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硕 士 学 位 论 文

优良啤酒酿造酵母菌株的选育

Screening of the Fine Brewer's Yeast Strain

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优良啤酒酿造酵母菌株的选育

摘 要

啤酒酿造酵母菌种的优劣对啤酒质量起着至关重要的作用，可以说酵母是啤酒的灵魂。因此选育性能优良的啤酒酿造酵母菌种是啤酒工业的一项重要任务。本研究以啤酒酵母菌株 BW 和 H38 为出发菌株，通过物理化学因子处理，筛选优良的啤酒酵母菌株，并为原生质体融合提供直接亲本；运用原生质体融合技术，构建和筛选优良的啤酒酵母菌株。

菌株 BW 经亚硝酸 （0.04 mol/L 处理 10 min，致死率 82％） 诱变，筛选得到一株发酵液中高级醇含量较低的菌株 N1。该菌株在 12℃下三角瓶发酵 8 d，发酵液中的双乙酰和高级醇含量分别为 0.1000 mg/L 和 87.68 mg/L，比出发菌株 BW 的（0.1230 mg/L 和 115.04 mg/L）分别降低 18.7％和 24.3%。菌株 N1 经紫外线（功率 30 W、灯距 26 cm、垂直照射 10 min，致死率 95.3%）诱变，筛选得到一株发酵特性较优良的菌株 UN41。该菌株在 12℃下三角瓶发酵 8 d，发酵度为 65.0%。发酵液中双乙酰、高级醇和乙醛的含量分别为 0.0768 mg/L、92.15 mg/L 和 9.33 mg/L，比出发菌株 BW 的分别降低 37.6%、19.9%和 27.6%。

非凝絮性的菌株 H38 经 He-Ne 激光和氯化锂（激光照射 30 min，氯化锂浓度 0.3%，致死率 70.2%）复合诱变，筛选得到 2 株发酵特性较优良的菌株 HL28 和 HL29。在 12℃下三角瓶发酵 8 d，这两株菌的凝絮性较强，本斯值分别为 3.2 mL 和 2.2 mL，发酵度分别为 66.3%和 66.7%，双乙酰含量分别为 0.0716 mg/L 和 0.0635 mg/L，比出发菌株的分别降低 13.2%和 23.0%；高级醇含量分别为 66.67 mg/L 和 62.70 mg/L，比出发菌株的分别降低 8.9%和 14.4%；乙醛的含量（8.80 mg/L 和 8.14 mg/L）虽然比出发菌株

的略高，但仍然低于阈值10 mg/L。

运用正交试验确定了菌株N1和H38原生质体的形成和再生的条件。结果表明，经EDTA一巯基乙醇脱壁预处理剂处理10 min后，菌株N1细胞在30℃下，用1% 蜗牛酶处理10 min，其原生质体形成率和再生率分别为99.3%和49.7%；菌株H38的细胞在25℃下，用0.5% 蜗牛酶处理15 min，其原生质体形成率和再生率分别为99.9%和14.8%。

确定了菌株N1原生质体热灭活的条件为55℃温育25 min。用营养缺陷型菌株X6-20原生质体与灭活的营养型互补的菌株N1原生质体融合构建新菌株。在PEG（MW 6000）30%、Ca^{2+} 10 mmol/L的条件下，融合30 min，两个亲株的融合率为2.7×10^{-6}。

根据直接亲本N1和X6-20营养特性，确定了融合子检出培养基。在N1和X6-20融合的融合子检出培养基上挑取21株菌。通过三角瓶低温发酵，得到2株优良的菌株GR5和GR8。在12℃下三角瓶发酵8 d，这两株菌的本斯值分别为2.7 mL和3.0 mL；发酵度分别为65.3%和66.5%；发酵液中的双乙酰含量分别为0.0568 mg/L和0.0583 mg/L；高级醇含量分别为87.79 mg/L和103.85 mg/L；乙醛含量分别为11.21 mg/L和9.66 mg/L。

对菌株GR5进行500 L罐发酵，结果表明，在发酵至第8 d时，GR5发酵液的发酵度为69.2%，双乙酰、高级醇和乙醛的含量分别为0.0498 mg/L、74.4 mg/L，和6.34 mg/L。

对融合株GR5进行了120吨罐的发酵试验。结果表明，融合株GR5发酵的啤酒的口感较柔和、协调、后味较干净、略带酯香味，该菌株具有很好的应用前景。

用菌株H38的原生质体与灭活的N1的原生质体融合构建新菌株。运用正交试验法，确定了菌株N1和H38原生质体融合的最适条件为：PEG（MW6000）25%、$CaCl_2$ 30 mmoL/L、作用时间40 min、作用温度35℃。

在该条件下两个亲株的融合率为 2.58×10^{-5}。

根据直接亲本 N1 和 H38 抗制霉菌素的特性，确定了融合子检出培养基。在融合子检出培养基上挑取 51 株菌。通过三角瓶低温发酵，得到 3 株优良的菌株 HN31-3、HN31-6 和 HN40-5。在 12℃下三角瓶发酵 8 d，菌株 HN31-3 的本斯值为 2.8 mL，发酵液的发酵度为 67.0%，双乙酰、高级醇和乙醛的含量分别为 0.0778 mg/L、61.90 mg/L 和 8.53 mg/L；菌株 HN31-6 的本斯值为 3.2 mL，发酵液的发酵度为 66.9%，双乙酰、高级醇和乙醛的含量分别为 0.0627 mg/L、60.12 mg/L 和 9.05 mg/L；菌株 HN40-5 的本斯值为 2.8 mL，发酵液的发酵度为 67.6%；双乙酰、高级醇和乙醛的含量分别为 0.0838 mg/L、85.97 mg/L 和 7.31 mg/L。

对菌株 HN31-3、HN31-6 和 HN40-5 进行 500 L 罐发酵。结果表明，在发酵至第 8 d 时，融合株 HN31-3、HN31-6 和 HN40-5 的发酵度分别为 69.2%、69.7%和 67.7%；发酵液中双乙酰的含量分别为 0.0503 mg/L、0.0583 mg/L 和 0.0302 mg/L；高级醇的含量分别为 57.7 mg/L、62.5 mg/L 和 60.8 mg/L；乙醛的含量分别为 7.17 mg/L、6.07 mg/L 和 2.41 mg/L。这三个融合株发酵的啤酒口感均较好，具有很好的应用前景。

测定了菌株 GR5、GR8、 HN31-3、HN31-6 和 HN40-5 的细胞大小、DNA 含量和生物量以及连续传 10 代后测定它们的主要发酵特性，结果表明，这 5 个菌株是融合株，它们的发酵特性稳定。

关键词： 啤酒酿造酵母；诱变育种；原生质体融合；发酵特性；中试

Screening of the Fine Brewer's Yeast Strain

Abstract

The brewer's yeast strain is the base and key of the beer brewage production. To improve the beer quality and yield, in the first, an excellent yeast strain must be selected. In the study, strain *Saccharomyces cerevisiae* BW and H38 were used as the original strains. After treatment with physical and chemical mutagen and selection, the fine mutants or mutants with genetic markers used for the protoplast fusion were obtained. Protoplast fusion technique was applied to construct the fine brewer's yeast strains.

The strain N1 was obtained from strain BW after treatment with 0.04 mol/L HNO_2 for 10 min (death rate 82%) and selection. The content of diacetyl and higher alcohol in the fermented liquid of strain N1 in flask experiment (under the fermentation condition of 500 mL flask with 300 mL 12^{o}BX wort at 12^{o}C for 8 days, the same below) were 0.1000 mg/L and 87.68 mg/L, 18.7% and 24.3% lower than that of the original strain BW, respectively. The strain UN41 was obtained from strain N1 after treatment with UV (death rate 95.3%) and selection. Fermentation degree of strain UN41 was 65%, the content of diacetyl, higher alcohol and acetaldehyde in the fermented liquid of strain UN41 in flask experiment were 0.0768 mg/L, 92.15 mg/L and 9.33 mg/L, 37.6%、19.9% and 27.6% lower than that of the original strain BW, respectively.

Strain HL28 and HL29 were obtained from the non -flocculence original strain H38 after combining mutagenesis with laser (30 min) and LiCl (0.3%, death rate 70.2%) and screening. The Burns values of the strain HL28 and HL29

were 3.2 mL and 2.2 mL, respectively. Fermentation degree in the fermented liquid of strain HL28 and HL29 in flask experiment were 66.3% and 66.7%, respectively. Diacetyl content of strain HL28 and HL29 were 0.0716 mg/L and 0.0615 mg/L, 13.2% and 23.0% lower than that of the original strain H38, respectively. Higher alcohol content of strain HL28 and HL29 were 66.67 mg/L and 62.70 mg/L, 8.9% and 14.4% lower than that of the original strain H38, respectively. Acetaldehyde content of strain HL28 and HL29 were little higher than that of the strain H38, but still lower than the threshold of 10 mg/L.

The conditions of the protoplast formation and regeneration of strain N1 and H38 were investigated with orthogonal experiment. The results showed that after pretreatment with 0.1％ mercaptoethanol-EDTA for 10 min, the efficiency of protoplast formation and regeneration of strain N1 were respectively 99.3% and 49.7% under the condition of 1％ snail enzyme, 30℃ for 10 min reaction; those of strain H38 were respectively 99.9% and 14.8% under the condition of 0.5％ snail enzyme, 25℃ for 15 min reaction.

The death rate of protoplast of strain N1 was 100% after incubating it at 55℃ for 25 min. The protoplast fusants were constructed with the protoplasts of auxotrophic mutant X6-20 and heat-inactivated N1. The fusion frequency was 2.7×10^{-6} under the conditions of PEG (MW = 6000) 30%, Ca^{2+} 10 mmol/L and reaction time of 30 min.

The anti -mycostatin character of strain N1 was obtained by experiment. The protoplast fusants were constructed with the protoplast of strain H38 and heat-inactivated N1. The conditions of protoplasts fusion between N1 and H38 were researched with orthogonal experiment. The results indicated that the optimal reaction conditions were as following: PEG (MW = 6000) 25%, Ca^{2+} 30 mmol/L ,

temperature 35°C and reaction time of 40 min. Under the conditions, the highest fusion frequency was 2.58×10^5.

The selection medium of fusants was established according to nutrient properties of the parental strains N1 and X6-20. 21 fusants were obtained from the selection medium of fusants, and named for GR1,GR2…GR21. Two fine strains GR5 and GR8 were obtained after selection. Burns value of strain GR5 and GR8 were 2.7 mL and 3.0 mL, respectively; Fermentation degree in the fermented liquid of strain GR5 and GR8 in the flask experiment were 65.3% and 66.5%, respectively; Diacetyl content of strain GR5 and GR8 were 0.0568 mg/L and 0.0583 mg/L, respectively. Higher alcohol content of strain GR5 and GR8 were 87.79 mg/L and 103.85 mg/L, respectively. Acetaldehyde content of strain GR5 and GR8 were 11.21 mg/L and 9.66 mg/L, respectively.

The fermentation tests of strain GR5 was conducted in the 500 L fermentor. The results after the fermentation for 8 days in the fermented liquid indicated that formentation degree of strain GR5 was 69.2%, the content of diacetyl, acetaldehyde and total higher alcohols were 0.0498 mg/L, 6.34 mg/L and 74.4 mg/L, respectively.

The fermentation tests of strain GR5 were carried out in the 120 t fermentor. The results indicated that the beer produced by strain GR5 was harmony. The strain GR5 has a well applied prospect in the beer brewing.

The fusants selection medium was established according to anti -mycostatin properties of the parental strains N1 and H38. 51 fusants were obtained from the selection medium of fusants between N1 and H38, and named for HN1,HN2…HN51. Three fine strains HN31-3, HN31-6 and HN40-5 were obtained after selection in flask experiment. Fermentation degree in the fermented

liquid of strain HN31-3, HN31-6 and HN40-5 were 67.0%, 66.9% and 67.6%, respectively; Burns value of strain HN31-3, HN31-6 and HN40-5 were 2.8 mL, 3.2 mL and 2.8 mL, respectively; Diacetyl content of strain HN31-3, HN31-6 and HN40-5 were 0.0778 mg/L, 0.0627 mg/L and 0.0838 mg/L, respectively; Higher alcohol content of strain HN31-3, HN31-6 and HN40-5 were 61.90 mg/L, 60.12 mg/L and 85.97 mg/L, respectively; Acetaldehyde content of strain HN31-3, HN31-6 and HN40-5 were 8.53 mg/L, 9.05 mg/L and 7.31 mg/L, respectively.

The fermentation tests of strain HN31-3、HN31-6 and HN40-5 were conducted in the 500 L fermentor. The results after fermentation for 8 days indicated that formentation degree in the fermented liquid of strain HN31-3、HN31-6 and HN40-5 were 69.2%, 69.7% and 67.7%, respectively; Diacetyl content of strain HN31-3、HN31-6 and HN40-5 were 0.0503 mg/L, 0.0583 mg/L and 0.0302 mg/L, respectively; Total higher alcohols content of strain HN31-3、HN31-6 and HN40-5 were 57.7 mg/L、62.5 mg/L and 60.8 mg/L；Acetaldehyde content of strain HN31-3、HN31-6 and HN40-5 were 7.17 mg/L、6.07 mg/L and 2.41 mg/L.

The cell volume, biomass, DNA content of strain GR5, GR8, HN31-3, HN31-6 and HN40-5 were measured in comparison with their parental strains and the results showed that strain GR5, GR8, HN31-3, HN31-6 and HN40-5 were fusants. The stability of these strains were also studied. The results showed that stability of these strains were excellent. The conditions influencing flocculence of strain H38 were primarily studied.

Key words: brewer's yeast; mutagenesis breeding; protoplast fusion; formentational property; pilot scale

前 言

一 啤酒的营养作用

啤酒是一种酒精含量很低的饮料，它不仅干冽爽口，还含有蛋白质、碳水化合物、维生素、矿物质等丰富的营养成分，是一种平衡性很好的饮料。啤酒的蛋白质中含有 12%～22%的必需氨基酸，维生素也较丰富，有 B_1、B_2、B_{12} 以及烟酸等，矿物质以钙和磷为多。因此，早在 1971 年国际营养学会就将啤酒列为营养食品，人们也常把啤酒称为“液体面包”。在德国有的把啤酒列入病人膳食中；而在英国有些妇产科医院则把啤酒作为产妇必喝的饮料[1]。

啤酒不仅含有人体所必需的营养成分，啤酒的风味即啤酒的香气、苦味、碳酸气体等则形成了啤酒独特的嗜好特性。啤酒的风味除了使人们的感官得到一种愉悦的享受外，这些风味成分还与其他成分共同构成了啤酒的生理功能。适度的饮用啤酒能够缓解精神紧张、消除精神压力，同时还能促进胃液分泌、增进食欲。由于啤酒具有利尿作用，因此，啤酒对肾脏及尿道结石也有一定的疗效作用。

啤酒是一种历史悠久的低酒精饮料，自古就被认为是一种营养丰富且安全性很高的天然饮料。作为一种饮料，啤酒具有双重性质，既能致醉，也能使人解渴，因此，啤酒是一种具有酒和饮料双重功能的广范围的饮品[2]。

二 啤酒酿造业在国民经济中的重要作用和发展前景

啤酒作为具有悠久历史传统的产品和成熟的行业，在我国得到快速发展。产量从 20 世纪 80 年代初的 7，8 十万吨到 20 世纪 90 年代初的 600 多万吨，再到 2002 年的 2300 多万吨，逐年大幅度提高；生产企业也经历了由数量的剧增和自身规模的扩大阶段到企业的规模化、集团化的发展阶段。