Research article

Acid whey as a medium for cultivation of conventional and non-conventional yeasts

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Abstract: *The aim of this study was to investigate the capacity of different yeast strains to grow on acid whey and assimilate different carbon sources. Twenty different yeasts (with GRAS status) were tested, sourced from the LOCK105 Culture Collection (Poland) and the National Collection of Yeast Cultures (Great Britain). The acid whey, from cows was characterized in terms of it main chemical components (carbohydrates, organic acids, aminoacids, FAN, total nitrogen) using HPLC techniques and chemical methods. The best producer of biomass (of conventional yeast strains) was S. cerevisiae Ja64 (biomass yield 1.16 g of dry mass/l), while among non-conventional yeasts the highest increase of biomass showed the strain D. hansenii, reaching the biomass yield of 1.12 g of dry mass/l.*

Keywords: *acid whey, yeasts, growth, assimilation.*

Introduction

Whey is a by-product of the dairy industry, and for years has been used either as animal feed or disposed of as waste. It can be problematic to dispose of whey for two main reasons. Firstly, its BOD5 (the amount of O2 in milligrams required to oxidize the organic load per liter of whey, within five days) is high, at around 35.000-55.000 mg O_2/l. This makes waste water treatment expensive, particularly in the case of small cheese plants. The second point is related to the quantity of whey produced annually: over 160.000.000 tons worldwide. Given the logistical, economic and environmental costs, it is clearly preferable to find ways of utilizing whey. One solution that has been proposed is to produce ethanol on original whey containing a small percentage of lactose. However, this process demands a large amount of energy, resulting in high costs, because of the low ethanol yield [1]. The cultivation of microorganisms on cheese whey has been offered as an alternative [2, 3], since this can reduce its BOD by 90-95% [4], resulting in high added-value bio-ingredients for the food industry [5]. Besides the basic sugars lactose and galactose, whey also contains vitamins and minerals which improve the physiological activity of cultivated cells. The yield of lactose

to biomass can reach 50-57% [6], and can be optimized by supplementing the culture media with 0.1-5% yeast extract [7, 8].

Yeast strains can be divided into three groups on the basis of lactose uptake. Group I contains yeasts able to ferment lactose, glucose and galactose, capable of extracellular lactose hydrolysis and of apparent facilitated diffusion of glucose and galactose. Group II consists of yeasts which are unable to ferment lactose, while glucose and galactose are fermented and transported by an apparent proton symport. Group III contains strains capable of fermenting lactose, glucose and galactose, transporting lactose by an apparent proton symport mechanism and of extracellular hydrolysis of lactose. The transport modes for glucose and galactose are variable [9].

To achieve good utilization of sugars from whey, it is especially important to choose a microbial strain with suitable physiological characteristics. Therefore, suitable yeast strains should be evaluated not only on the basis of different assimilation/fermentation criteria, but also according to their aroma profiles and sensitivity towards changeable environmental conditions.

This work presents the results of a study into the capacity of different yeast strains to grow on acid whey and assimilate different carbon sources. The twenty yeast strains with GRAS status had been tested in previous research to show their potential for cultivation on acid whey [10, 11].

Experimental

Yeasts

Half of the yeasts (10) were conventional, fermenting strains, belonging to the genus *Saccharomyces* (brewery, baker's, wine or distillery yeasts). The other non-conventional yeasts belonged to the genera: *Kluyveromyces, Candida, Pichia, Debaryomyces, Dekkera* and *Wickerhamomyces*. They were from the food industry as well as from two culture collections: LOCK105 Culture Collection (Poland) and the National Collection of Yeast Cultures (UK). The strains were maintained by monthly transfers on 2% malt extract agar slants and stored at 4^{o}C.

Cultivation

The basal culture medium was acid whey from cow milk, filtered and sterilized using microfiltration with 0,45-µm-pore-size membranes (Millipore. The natural pH of the acid whey was 4.0. However, acid whey after pH correction to 5.0 was also used.

The inoculum (~ 5×10^7 CFU/ml) was the yeast cultures after cultivation in 25 ml Erlenmeyer flasks containing 10 ml wort broth (Merck) at 25^{o}C for 48 h on a rotary shaker at 110 rpm. Acid whey was inoculated (1% v/v) and incubated on a rotary shaker at 110 rpm at 25^{o}C for 48 h.

Analytical methods

Acid whey characteristics

Acid whey was characterized on the basis of its main chemical components including: carbohydrates, organic acids, amino-acids, free amino nitrogen (FAN) and total nitrogen concentration. The carbohydrate profiles was determined using enzymatic method (K-LACGAR 03/14 Megazyme). Free amino nitrogen concentration (FAN) was determined based on the color reaction of amino acids with ninhydrin and by absorbance measurements at a wavelength of 570 nm (Multiskan GO Microplate Spectrophotometer, Thermo Fisher Scientific) [12]. Total nitrogen concentration was measured using the Kjeldahl method [13]. The free amino acid content was evaluated using the HPLC method. For this assay, centrifugal ultrafiltration (Amicon Ultra-4 Centrifugal Filter Units, Merck) of whole acid whey was used to eliminate proteins exceeding a molecular weight 3 kDa. The samples (5 µl) were then transferred into glass tubes and evaporated to dryness in a vacuum Pico Tag Workstation (Waters). The free amino acids present in the dried sediment were converted into phenylthiocarbamide (PTC) derivatives [14]. The obtained compounds were dissolved in amino acid solvent, added in portions of (200 ml), and then 5µl of the solutions were analyzed using high pressure liquid chromatography (HPLC). Used equipment and reagents: Thermo Finnigan Surveyor HPLC System, diluent WAT088119, eluent A WAT052890, eluent B WAT088112, Pico Column 3.9x300 mm, time of analysis 20 minutes; Waters. Quantitative calibration had been performed using six different concentrations of amino acid standards.

Yeast biomass concentration

Yeast concentration was estimated by measuring the optical density at 540 nm (Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer, Thermo Fisher Scientific) and relating the readings to biomass dry weight with a calibration curve.

All experiments were conducted in triplicates and the standard deviation was calculated.

Results and Discussion

Whey is the raw material, which has still not been used enough. Whey contains galactose, lactose, vitamins and minerals which improve the physiological activity of yeast cells [1]. The aim of this study was to evaluate the ability of the growth of selected yeast in a medium composed of whey – a waste product of the dairy industry.

The whey samples used in this work contained lactose as the main carbohydrate (9.74 g/l), but substantial amounts of galactose (0.84 g/l) was also detected. These compounds may also be used by yeasts as their carbon source, maximizing the use of different sources of carbon and energy, and reducing the amount of waste. The concentration of total nitrogen in the tested acid whey was 588 mgN/l, of which the free amino acid (FAN) content comprised 101 mgN/l.

The whey also contained amino-acids – valuable sources of carbon and nitrogen for yeast strains. Six different amino acids were found: glutamine, proline, methionine, cysteine, isoleucine and leucine. The highest concentration was noted in cysteine at 0.49 mg/ml, the lowest for leucine at 0.02 mg/ml (Figure 1).

All amino-acids tested stimulated the growth of the yeast strains as well as being precursors of aroma compounds. Three of the detected compounds, methionine, isoleucine and leucine, are in addition exogenous amino-acids suitable for human consumption.

To reach a good utilization of whey, it is particularly important to choose a strain of yeast with suitable physiological characteristics. Biomass concentration of conventional yeast strains *Saccharomyces* spp. and non-conventional yeasts are shown in Figures 2 and 3, respectively.

Among tested conventional yeasts, strain Ja64 achieved the highest biomass concentration (1.16 g/l). Additionally, *Saccharomyces* L, TT, Elblag strains produced biomass above 1 mg / mL of acid whey. It was also noted that the strain *S. cerevisiae* 1183 was the weakest producer of biomass from acid whey.

The strain *D.hansenii* reached the highest yield of biomass (1.12 g/l) among non-conventional yeasts. Also strains belonging to *K.marxianus* 179 and 0028 showed high production of biomass in culture medium. The lowest biomass yield was obtained for *D.bruxellensis* C2 strain.

Generally, the conventional yeasts *Saccharomyces* spp. showed a greater yield of yeast biomass than the second group of tested yeasts. Patelski et al. [15] studied the same yeast species during cultivation in the sugar beet pulp hydrolysate supplemented with magnesium and nitrogen compounds. The concentration of the yeast biomass was then about 9 g d.m./l. It was 9 times more in comparison to the results obtained for the best producer biomass from acid whey. Despite this fact, we may state that tested acid whey, rich in both carbohydrates and nitrogen compounds, is a good substrate for yeast growth. On yeast biomass production, one variable is of major importance: carbohydrate concentration in the broth. *S.cerevisiae* and many other yeasts may thrive on a variety of carbon sources, but glucose and fructose are the preferred ones. When one of these sugars is present, the enzymes required for the utilization of alternative carbon sources are synthesized at low rates or not at all. This phenomenon is known as carbon catabolite repression [16]. Acid whey usually contains lactose and galactose only. Therefore, the capacity of yeast strain to grow on acid whey should take into account its assimilation profiles. Among disaccharides, lactose is one of the most refractory carbon substrate to most of the yeasts. According to the literature, only few yeast species are lactose positive [17]. The results presented in Table 2 showed that the utilization rates of the saccharides for conventional yeasts varied and were in the range 29-93%. Similar results were noted in the case of non-conventional yeasts, where the use of saccharides ranged from 38% to 84%. Galactose, as a monosaccharide, is easily assimilated by yeasts. In the study conducted by Champagne et al. [18]

S. cerevisiae assimilated galactose much more rapidly than other carbon sources. On the other hand, non-conventional yeasts assimilated lactose better than conventional ones. According to the literature, *K. marxianus* strains are able to assimilate lactose as a carbon source, that makes them good producers of biomass in these culture media [19].

Table 1. Yeasts used in the selection studies

No	Name	Origin
1	*Saccharomyces cerevisiae* (TT)	LOCK
2	*Saccharomyces cerevisiae* (1183)	NCYC
3	*Saccharomyces cerevisiae* (Ja64)	LOCK
4	*Saccharomyces cerevisiae* (Ethanol Red)	Lesaffre
5	*Saccharomyces cerevisiae* (BC16a)	LOCK
6	*Saccharomyces pastorianus* (Elblag)	LOCK
7	*Saccharomyces pastorianus* (1116)	NCYC
8	*Saccharomyces cerevisiae* (L)	LOCK
9	*Saccharomyces cerevisiae* Tokay	LOCK
10	*Saccharomyces cerevisiae* (winery – Lalvin V1116)	Lallemand
11	*Wickerhamomyces anomalus* (C1)	LOCK
12	*Dekkera bruxellensis* (C2)	LOCK
13	*Kluyveromyces marxianus* (0026)	LOCK
14	*Kluyveromyces marxianus* (0028)	LOCK
15	*Kluyveromyces lactis*	LOCK
16	*Candida utilis*	LOCK
17	*Pichia angusta* (495)	NCYC
18	*Debaryomyces hansenii*	LOCK
19	*Kluyveromyces marxianus* (179)	NCYC
20	*Pichia stipitis* (1541)	NCYC

Table 2. Utilization of saccharides by yeast during the process

	Yeast strain	Utilization [%]	
		Lactose	Galactose
Conventional yeast	*S. cerevisiae TT*	34	33
	S. cerevisiae NCYC 1183	51	91
	S. cerevisiae Ja64	39	91
	S. cerevisiae BC16a	55	88
	S. cerevisiae Etanol Red Lesaffre	36	29
	S. cerevisiae Elbląg	67	82
	S. cerevisiae NCYC 1116	69	55
	S. cerevisiae L	81	92
	S. cerevisiae Tokay	84	90
	S. cerevisiae (winery-LalvinV1116)	72	93
non-conventional yeast	*W. anomalus* C1	48	52
	D. bruxellensis C2	58	54
	K. marxianus 0026	52	53
	K. marxianus 0028	54	48
	K. lactis	84	76
	C .utilis	40	38
	P. angusta NCYC 495	54	50
	D. hansenii LOCK	67	45
	K. marxianus NCYC 179	74	63
	P. stipitis NCYC 1541	65	84

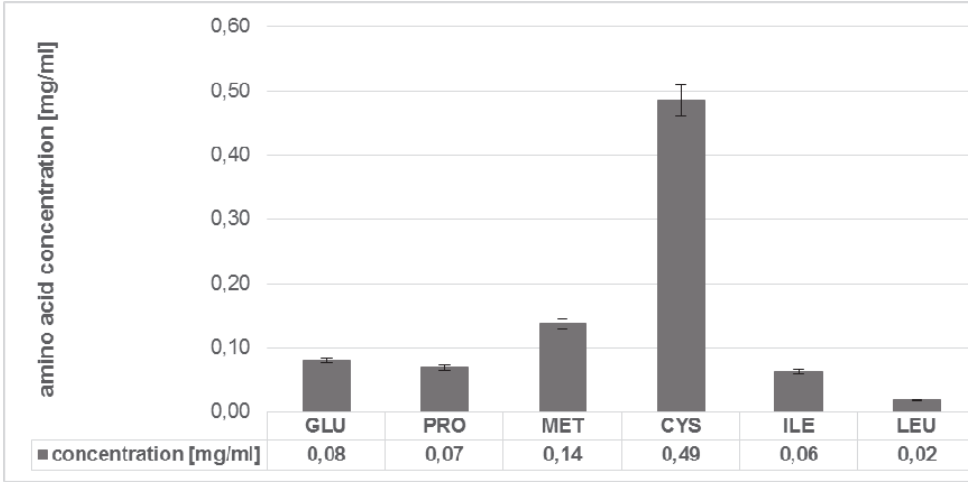

Figure 1. Amino-acid profile in whey culture medium

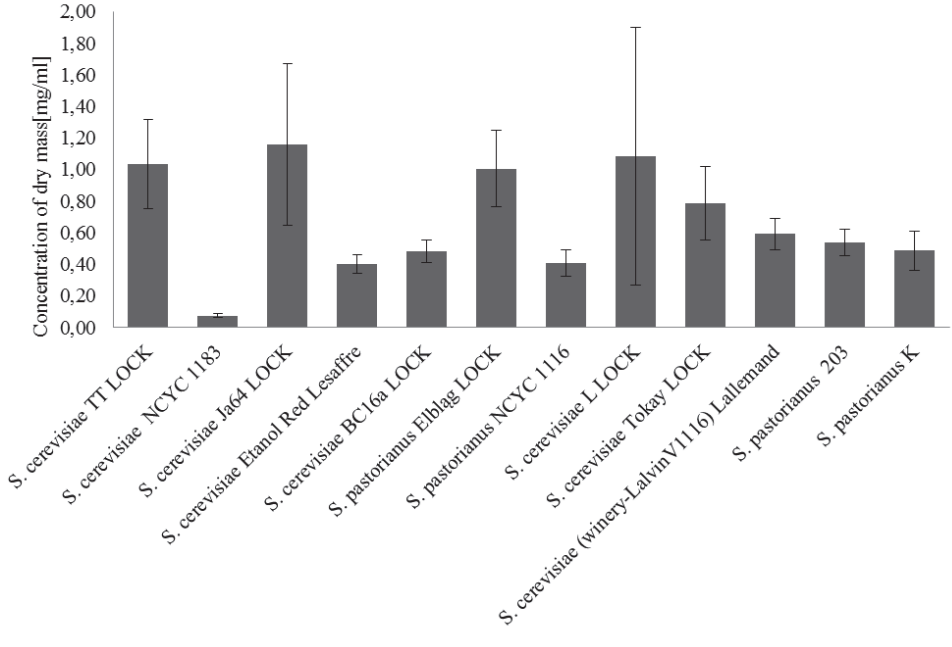

Figure 2. Biomass concentration of conventional yeast strains

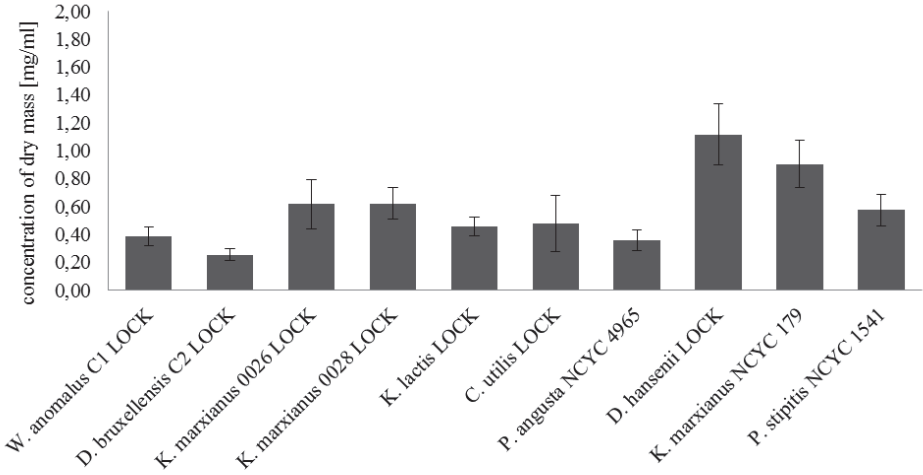

Figure 3. Biomass concentration of non-conventional yeast strains

Conclusions

The outcomes of our studies indicated that the biomass yield of yeasts cultivated in acid whey depends on the assimilation profiles of each strain. Acid whey, due to carbohydrate and nitrogen compounds, created favorable conditions to growth for some conventional and non-conventional strains. *K. marxianus, D. hansenii* as well as *S. cerevisiae* have the potential to be used for the production of yeast biomass on acid whey. These strains may be used as both monocultures and mixed populations to enhance the utilization of acid whey as a waste material. Utilization of non-conventional yeasts with broad assimilation spectra can allow to obtain yeast biomass rich in protein and amino acids, and other valuable products. However, further trials with these strains in mono- and mixed cultures are required.

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