PROMOTION OF L-TRYPTOPHAN PRODUCTION BY MUTATION OF CORYNEBACTERIUM GLUTAMICUM

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ABSTRACT

L-TRYPTOPHAN IS NOT ABUNDANT IN THE NATURAL WORLD.IT MAINLY EXISTS IN THE FOOD OF ANIMAL TYPE SUCH AS MEAT, EGG, AND MILK. FOR THE FOOD OF PLANT TYPE, EXCEPT POTATOES, THE CONTE -NT OF L-TRYPTOPHAN IS VERY SCARCE ESPECIALLY IN GRAINS LIKE RICE AND WHEAT.FOUR METHODS , INCLUDING HYDROLYSIS OF PROTEIN, CHEMICAL SYNTHESIS, ENZYMATIC TRANSFORMATION, AND FERMENT -ATION, ARE USED TO PRODUCE L-TRYPTOPHAN. AMONG THEM, FERMENTATION IS MOST FREQUENTLY USED BECAUSE IT COULD USE LOW-PRICED CARBON AND NITROGEN SOURCES.THEREFORE, FERMENTATION IS THE METHOD USED IN THIS STUDY TO PRODUCE L-TRYPTOPHAN. CORYNEBACTERIUM GLUTAMICUM CCRC12511WAS THE FIRST STRAIN TO BEGIN.UNFORTUNATELY,THIS STRAIN APPEARED TO PRODUCE NO L-TRYPTOPHAN.TH -EREFORE,MUTATION OF THIS STRAIN WAS THEN CONSIDERED TO IMPROVE THE L-TRYPTOPHAN YIELD.NTG (N-METHYL-N'- NITRO-N-NITROSOGUANIDINE) WAS THEN USED AS THE MUTANT REAGENT TO TREAT THE O -RIGINAL STRAIN.THROUGH A SERIES OF SCREENING EXPERIMENTS,SEVERAL POTENTIAL STRAINS WERE SE -LECTED.UNFORTUNATELY,THE MUTATED STRAINS STILL SHOWED NO EVIDENCE TO PRODUCE L-TRYPTOPHAN .THEREFORE,THE STRAIN OF CORYNEBACTERIUM GLUTAMICUM CCRC 11631 WAS THEN SELECTED TO REPLAC -E CCRC 12511.HOWEVER,THE STRAIN OF CCRC 11631 DID NOT PRODUCE L-TRYPTOPHAN EITHER.THEN,TH -E STRAIN OF CCRC 11631 WAS MUTATED.ONE OF THE MUTATED STRAINS FROM CCRC 11631,NAMELY NO. 195, WAS SELECTED AS A POTENTIAL CANDIDATE FOR PRODUCING L-TRYPTOPHAN. AN EXPERIMENTAL DESIGN METHOD WAS USED TO STUDY THE OPTIMAL CULTIVATING CONDITION FOR MU -TATED STRAIN NO.195.THE THREE MAJOR FACTORS,INCLUDING THE CONCENTRATION OF GLUCOSE,THE TY -PE OF NITROGEN SOURCES AND THE CULTIVATION TIME, WERE CONSIDERED.THROUGH THE ANALYSIS OF ANOVA (ANALYSIS OF VARIANCE), EXPERIMENTAL RESULTS SHOWED THAT THE INITIAL GLUCOSE CONCENT -RATION,THE ORGANIC NITROGEN TYPE AND THE CULTIVATION TIME ALL HAD A SIGNIFICANT EFFECT TO THE YIELD OF BIOMASS.THE INITIAL GLUCOSE CONCENTRATION AND THE CULTIVATION TIME HAD A SIGN -IFICANT EFFECT TO THE YIELD OF L-TRYPTOPHAN PRODUCTION, AND THE CULTIVATION TIME HAD NOT. EXPERIMENTAL RESULTS SHOWED THAT THE OPTIMAL CULTIVATING CONDITION IS AS FOLLOWS: THE INIT -IAL GLUCOSE CONCENTRATION 40 G/L,THE ORGANIC NITROGEN TYPE CORN STEEP LIQUOR AND THE CULT -IVATION TIME 72 H.THE CONCENTRATION OF L-TRYPTOPHAN COULD REACH 8.347 MG/L AFTER 72 H OF CULTIVATION OF STRAIN NO. 195 IN A SHAKER WITH A SPEED OF 150 RPM AND 30 ℃.LATER, A JAR F -ERMENTOR OF 3 L WAS USED TO CULTIVATE STRAIN NO. 195 UNDER 30 ℃ AND PH 7.0. THE CONCENTR -ATION OF L-TRYPTOPHAN COULD REACH 13.5 MG/L AFTER 72 H CULTIVATION IN THE FERMENTOR WITH A WORKING VOLUME OF 2 L AND A STIRRING SPEED OF 250 RPM.

Keywords : L-TRYPTOPHAN, MUTANT, FERMENTATION, CORYNEBACTERIUM GLUTAMICUM

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REFERENCES

1.王文憲（1991）生物化學要論，P. 299，合記圖書出版社，台北。 2.李宛儒（1996）利用BREVIBACTERIUM DIVARICATUM 營養要求性變異株發酵生產色胺酸之研究，國立 台灣大學農業化學研究所，碩士論文。 3.黃伯超與游素玲（1992）營養學精要， P. 148 - 152， P. 720，健康文化事業，台北。 4.黃清龍(1998) 生物技術的發展與應用，P. 52 - 63，九州圖書文物有限公司，台北。 5.張為憲（1984）高等食品化學， P. 67 - 103，華香園出版社，台北。 6.張雪華、黃種金、徐敬衡及華傑 (1994) 胺基酸產業趨勢分析， P.5，食品工業研究所，新竹。 7.劉英俊（1996）最新微生物應用工業， P. 81 - 82，中央圖書出版社，台北。 8.蘇遠志與黃世佑 (1994) 微生物化學工程， P. 243 - 256，華香園出版社，台北。 9.ADELBERG, E. A., AND J. W. MEYERS (1953) MODIFICATION OF THE PENICILLIN TECHNIQUE FOR THE SELECTION OF AUXOTROPHIC BACTERIA, J. BACTERIOL., 65:348 - 353. 10.GISH, K., AND C. YANOFSKY （1993）INHIBITION OF EXPRESSION OF THE TRYPTOPHANASE OPERON ON ESCHERICHIA COLI BY EXTRACHROMOSOMAL COPIES OF THE TNA LEADER REGION, J. BACTERIOL., 175(11):3380 - 3387. 11.HAGINO, H., AND K. NAKAOYMA（1975）L-TRYPTOPHAN PRODUCTION BY ANALOG-RESISTANT MUTANTS DERIVED FROM A PHENYLALANINE AND TYROSINE DOUBLE AUXOTROPH OF CORYNEBACTERIUM GLUTAMIC -UM, AGRI. BIO. CHEM., 39（2）:343 - 349. 12.HWANG, S. O., G. H. GIL, Y. J. CHOI, K. R. KSNG, J. K. LEE AND J. C. BAE（1985）THE FER -MENTATION PROCESS FOR L-PHENYLALANINE PRODUCTION ON USING AN AUXOTROPHIC REGULATORY MU -TANT OF ESCHERICHIA COLI, APPL. MICROBIOL. BIOTECHNOL., 22:108 - 113. 13.IKEDA, M., K. NAKANISHI, K. KINO AND R. KATSUMATA（1994）FERMENTATIVE PRODUCTION OF TRY -PTOPHAN BY A STABLE RECOMBINANT STRAIN OF CORYNEBACTERIUM GLUTAMICUM WITH A MODIFIED S -ERINE-BIOSYNTHETIC PATHWAY, BIOSCI. BIOTECH. BIOCHEM., 58（4）:674 - 678. 14.IKEDA, M., AND R. KATSUMATA（1995）TRYPTOPHAN PRODUCTION BY TRANSPORT MUTANTS OF CORYNE -BACTERIUM GLUTAMICUM, BIOSCI. BIOTECH. BIOCHEM., 59(8):1600 - 1602. 15.KUPFER, D. AND D.E. ATKINSON （1964）QUANTITATIVE METHOD FOR DETERMINATION OF INDOLE,TR -YPTOPHAN AND ANTHRANILIC ACID IN THE SAME ALIQUOT, ANAL. BIOCHEM., 8:82 - 94. 16.MARTIN, J. F., R. SANTAMARIA, H. SANDOVAL, G. DEL REAL, L. M. MATEOS AND J. A. AGUILAR -(1985) CLONING SYSTEMS IN AMINO ACID-PRODUCING CORYNEBACTERIA, BIO/TECHNOLOGY, 5(7) :137 - 146. 17.MATHEWS, C.K. AND K.E. VAN HOLDE (1990) BIOCHEMISTY, P. 716-726, THE BENJAMM/CUMMINGS -PUBLISHING, REDWOOD CITY, CA. 18.SHIIO, I., H. SATO AND M. NAKAGAWA （1972）L-TRYPTOPHAN PRODUCTION BY 5-METHYL- TRYPTOP -HAN- RESISTANT MUTANT OF GLUTAMATE-PRODUCING BACTERIA, AGRI. BIO. CHEM., 36（13）:2315 - 2322. 19.SHIIO, I., S. I. SUGIMOTO AND M. NAKAGAWA （1975）PRODUCTION OF L-TRYPTOPHAN BY MUTANT BREVIBACTERIUM FLAVUM RESISTANT TO BOTH TRYPTOPHAN AND PHENYLALANINE ANALOGUES, AGRIC. BIOL. CHEM., 39（3）:627 - 635. 20.SHIIO, I., S. I. SUGIMOTO AND K. KAVAMURA（1988）BREEDING OF PHENYLALANINE-PRODUCING BR -EVIBACTERIUM FLAVUM STRAINS BY REMOVING FEEDBACK REGULATION OF BOTH THE TWO KEY ENZYME -S IN ITS BIOSYNTHESIS, AGRI. BIO. CHEM., 52（9）:2247 - 2253. 21.SU, Y. C., AND K. YAMADA (1960) STUDIES ON L-GLUTAMIC ACID PRODUCING STRAIN AND ITS TAX -ONAMICAL STUDIES, BULL. AGRI. CHEM. SOC. JPN., 24(1):69 - 74. 22.SUGIMOTO, S. I., AND I. SHIIO（1977）ENZYMS OF THE TRYPTOPHAN SYNTHETIC PATHWAY IN BREV -IBACTERIUM FLAVUM, J. BIOCHEM., 81:823 - 833. 23.WINDHOLZ, M. (1983) THE MERCK INDEX-AN ENCYCLOPEDIA OF CHEMICALS AND DRUGS, 10TH ED., P .9601, MERCK, NJ. 24.YOKOTA, A. AND S. TAKAO (1984) CONVERSION OF PYRUVIC-ACID FERMENTATION TO TRYPTOPHAN PR -ODUCTION BY THE COMBINATION OF PYRUVIC-ACID PRODUCING MICROORGANISMS AND ENTEROBACTER AEROGENE HAVING HIGH TRYPTOPHAN ACTIVITY, AGRIC. BIOL. CHEM., 48(11):2663 - 2668.