanol Production by Environmental and Internal Factors

Yeasts are the most commonly used microorganisms that can produce ethanol concentrations as high as 18% of the fermentation broth [16]. Several types of yeast strains are available in the market worldwide and are commonly used in traditional fermentation processes to produce various types of alcohol [17, 18]. The well-known selected strains for this purpose are S. cerevisiae [19], S. ellipsoideus [20, 21], S. pastorianus [22, 23], Kluyveromyces fragilis [24, 25], Candida Pseudotropicalis [26, 27], and Schizosaccharomyces pombe [28, 29]. Nearly 80% of ethanol is produced by anaerobic fermentation of different sugar sources by S. cerevisiae [30-32]. In this study, in order to optimize the conditions for maximum ethanol production by the selected yeast isolate, Hanseniaspora opuntiae

MK 460485 was used. This strain is a new strain of yeast that has been discovered and identified by the authors of this article [33]. In subsequent studies, it was proved that this strain has great potential for the production of ethanol [34]. We used the experimental design method using the Response Surface Method. This method allows you to get results on optimizing production conditions of increased reliability while saving time and materials, significantly reducing the number of tests and analyzes. For this purpose, firstly, we used the plackett-burman method, in which the effective factors were checked by the Minitab© software application. It should be mentioned that, since in the methods of designing experiments it is essential to take into account two values for each factor, in order to study the effect on the volume of ethanol produced by such important factors as a carbon source and a nitrogen source, we first conducted a one-factor experiment with varying one factor - time.

Screening for Factors Affecting Ethanol Fermentation

At this stage, we used the results of ethanol production optimization with varying the following factors: the use of various sources of carbon and nitrogen (with varying time factors); the influence of the two most effective sources of carbon (sucrose and glucose) and nitrogen (ammonium sulfate and ammonium chloride); the results of five concentrations of the carbon source (range 1-10%) and five concentrations of the nitrogen source (range 1-5%) at temperature ranges of 25-37 ° C and at pH ranges of 4-7; and the effect of inoculum solution concentration values. All factors were analyzed using the Minitab 18.1© software through the plackett-burman method.

After developing an experimental design using the Plackett-Burman method, each of the experiments provided by the application was carried out under laboratory conditions. Further, by screening and analyzing the results obtained, the most significant factors affecting the efficiency of ethanol production by yeast were identified.

Optimization of Factors Affecting Ethanol Production

After identifying effective factors on ethanol production, the optimization of each of the factors separately and their interactions were investigated using the so-called surface response method or RSM method. The surface response method (RSM) is a combination of mathematical and statistical methods used to study the relationship between one or more responses with a set of factors. It makes it possible to find the optimal values of factors in the desired area with a minimum number of tests. One subsection of the RSM method is the Box-Behnken or BBD design method.

At this stage of the study, to optimize the values of the factors selected by the Plackett-Brman method, we developed an experimental design for three key factors. These are carbon source, temperature, and pH.

After the test of each series of experiments presented by the software, the corresponding studies were conducted in the laboratory. Then, by analyzing the results of each test, the best conditions were provided in the next series of experiments. Further, comparing the laboratory results with the obtained software, the optimal fermentation conditions were determined, which were checked in a laboratory environment.

Results

In the present study, in order to increase the accuracy and also to create statistical diversity in the study, we used four different sources (Wheat bran, Rice-based medium, mashed potatoes, and corn stover) to evaluate the amount of lovastatin production by Hanseniaspora opuntiae MK-460485 (Fig. 1).

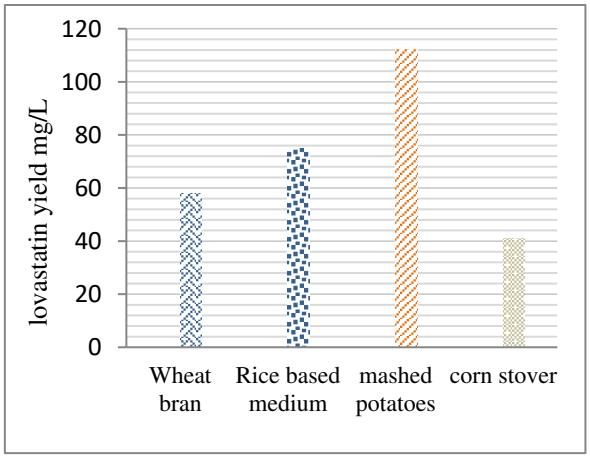

Fig. 1: The Rate of Lovastatin Production in the Presence of Different Sources in the Culture Medium

Along with the study of lovastatin production by Aspergillus terreus in the presence of wheat bran, Jaivel and Marimuthu (2010), showed that this microorganism produces 983.3 µg/g lovastatin [35]. One study carried out by Panda et al. in 2009 conducted on lovastatin production by Monascus purpureus in the presence of Rice-based medium, indicated that M.purpureus produces 3.42 mg lovastatin in every g of medium culture [36]. Another study carried out by Samiee et al. (2003) on Aspergillus terreus had shown 55 mg/L lovastatin yield [37]. The present study showed that the studied species have a very good ability to produce lovastatin, especially in the presence of mashed potatoes (up to 112 mg/l). The comparison of the results of the present study with other studies conducted in this field indicates that the hanseniaspora opuntiae mk 460485 has a completely acceptable performance compared to many other species that are used as strong species in the production of lovastatin.

Results of Optimization of Ethanol Production by Environmental Factors

An analysis of the results of laboratory tests performed using the Plackettt-Burman application package of the Minitab software allows us to conclude with a 95% probability ($\alpha = 0.05$) of the most effective factors affecting the production of ethanol with this new strain. According to the results, the main factors influencing the ethanol production by the H.opuntiae MK 460485 strain are sources of nitrogen, carbon, and also temperature. Unlike the mentioned factors, the pH value is less significant, since its change in the range from 3 to 7 does not significantly affect the amount of ethanol released by the yeast strain Hanseniaspora opuntiae MK 460485 (Fig. 2).

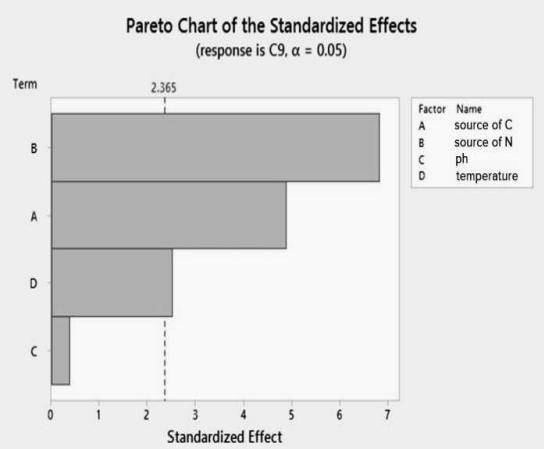

Fig. 2: The Influence of the Measured Factors on the Efficiency of Ethanol Production by the Derived Strain of Hanseniaspora opuntiae MK 460485

Optimization of the Composition of the Nutrient Medium by RSM

This stage of the study aimed to establish the best combination of nutrients in the culture medium, in which optimal conditions for ethanol production will be ensured by culturing the strain Hanseniaspora opuntiae MK 460485, i.e. designing the composition of the nutrient medium for maximum alcohol production in an enzymatic manner.

After determining the effective factors for the production of ethanol by the statistical method of Plackett-Burman, at the next stage, the minimum, maximum, and zero levels of each of the factors were determined. The experiment plan for optimizing the ethanol production objective function was developed using the CCD method (Central composite or Central Mix Design), which involved the use of Design Expert 7.0.0.© software. Taking into account the maximum level of tolerance for the growth of yeast isolates at a temperature of 42 °C, quantitative temperature levels were chosen for optimization experiments: 25 ° C (minimum level or -1) and 37 ° C (maximum level or +1). According to the experimental results obtained above, molasses with a concentration in the nutrient medium from 20 to 100% was used as a carbon source. When optimizing the source of nitrogen, which was taken as ammonium nitrate (see the rationale above), its concentration varied from 1 g/l to 5 g/l. Levels of factors (maximum, central or zero, minimum) are given in Table 1.

Table 1. Substantiated Levels of Variation of Factors of the Optimization Experiment for the Production of Ethanol Hanseniaspora Opuntiae MK 460485.

Variable Factors	Coded Factor Levels		
	-1 (min)	0 (cen)	1 (max)
Molasses (%)	20	55	90
Ammonium Nitrate (g/l)	1	3	5
Temperature (°C)	25	31	37

Next, an experiment plan was developed, including 20 experiments following the variation of all combinations of selected factors by their levels. From among these 20 experiments, 16 were decentralized tests and 4-center tests (Response Surface Method).

The order of the experiments was adopted taking into account the principle of randomness. As the optimization parameter, the amount of ethanol produced was selected by the Hanseniaspora opuntiae Mk 460485 strain, which was established using GC analysis (gas chromatography).

All developed experiments were performed in laboratory conditions with a combination of factor values according to the plan shown in Table 2.

As can be seen from the data in Table 2, the best results for the production of ethanol were obtained in experiments No. 2 and No. 6. At the next stage of the studies, statistically significant factors for the production of ethanol by the Hanseniaspora opuntiae MK 460485 strain were determined using the ANOVA analysis method (Analysis of Variance), which allows us to eliminate insignificant factors by calculating the P-value (p-criterion or the probability of obtaining a distribution of value values). Moreover, factors in which the p-test was less than the values of α (i.e., 0.01) were considered as effective factors significantly increasing the production of ethanol. Factors with a p-criterion greater than 0.01 were excluded from further consideration. The values of the p-criterion and the results of the analysis of factors carried out with its use are given in Table 2.

Table 2. The Values of the Factor P-value (p-test) in the analysis of the Significance of Factors for the Production of Ethanol

Nutrient Composition Models	DF	Dispersion (st.d)	F-Value	P-Value
	9	12.506	42.68	0.0001
A-Molasses	1	0.7704	23.67	0.0001
B-Amm.Nitra	1	0.7904	23.67	0.001
C-Temp	3	3.7301	38.19	0.001
A*B	1	1.1100	34.10	0.006
A*C	1	1.5602	30.53	0.007
B*C	1	2.3568	39.17	0.01

After determining the factors affecting the production of ethanol by an isolated strain of Hanseniaspora opuntiae MK 460485, using the software, we determined the coefficients of the statistical equation, which indicate the influence of each of the factors on the amount of ethanol produced.

The obtained equations adequately correspond to the experimental results:

$$R1 = 22,87 + 3,00 * Molasses + 1,03 * Ammonium\ nitrate - 0,38 * Temperature - 0,63 * Molasses * Ammonium\ nitrate + 0,073\ molasses * Temperature + 0,085 * Ammonium\ nitrate * Temperature - 0,365 * Molasses^2 - 1,63 * Ammonium\ nitrate^2 - ,06 * Temperature^2$$

The values of the coefficients R2 and R2 (adj) for the obtained ethanol production equation are 0.9652 and 0.9339, respectively, which indicates the statistical significance of the model and its adequacy to practical values. The P-value (p-criterion) for (lack of fit) is 0.140 in the resulting equation, i.e. above 0.05. This means that the equation is significant and may be applicable in the practice of predicting results, while probabilistic errors can be ignored. Based on the calculations of dispersions (random variances) produced by the software, the effects of the interactions of effective factors on ethanol production are graphically illustrated in Figures 3 and 5.

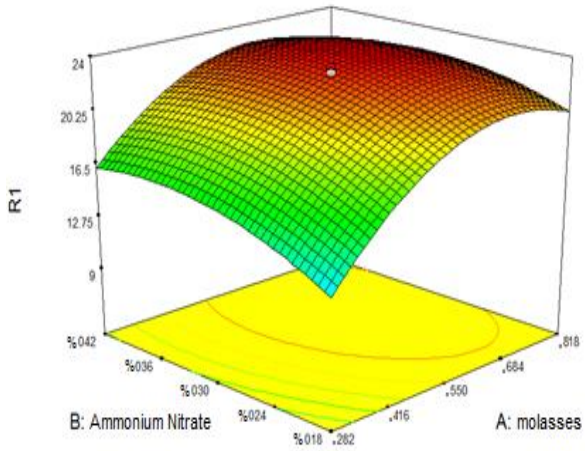

Fig. 3 Three-dimensional Response of the Interaction of Factors of the Amount of Molasses and Ammonium Nitrate to the Output of Ethanol Produced

As shown in Figures 3 and 4, an increase in molasses concentration to 56.5% leads to an increase in ethanol production, but after that, the amount of alcohol decreases with an increase in its concentration. Similar trends in ethanol production were identified in the analysis of the influence of interactions of ammonium nitrate concentration factors (Figures 3 and Figures 4) and temperature factors (Figures 4 and Figures 5). Thus, an increase in temperature in the range from 29 to 31 °C when interacting with the amount of ammonium nitrate in the range from 2.8 to 3.2 g/l, contributes to an increase in ethanol production.

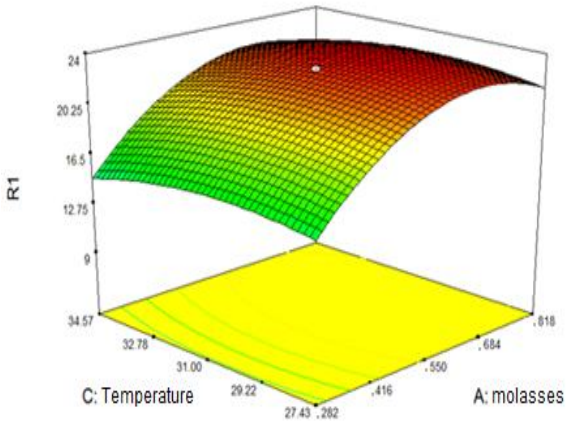

Fig. 4 Three-dimensional Response of the Interaction of Temperature Factors and Molasses Quantity to the Output of Ethanol Produced

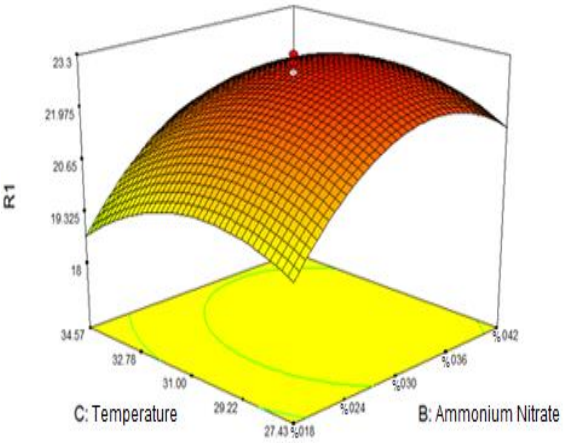

Fig. 5 Three-dimensional Response of the Interaction of Temperature Factors and the Amount of Ammonium Nitrate to the Output of Ethanol Produced

It should be noted that in each graph the interactions of two factors are illustrated, and the remaining three factors are fixed at a central point. An increase in the values of these factors above the range during the interaction of both factors leads to a decrease in ethanol production. Under optimal conditions, the amount of carbon source, as well as the temperature and concentration of the nitrogen source, are in the middle of this range. In other words, an increase or decrease in these factors relative to the middle leads to a decrease in ethanol production.

In conclusion, the optimal cultivation conditions of the strain Hanseniaspora opuntiae MK 460485 were identified based on an equation obtained by software to optimize the production of ethanol within the region of the model. The optimal factor values for ethanol production by the Hanseniaspora opuntiae 460485 strain are shown.

To verify the established optimal values of the factors, special studies were conducted on the production of ethanol using these

cultivation conditions. Finally, the amounts of ethanol obtained experimentally in triplicate were 19.56%, 19.95%, 19.3%, respectively. This indicates the coincidence of experimental data with the result of the program forecast, which amounted to 19.6%, and shows the adequacy of the model and the reliability of the optimal cultivation conditions for the cultivation of the Hanseniaspora opuntiae MK 460485 strain calculated on it.

The maximum ethanol production (19.6%) was obtained under the following cultivation conditions: molasses concentration - 56.5%, ammonium nitrate concentration - 3 g/l, and temperature - 30.41 ° C.

Similar results were obtained by Hoda et al. using the statistical response surface analysis method (RSM by CCD method) to optimize the cultivation of Saccharomyces cerevisiae yeast (PTCC 24860) in molasses of sugar beets in the production of ethanol. In this case, the optimal cultivation conditions were: pH 5.3, incubation time - 24 hours, glucose content - 35%, and the content of chloride nitrate (NH4Cl) that was 1.5 g/l [38].

Karuppaiya 2009 and others, using a similar method to optimize the cultivation conditions of Zymomonas mobilis yeast when using apple juice as a carbon source, obtained the following optimal conditions for maximum ethanol yield: pH 4.5, temperature - 32 °C, and fermentation time that was 37 hours. Under these conditions, ethanol production in the culture medium was 12.64% [39].

Conclusion

The comparison of the results of the present study with other studies conducted in this field shows that the hanseniaspora opuntiae mk 460485 has a completely acceptable performance compared to many other species that are used as strong species in the production of lovastatin. The main factors of ethanol fermentation were screened and optimized based on the mathematical method of experiment planning using the Minitab© program and the Plackett-Burman package. It was shown that the main factors of ethanol production by this strain are sources of carbon, nitrogen, and temperature, and the pH does not have a significant effect. A mathematical model has been obtained that relates the amount of ethanol produced and the main factors of ethanol fermentation, based on which the optimal cultivation conditions of the strain are established: molasses concentration 56.5% ammonium nitrate 3 g/l, and cultivation temperature of 30.4 °C. Ethanol production under optimal fermentation conditions was 19.6%.

Acknowledgements

The authors of the present article all contributed almost equally to the preparation of this article. The authors would like to thank Prof. Morovvati and Dr. Romiani for their technical assistance. We also thank Prof. Sawari and the Iranian Biological Resource Center (IBRC) for their scientific guidance.

References

1. Valera HR., Gomes J., Lakshmi S., Gururaja R. Suryanarayan S, Kumar D. Lovastatin production by solid-state fermentation using Aspergillus flavipes. Enz Microb Tech. 2005; (37): 521-526.
2. Miyake T., Uchitomi K., Zhang M Y. Effect of the principle nutrients on lovastatin production by Monascus pilosus. Biosci Biotechnol Biochem. 2006; (70):1154-1159.
3. Lee HY, Kim YS. Identification of amino acid residues in the carboxyl terminus required for malonate-responsive transcriptional regulation of MatR in Rhizobium leguminosarum bv. trifolii. BMB Reports. 2001;34(4):305-9.
4. Pandey A., Soccol CR., Rodrigoez-Leon, Nigam P. Solid-state fermentation in biotechnology - Fundamentals and Applications 1st ed. New Delhi India: Asiatech Publications Inc 2001.
5. Wei PL, Xu ZN, Cen PL. Lovastatin production by Aspergillus terreus in solid-state fermentation. Journal of Zhejiang University-Science A. 2007 Aug 1;8(9):1521-6.
6. Bizukojc M, Ledakowicz S. Physiological, morphological and kinetic aspects of lovastatin biosynthesis by Aspergillus terreus. Biotechnology journal. 2009 May;4(5):647-64.
7. Moore RN, Bigam G, Chan JK, Hogg AM, Nakashima TT, Vederas JC. Biosynthesis of the hypocholesterolemic agent mevinolin by Aspergillus terreus. Determination of the origin of carbon, hydrogen, and oxygen atoms by carbon-13 NMR and mass spectrometry. Journal of the American Chemical Society. 1985 Jun;107(12):3694-701.
8. Mol MJ, Erkelens DW, Leuven JA, Schouten JA, Stalenhoef AF. Simvastatin (MK-733): a potent cholesterol synthesis inhibitor in heterozygous familial hypercholesterolaemia. Atherosclerosis. 1988 Feb 1;69(2-3):131-7.
9. Barrios-González J, Miranda RU. Biotechnological production and applications of statins. Applied microbiology and biotechnology. 2010 Jan 1;85(4):869-83.
10. Sugiawan Y, Managi S. New evidence of energy-growth nexus from inclusive wealth. Renewable and Sustainable Energy Reviews. 2019 Apr 1;103:40-8.
11. Coady D, Parry I, Sears L, Shang B. How large are global fossil fuel subsidies?. World development. 2017 Mar 1;91:11-27.
12. Lott MC, Pye S, Dodds PE. Quantifying the co-impacts of energy sector decarbonisation on outdoor air pollution in the United Kingdom. Energy Policy. 2017 Feb 1;101:42-51.
13. Saïdane-Bchir F, El Falleh A, Ghabbarou E, Hamdi M. 3rd generation bioethanol production from microalgae isolated from slaughterhouse wastewater. Waste and Biomass Valorization. 2016 Oct 1;7(5):1041-6.

14. Goh CS, Lee KT. A visionary and conceptual macroalgae-based third-generation bioethanol (TGB) biorefinery in Sabah, Malaysia as an underlay for renewable and sustainable development. Renewable and Sustainable Energy Reviews. 2010 Feb 1;14(2):842-8.
15. Singh A, Nigam PS, Murphy JD. Renewable fuels from algae: an answer to debatable land based fuels. Bioresource technology. 2011 Jan 1;102(1):10-6.
16. Balat M, Balat H, Öz C. Progress in bioethanol processing. Progress in energy and combustion science. 2008 Oct 1;34(5):551-73.
17. Hossain N, Jalil R. Sugar and bioethanol production from oil palm trunk (OPT). Asia Pacific Journal of Energy and Environment. 2015; 2(2):81–84.
18. Cheng C., Hani H., Ismail K. Production of Bioethanol from oil palm empty fruit bunch, J. Meteorol. Soc. 2011; (52): 69–72.
19. Lam FH, Ghaderi A, Fink GR, Stephanopoulos G. Engineering alcohol tolerance in yeast. Science. 2014 Oct 3;346(6205):71-5.
20. Nikolić S, Mojović L, Pejin D, Rakin M, Vukašinović M. Production of bioethanol from corn meal hydrolyzates by free and immobilized cells of Saccharomyces cerevisiae var. ellipsoideus. biomass and bioenergy. 2010 Oct 1;34(10):1449-56.
21. Nikolić S, Mojović L, Djukić-Vuković A. Possibilities of improving the bioethanol production from cornmeal by yeast Saccharomyces cerevisiae var. ellipsoideus. InCauses, Impacts and Solutions to Global Warming 2013 (pp. 627-642). Springer, New York, NY.
22. Gonçalves P, de Sousa HR, Spencer-Martins I. FSY1, a novel gene encoding a specific fructose/H+ symporter in the type strain ofSaccharomyces carlsbergensis. Journal of bacteriology. 2000 Oct 1;182(19):5628-30.
23. Dunn B, Sherlock G. Reconstruction of the genome origins and evolution of the hybrid lager yeast Saccharomyces pastorianus. Genome Research. 2008 Oct 1;18(10):1610-23.
24. Dragone G, Mussatto SI, e Silva JB, Teixeira JA. Optimal fermentation conditions for maximizing the ethanol production by Kluyveromyces fragilis from cheese whey powder. biomass and bioenergy. 2011 May 1;35(5):1977-82.
25. Ferrari MD, Loperena L, Varela H. Ethanol production from concentrated whey permeate using a fed-batch culture ofKluyveromyces fragilis. Biotechnology letters. 1994 Feb 1;16(2):205-10.
26. Ghaly AE, El-Taweel AA. Effect of micro-aeration on the growth of Candida pseudotropicalis and production of ethanol during batch fermentation of cheese whey. Bioresource Technology. 1995 Jan 1;52(3):203-17.
27. Kargi F, Ozmıhcı S. Utilization of cheese whey powder (CWP) for ethanol fermentations: Effects of operating parameters. Enzyme and Microbial Technology. 2006 Mar 2;38(5):711-8.
28. Choi GW, Um HJ, Kim MN, Kim Y, Kang HW, Chung BW, Kim YH. Isolation and characterization of ethanol-producing Schizosaccharomyces pombe CHFY0201. Journal of microbiology and biotechnology. 2010;20(4):828-34.
29. Domizio P, Liu Y, Bisson LF, Barile D. Cell wall polysaccharides released during the alcoholic fermentation by Schizosaccharomyces pombe and S. japonicus: quantification and characterization. Food microbiology. 2017 Feb 1;61:136-49.
30. McKendry P. Energy production from biomass (part 2): conversion technologies. Bioresource technology. 2002 May 1;83(1):47-54.
31. Rolz C, De Leon R. Ethanol fermentation from sugarcane at different maturities. Industrial crops and products. 2011 Mar 1;33(2):333-7.
32. Bai FW, Anderson WA, Moo-Young M. Ethanol fermentation technologies from sugar and starch feedstocks. Biotechnology advances. 2008 Jan 1;26(1):89-105.
33. https://www.ncbi.nlm.nih.gov/nuccore/1583101516
34. Keshtkar, S., Mezenova, O.Ya., Hosseini, S., Romiani, E. Isolation and Identification of the Hanseniaspora opuntiae MK 460485 as an efficient strain for ethanol production, J. Processes and Food Production Equipment. 2019;(1): 10-19.
35. Jaivel N., Marimuthu P. Optimization of lovastatin production in solid state fermentation by Aspergillus terreus. Int J Eng Sci Technol. 2010; (2): 2730-2733.
36. Panda BP., Javes S., Ali M. Engineering rice based medium for production of lovastatin with Monascus purpureus. Czech J Food Sci. 2009; (27): 352-368.
37. Samiee SM, Moazami N, Haghighi S, Aziz Mohseni F, Mirdamadi S, Bakhtiari MR. Screening of lovastatin production by filamentous fungi. Iranian Biomedical Journal. 2003 Jan 10;7(1):29-33.
38. Shafaghat H, Najafpour GD, Rezaei SP, Sharifzadeh M. Optimal growth of Saccharomyces cerevisiae (PTCC 24860) on pretreated molasses for ethanol production: Application of response surface methodology. Chemical Industry and Chemical Engineering Quarterly. 2010;16(2):199-206.
39. Karuppaiya M., Sasikumar E., Viruthagiri T., Vijayagopal V. Optimization of process parameters using response surface methodology (RSM) for ethanol production from waste cashew apple juice using Zymomonas mobilis. Chem. Eng. Comm. 2009; (196): 1-11.