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THIAISOLEUCINE RESISTANT MUTANTS IN SACCHAROMYCES CARLSBERGENSIS INCREASE THE CONTENT OF D-AMYL ALCOHOL IN BEER

by

JØRN DALGAARD MIKKELSEN[1], POUL SIGSGAARD[2], ARNE OLSEN[2], KENNETH ERDAL[2], MORTEN C. KIELLAND-BRANDT[1], and JENS G. LITSKE PETERSEN[1]

[1]Department of Physiology, Carlsberg Laboratory and [2]Department of Brewing Chemistry, Carlsberg Research Laboratory, Gamle Carlsberg Vej 10, DK-2500 Copenhagen, Valby

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Four presumably dominant thiaisoleucine resistant mutants of a brewing strain of Saccharomyces carlsbergensis were previously shown to produce more D-amyl alcohol than the parent strain when grown in synthetic minimal medium with aeration. In the present work an increased production of D-amyl alcohol was also found in fermenting wort under brewing conditions resulting in a beer with an altered ratio between D-amyl alcohol and isoamyl alcohol. The synthesis of 2,3-pentanedione plus its precursor, α-aceto-α-hydroxybutyrate, was generally higher in the mutants. The mutants showed a somewhat lower rate of increase in cell concentration during fermentation than the parent strain. With respect to other analytical data and taste, the three mutants isolated after moderate mutagenesis did not show deviations outside the range of the parent. One mutant obtained from a more heavily mutagenized culture deviated in several respects.

1. INTRODUCTION

An earlier report (6) described the isolation and some characteristics of thiaisoleucine resistant mutants in a non-mating, non-sporulating Saccharomyces carlsbergensis brewing strain. In comparison to the wild type the mutants had a threonine deaminase with a lower sensitivity to feed-back inhibition by L-isoleucine. All four

Abbreviation: GLC = gas-liquid chromatography.

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studied mutants synthesized 2–5 times more 2-methyl-1-butanol (D-amyl alcohol) than the parent strain during aerobic growth in minimal medium. In the present study, pilot beer fermentations with the four mutants and their parent strain were carried out in order to find out whether increased production of D-amyl alcohol would take place also in wort and whether formation of vicinal diketones and their precursors would be affected by the mutations.

2. MATERIALS AND METHODS

2.1. Strains

The strain referred to as parent is the brewing strain of Saccharomyces carlsbergensis used in previous studies (4, 6). The thiaisoleucine resistant mutants C77–T226, C77–T230 and C77–T232 (6) were derived from the parent strain by selection after mutagenesis to a survival of 10%, while C77–T70 was derived from a culture mutagenized to a survival of 0.01%.

2.2. Pilot brewing and analyses of yeast and beer

Propagation of yeast in sterile hopped pilsner wort and pilot beer brewings using the five strains, as well as analyses of yeast and beer, were carried out as described earlier (1, 2, 4). Vicinal diketones and their precursors were measured by gas-liquid chromatography (GLC) as described by HAUKELI and LIE (5). D-amyl alcohol, isoamyl alcohol and isobutanol in bottled beer were determined by GLC as described (6). However, because of the larger amounts of fusel alcohols present in beer, chloroform extractions were not necessary. Instead, 5–20 µl of beer were injected directly into the chromatograph.

3. RESULTS

3.1. Fermentation

The four mutants and the parent strain were propagated on wort and added to 28 kg of wort at a pitching rate of 10×10^6 cells per ml. Propagation and fermentation were carried out in duplicate for the parent strain (fermentations a and b), while the mutants were investigated in single experiments. The fermentations were followed by counting cells in suspension (Figure 1). The cell concentration of the parent strain is seen to increase faster than the mutants, reaching 50×10^6 cells per ml in 6 days. The mutants C77–T70, C77–T226 and C77–T230 increased to about 30×10^6 cells per ml, while C77–T232 was intermediate. The brews of the parent and mutant strains were cooled after 144 and 160 hours, respectively, and racked after 160 and 168 hours. During the last day of fermentation the cell concentration of C77–T70 dropped, while that of the 3 other mutants continued to increase. This behaviour of C77–T70 was a result of increased flocculence during fermentation and storage, since total yield of yeast was not similarly affected.

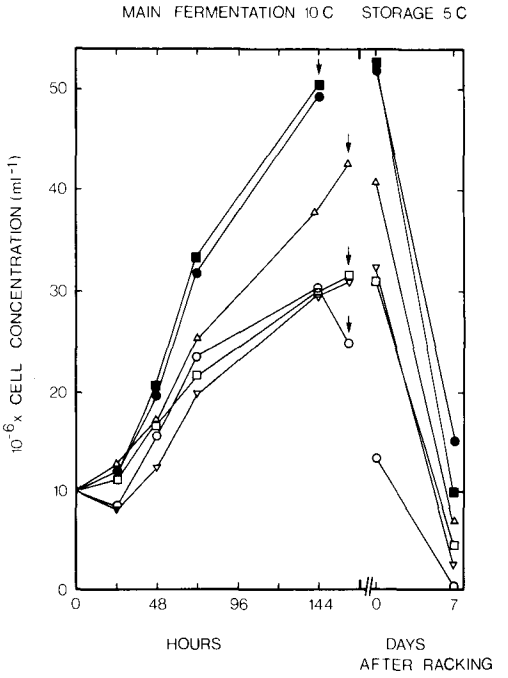

Figure 1. Cell concentration in suspension during main fermentation and storage. ■, parent strain, fermentation a; ●, parent strain, fermentation b; ○, mutant C77–T70; □, C77–T226; ▽, C77–T230; △, C77–T232. Arrows designate the time of cooling.

3.2. Analytical data of the pilot brews

Analyses of the final beer of the six brews are given in Table I. The taste panel described the brew (a) of the parent strain and that of C77–T230 as normal, brew (b) of the parent strain

Table I.

Comparison of pilot brews with 4 thiaisoleucine resistant mutants and their parent strain.

Analysis of bottled beer	Parent Fermentation a	Parent Fermentation b	Mutants C77–T70	Mutants C77–T226	Mutants C77–T230	Mutants C77–T232
Original extract, % Plato	10.4	10.4	10.3	10.4	10.3	10.4
Alcohol, % wt/wt	3.57	3.62	3.28	3.62	3.60	3.56
Attenuation, real, %	67	68	62	68	68	67
Bitterness, EBU	19	22	20	19	21	19
pH	4.09	4.13	4.12	4.11	4.08	4.10
Viscosity, mPa·s	1.52	1.51	1.52	1.51	1.51	1.51
Head retention, s	88	87	85	91	90	89
Initial haze, EBC	0.4	0.4	0.4	0.4	0.4	0.4
Total haze, 0 °C after 5 days at 60 °C, EBC	6.4	6.4	6.5	6.6	7.4	6.3
Colour, EBC	5.5	5.0	5.5	5.0	5.5	5.0
CO_2, % wt/wt	0.50	0.52	0.50	0.53	0.54	0.50
Total N, $mg \cdot l^{-1}$	380	380	420	400	380	380
Characterization by Taste Panel (average score) [a]	2.4	1.6	0.9	1.7	2.3	1.6

[a] 3 = normal, 2 = acceptable, 1 = not acceptable, 0 = with grave faults

Table II.

Fusel alcohols in final beer (ppm)[a].

Strain	Isobutanol	D-amyl alcohol	Isoamyl alcohol	D-amyl/Isoamyl
Parent, fermentation a	9.5 ± 0.7	13.0 ± 0.8	46.7 ± 2.2	0.273 ± 0.006
Parent, fermentation b	9.0 ± 0.4	12.7 ± 0.8	43.7 ± 1.1	0.290 ± 0.008
C77–T70	10.9 ± 0.6	22.3 ± 1.2	40.1 ± 1.9	0.555 ± 0.013
C77–T226	8.9 ± 0.3	16.7 ± 0.8	43.6 ± 0.9	0.382 ± 0.012
C77–T230	8.5 ± 1.1	15.6 ± 0.6	47.6 ± 1.9	0.317 ± 0.015
C77–T232	8.0 ± 0.6	20.1 ± 0.7	47.6 ± 0.6	0.420 ± 0.010

[a] The figures represent mean and standard deviation from 3 injections into the gas chromatograph.

and those of C77–T226 and C77–T232 as not quite satisfactory and that of C77–T70 as not acceptable and diacetyl like. The analytical data are very similar for the parent strain and the mutants C77–T226, C77–T230 and C77–T232, while C77–T70 has a lower attenuation.

Table II shows the concentrations of three fusel alcohols in the six beers. Increased amounts of D-amyl alcohol were found with the 4 mutants as compared to the parent strain, both when expressed in absolute amounts and as the ratio to isoamyl alcohol. The concentration of isobutanol was similar in all 6 beers.

In Table III are given the contents of vicinal diketones and their precursors at racking and in the final beer. Low levels of free diacetyl and 2,3-pentanedione were found with the mutants C77–T70 and C77–T226 at racking. In the final beer similar low levels of the free diketones were found in all brews. The contents of α-acetolactate and α-aceto-α-hydroxybutyrate decreased in all brews during lagering. However, beer from C77–T70 had higher levels of the two acids than the parent, while the other mutants showed levels as low as the parent. The amounts of 2,3-pentanedione plus its precursor, α-aceto-α-hydroxybutyrate, were generally higher in the mutants than in the parent. When comparing the individual mutants, the increase in 2,3-pentanedione and its precursor (Table III) correlated with the increase in D-amyl alcohol (Table II).

Table III.

Vicinal diketones and their precursors at racking and in the final beer (ppm).

	Parent Fermentation		Mutants			
	a	b	C77–T70	C77–T226	C77–T230	C77–T232
At racking:						
Diacetyl	0.31	0.19	0.05	0.06	0.24	0.36
2,3-pentanedione	0.08	0.07	0.01	0.03	0.08	0.12
α-acetolactate	0.50	0.67	0.58	0.79	0.77	0.38
α-aceto-α-hydroxybutyrate	0.25	0.26	0.41	0.45	0.29	0.20
Final beer:						
Diacetyl	0.06	0.05	0.05	0.05	0.06	0.05
2,3-pentanedione	0.02	0.02	0.04	0.03	0.02	0.02
α-acetolactate	0.073	0.064	0.127	0.046	0.063	0.081
α-aceto-α-hydroxybutyrate	0.006	0.007	0.035	0.007	0.007	0.013
2,3-pentanedione plus α-aceto-α-hydroxybutyrate	0.026	0.027	0.075	0.037	0.027	0.033

4. DISCUSSION

D-amyl alcohol is formed by decarboxylation and reduction of α-keto-β-methylvalerate, which is the last intermediate in the biosynthetic pathway converting threonine to isoleucine. The increased production by the mutants of D-amyl alcohol previously found to take place in minimal medium under aerobic conditions (6) was in the present study also observed in fermenting wort under brewing conditions. We take it to be an effect of the weaker feed-back inhibition of the pathway by isoleucine. Since the precursor for 2,3-pentanedione is another intermediate in this pathway, the observed increase of 2,3-pentanedione and its precursor may also be an effect of the weaker feed-back inhibition.

Threonine deaminase may play a direct role in the regulation of the synthesis of other enzymes in the pathways leading to isoleucine and valine (3). We have therefore considered the possibility that altered amounts of diacetyl and its precursor, α-acetolactate, may be attained by mutation to thiaisoleucine resistance. The present study has not given an answer to this question. The mutants C77–T70 and C77–T226 did have a decreased concentration of diacetyl at racking, but the amounts of its precursor α-acetolactate, was surprisingly high in the final beer from mutant C77–T70 as compared with the parent strain. It is not clear whether these alterations can be ascribed to the changes in the gene for threonine deaminase or to other mutations.

With regard to other analyses and taste of the beer, the mutants, except C77–T70, gave normal data compared to the parent. The mutants did not reach as high a cell concentration during the main fermentation as the parent strain, but a normal degree of fermentation was achieved. The deviating behaviour of C77–T70 is likely to be a result of mutations in other genes than the structural gene for threonine deaminase, since it was isolated after mutagenesis to a survival of about 10^{-4}.

In conclusion, we have found that mutations in brewer's yeast can cause alteration of the concentration of flavor constituents of the beer in a biochemically predicted way, without significantly altering other measured beer characteristics. The mutations are presumably dominant and may be of use as selective markers in the cross-breeding of asexual brewer's yeast with the aid of protoplast fusion.

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