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BREWING PERFORMANCE OF A YEAST AFTER PROLONGED GROWTH ON A SYNTHETIC MEDIUM

by

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Two clones of brewers yeast, grown for more than 300 cell generations on a synthetic minimal medium were propagated on wort and used in pilot brewing experiments. Parallel brews with wort cultured yeast were carried out for comparison. Analytical data showed no differences between beer brewed with yeast grown on minimal medium and wort cultured yeast. One out of the two clones grown on minimal medium gave a beer with a somewhat inferior taste.

1. INTRODUCTION

Yeast stocks used in beer fermentation are usually maintained either freeze-dried or on complex media (4), and to our knowledge there has been made no study of the brewing performance of yeast that has been grown for a long time on synthetic medium.

We have addressed ourselves to this question, since the development of breeding methods for brewers yeast may at many stages depend on selection for prototrophy on minimal media. It is not evident that prolonged growth on synthetic medium can be done without adverse effects on the brewing performance. The selection pressure may be quite different and result in the selection of unwanted variants formed by mutation or somatic crossing-over.

Here we report on the brewing performance of yeast grown for more than 300 cell generations on synthetic medium.

2. MATERIALS AND METHODS

2.1. Strain and media

The yeast was Saccharomyces carlsbergensis, Culture Collection of The Carlsberg Breweries, strain 244. Hopped pilsner wort was obtained from The Carlsberg Breweries. The synthetic minimal medium (5) contained per liter: 1 g $(NH_4)_2SO_4$, 0.875 g KH_2PO_4, 0.125 g K_2HPO_4, 0.500 g $MgSO_4 \cdot 7H_2O$, 0.10 g NaCl, 10µg $CuSO_4 \cdot 5H_2O$, 10 µg KI, 50µg $FeCl_3 \cdot 6H_2O$, 70 µg $ZnSO_4 \cdot 7H_2O$, 0.10 g $CaCl_2 \cdot 2H_2O$, 2 µg biotin, 400 µg thiamin, 400 µg pyridoxin, 2 mg inositol, 400 µg Ca-pantothenate, 20 g dextrose. Media for plates were solidified with 2% Bacto agar.

2.2. Prolonged growth on synthetic medium

Yeast cells were streaked on synthetic minimal medium, which was incubated at 30°C for two or three days. Cells from an isolated colony were transferred to a new minimal plate, and this procedure was repeated 18 times. Assuming at least 18 cell generations for the growth of a single cell into a colony this corresponds to more than 300 cell generations.

2.3. Propagation

Propagation for the pilot brewing was carried out by successive inoculations of larger and larger volumes of sterile wort: Ten ml were inoculated with approximately 10^6 yeast cells; after two days, when fermenting vigorously, the culture was transferred into 300 ml wort, which amount after four days was poured into 12 liter wort and allowed to ferment out (5 days). The temperature ranged from 27°C at the initial step to 20°C at the end of propagation. The temperature was then lowered to 10°C to allow the yeast to settle.

2.4. Pilot brewing

The pilot experiments were carried out in our pilot brewery (1). For each of the propagated yeast samples 28 kg portions of hopped pilsner wort (10.3% Plato) were pitched. After fermentation for one week at 10°C, the beer was racked into a 25 l storage tank with CO_2 pressure and placed in a refrigerated cabinet. During 6 weeks of storage, the beer temperature was decreased gradually from 5°C to -1°C. The beer was filtered through 20 x 20 cm Seitz K-7 sheets and the CO_2 content adjusted to 0.5%. Bottling and crowning were carried out manually, the bottles being knocked to reduce the air content in the headspace. The bottled beer was pasteurized at 62°C for 20 min.

2.5. Analyses of yeast and beer

The amount of yeast cells was counted on a Coulter Counter, Model D, and the percentage of dead cells determined with methylene blue.

Beer analyses were done according to ANALYTICA-EBC specifications (2) except for head retention, which was determined after BLOM (3).

3. RESULTS AND CONCLUSION

The yeast stock was streaked on wort agar, and two isolated colonies were taken for the parallel series of experiments outlined in Fig. 1. After more than 300 cell generations of growth on synthetic medium the yeast was propagated for pilot brewing, as was yeast from the original stock and from the two clones on the wort agar plates.

Since the fermentation temperature during the propagation was higher than that used in lager brewing, the physiological state of the yeast just after the propagation procedure could not be considered representative. In order to obtain a more representative pitching yeast, each of the five propagated yeast cultures was used in an adapting pilot brew and harvested after fermentation for pitching the experimental brew. Beer from the five adapting brews and the five experimental brews was bottled and analyzed. The results from the experimental brews are presented in Table I. The results from the adapting brews turned out to be very similar to these and are not described in detail. At pitching the experimental brews contained between 13 and 20 million cells per ml, of which between 15 and 25% were scored as dead.

The analytical data of the Table are all within

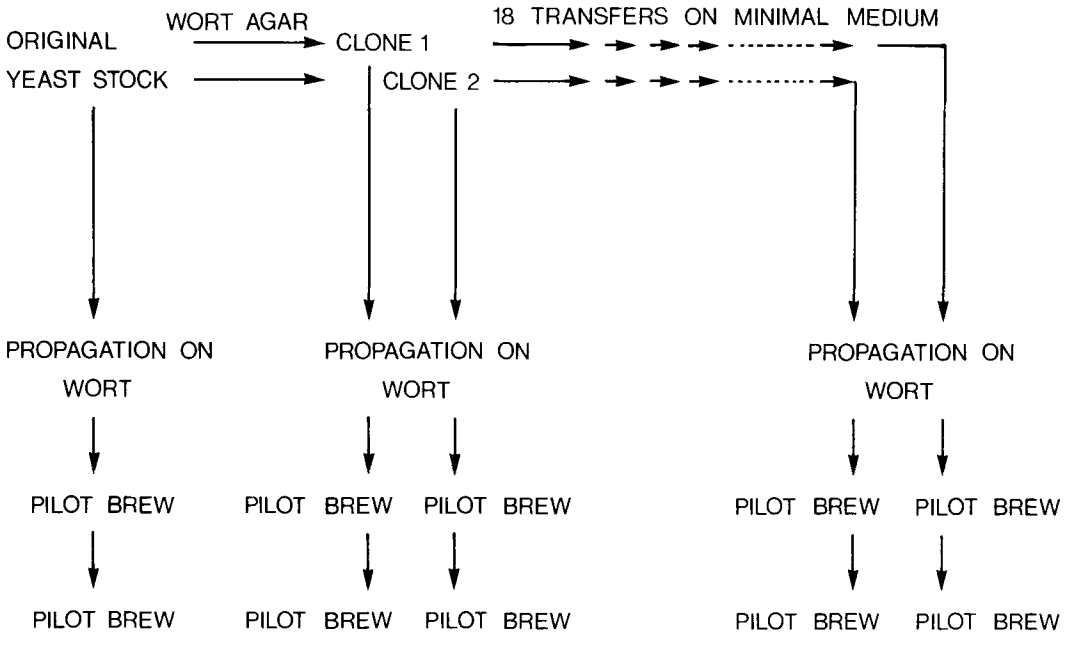

Figure 1. Flow diagram of the experiments.

Table I

Comparison of pilot brews with Saccharomyces carlsbergensis, strain 244, cultured on different media.

Analysis of the bottled beer	Original stock	Clone 1 on wort plate	Clone 2 on wort plate	Clone 1 on minimal medium	Clone 2 on minimal medium
Real extract, % Plato	3.81	3.86	3.86	4.07	3.92
Alcohol, % wt.	3.38	3.24	3.28	3.30	3.44
Attenuation, real %	63	62	62	61	63
Bitterness units	17	16	17	17	18
pH	4.2	4.2	4.2	4.3	4.2
Viscosity, mPa·s	1.57	1.55	1.55	1.57	1.57
Head retention, seconds	80	84	83	87	83
Diacetyl, ppm	0.13	0.15	0.13	0.12	0.16
SO_2, ppm	11	11	10	6	14
Total N mg/l	400	400	400	420	400
Flavour profile:	Within acceptable range of pilot brews	Within acceptable range of pilot brews		Within acceptable range of pilot brews	Slightly aberrant taste

the usual variation of pilot brews. The taste panel classified one of the beers brewed with the yeast grown on synthetic medium as slightly inferior and the other as normal.

The number of generations of the yeast cells on minimal medium in the present experiments exceeds that which would usually be involved in selection steps during breeding work. The results reveal that prolonged growth on minimal medium need not cause selection for inferior brewing performance. In breeding methods for brewers yeast selection steps on minimal or other synthetic media thus appear acceptable.

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