Chapter 10

EFFECT OF COMPLEX NUTRIENTS ON THE PRODUCTION OF ACETIC ACID BY

Saccharomyces cerevisiae Y_{Bmax}

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Availability and type of nutrient can exert strong physiological control over acetic acid production and cell growth. In addition to providing a sugar source for ethanol production the fermentation mash must also provide the secondary nutrients necessary for cell maintenance, growth & acetic acid production (529) Presence of some essential growth factors in complex nutrients of cheap natural products or byproducts was found to have the stimulatory effect as in the former case. The basic nutritional requirements of micro-organisms were a carbon source, an available nitrogen source, inorganic elements and trace metals and these were optimized in the previous chapter. But the proposed synthetic medium is not economical and is rather unusual for large scale acetic acid production. For economic reasons pure glucose & sucrose can seldom be used as the sole carbon source, except in processes which demand exact fermentation control. It has been suggested that nutritional deficiencies rather than ethanol toxicity are responsible for the premature stoppages of fermentation. Large amount of fermentable sugar is often incompletely used during fermentation, but improvement resulting from supplementation with lipids, proteins & vitamins has been reported. Significant improvement of alcoholic fermentation as a result of addition of unsaturated fatty acids & sterols or lipid protein complex are also there (530,531), their effect being attributed to the enhancement of resistance of yeast cells to ethanol. This higher tolerance has been related to a more favorable membrane composition in terms of fatty acids & sterols (532). Hence a complex medium has to be employed. The effect of complex nutrients from various plant and animal origin like yeast extract, beef extract, malt extract, wheat bran extract, rice bran extract, paddy soak liquor, peptone, corn steep liquor, soybean meal were studied to explore the synergistic effect of various nutrients present in complex nutrients. These complex media also served as inexpensive sources of nitrogen and carbon with several amino acids vitamins and minor amount of trace elements.

In laboratory tests very rapid cell growth & ethanol production & high yields are achieved with a glucose medium supplemented with NH_4Cl, $MgSO_4$, $CaCl_2$ & yeast extract (533). Yeast extract is the water soluble extract of autolysed yeast & contains all necessary yeast growth factors: amino acids, purines, pyrimidines & vitamins as well as minerals. They are produced from baker's yeast through autolysis at 50 – 55^{0}C or through plasmolysis in high concentration of NaCl. Total nitrogen contents of yeast extract ranges from 7.4 – 8.8% and contains various other micronutrients.The glycogen & trehalose of yeast cells are hydrolyzed to glucose during yeast extract production. The composition of yeast extract varies, partly because the substrates used for yeast cultivation affect the quality of the yeast extract (534-536).

Peptones (protein hydrolysates) can be utilized by many microorganisms but they are relatively expensive for industrial application. Sources of peptones include meat, casein, gelatin, keratin, peanut seeds, soy meal, cotton seeds & sunflower seeds. In several studies we have found that in general, supplementation of the yeast growth media, containing maltose, glucose or fructose, with a more complex structural nitrogen source such as peptone, induced higher biomass accumulation and ethanol production (537-544).

Aeration of yeast cells for 24 hrs. prior to being placed in 0.1% sodium acetate solution diminished sporulation, but this decrease was overcome by the addition of 0.1% yeast extract to the acetate solution. Cells starved by growth on Czapek solution agar +0.03% peptone formed very few ascospores in acetate solution. The addition of yeast extract or peptone in low content to the acetate solution increased the yield. However, the cells did not generate as many ascospores as well-nourished cells do in acetate solution (545).

Low-hydrolysis soy flour is also a prospective low-cost nutrient. Soybean meal, the residue from soybeans after the extraction of soybean oil, is a complex substrate. It is a complex mixture of protein (50% dry basis), carbohydrates (sucrose, stachyose, raffinose, arabinoglucan, arabinan & acidic oligosaccharides with 30% fibres), 1% residual fat & 1.8% lecithin. It is a good source of nutrients for industrial fermentation, especially for antibiotics (546) Soya meal is frequently used in antibiotic fermentation, catabolic regulation does not occur because of the slow catabolism of this complex nutrient (547). The lipid material in the soy meal could

contribute to the stability of cell wall during fermentation, reducing the rate of cell lysis & allowing better fermentation. No fructose was detected with low-hydrolysis soy flour as the nutrient. Soy flour is a relatively inexpensive & abundant source of protein with lipid which can be easily assimilated into cellular materials. Broth supplementation with soy flour can increase the final ethanol concentration & the rate of fermentation (548-550).

Corn steep liquor contains soluble components leached out from the corn during soaking. It is rich in organic nitrogen (44-46% protein on a dry matter basis), with about half of it as free amino acids (L- alanine, arginine, glutamic acid, isoleucine, threonine, vaine, phenyl alanine, methionine & cysteine); the balance is small peptides with very little protein intact (551). It contains several important vitamins, trace elements & lactic acid (10-30% dry basis) at relatively high amounts. It has been industrially used as a nutrient in ethanol fermentation & for production of pharmaceuticals (552).

An aqueous extract of malted barley is an excellent substrate for many fungi, yeasts & actinimycetes. Dry malt extract consists of about 90-92% carbohydrates, & is composed of hexoses (glucose, fructose), disaccharides (maltose, sucrose), trisachharides (maltotriose) & dextrins. Nitrogeneous substances present in malt extract include proteins, peptides, amino acids, purines, pyrimidines & vitamins (553).

Whole paddy saccharification and fermentation (WPSF) was experimentally investigated by Fujieda et al. to produce domestic bio-fuel or ethanol from rice for animal feed. Fermentation resulted in increased ethanol production, while simultaneous saccharification and fermentation (SSF) produced an ethanol concentration and yield of 9% and 85%, respectively, using Baker's dry yeast, which was more economical than using Shochu dry yeast (554).

Fermentation of heat-stabilized defatted rice bran with yeast yields a prebiotic composition that can promote the growth &/or activity of beneficial intestinal bacteria (probiotic) when consumed by an animal or a human. The prebiotic &/or probiotic compositions can afford substances having desirable health effects when consumed. The yeast fermented &/or probiotic fermented extracts & residues will have several neutraceutical compounds including protein, phenolics, phytic acid, arabinose,

bioactive isoflavones, dietary fibres & several others that will have health benefits (555).

Since complex nutrients are a source of carbohydrates, nitrogen, amino acids, vitamins, and minerals, the effect of complex nutrients was studied to determine to see if any of these nutrients are associated with the growth and production of acetic acid by *Sacharomyces cerevisiae* Y_{Bmax}.

MATERIALS & METHODS:

Medium & Cultural conditions: Production of acetic acid was carried out to determine the effect of different complex nutrients on *Sacharomyces cerevisiae* Y_{Bmax}. For this purpose, the 85ml of the basal medium used in 100ml Erlenmeyer flask contained glucose-10.0%, KH_2PO_4-0.1%, $(NH_4)_2SO_4$-0.5% and $MgSO_4$, $7H_2O$-0.05%, Fe^{2+}= 10µg/ml, Mn^{2+}=10µg/ml & Zn^{2+}=10µg/ml. pH was adjusted to 4.5. The yeast cells were harvested by washing the slants with sterile distilled water. The cell density was adjusted to 2.05×10^5 cells/ml. 5ml of cell suspension was used for the inoculation of the fermentation medium. Fermentation was carried out at 30°C at B.O.D. incubator for 96 hrs using 48 hour old culture of *Saccharomyces cerevisiae* Y_{Bmax}. For the objectives mentioned above, all the factors were kept constant except the one, to be optimized.

Preparation of Complex Nutrients: The above mentioned complex nutrients were sterilized separately and added to the basal medium aseptically in varying concentration (w/v). Solutions of yeast extract, beef extract, malt extract, meat extract, peptone & tryptone were prepared by dissolving the required amount of those materials in double distilled water separately, sterilized and added to the above mentioned fermentation medium.

Other complex nutrients were prepared by the following method –

Preparation of Wheat bran and Rice bran extract: 20 gms of mud-free bran was taken in 200 ml of hot distilled water (55^0C) in a 500 ml beaker. The suspension was kept at 55^0C for 18 hours. The extract was filtered through cotton, sterilized at 15 lbs/square inch pressure for 15 minutes and stored at 4^0C. The solid contents of wheat bran extracts and rice bran extracts were 11.54% and 1.08% respectively.

Preparation of Paddy soak liquor: 300 gm paddy was thoroughly washed with distilled water to remove mud and other extraneous materials adhering to paddy grains. The washed paddy was then dipped into 300 ml hot distilled water (55^0C) and was allowed to soak water for 2 hours when the grains swelled completely. The soaked water was then filtered through several layers of cotton. The solution then sterilized and stored at 4^0C. The solid content of paddy soak liquor was 2.1% of the extract.

Preparation of Corn steep liquor: 200 gms of maize was taken in 250 ml of double distilled water containing 0.52% potassium metabisulphite in a 1000 ml beaker and heated over a controlled bath for 48 hours at a temperature of 50 – 55°C. The corn steep solution was filtered off and then concentrated by evaporation under vacuum. The concentrated solution was sterilized at 15 lbs/square inch pressure for 15 minutes and stored at 40°C. The solid content was 2.68% of the extract.

Preparation of Soybean Meal Extract: 100 gm of soybean were made fat free by alcohol distillation. This soybean was_taken in a 1L beaker containing 300 ml of hot (55^0C) double distilled water. This_suspension was kept at 55^0C for 18 hours. The extract was then filtered through cotton and sterilized at 15 lbs / square inch pressure for 15 minutes and stored at 40°C._The solid content of soybean meal was 4.6% of the extract (556).

After preparation of the extracts, to investigate the effect of different complex nutrients, varying amounts of each of them were added to the fermentation medium.

RESULTS & DISCUSSIONS:

Effect of Peptone on Acetic Acid production by an ethanol & temperature resistant *Saccharomyces cerevisiae* Y_{Bmax} :

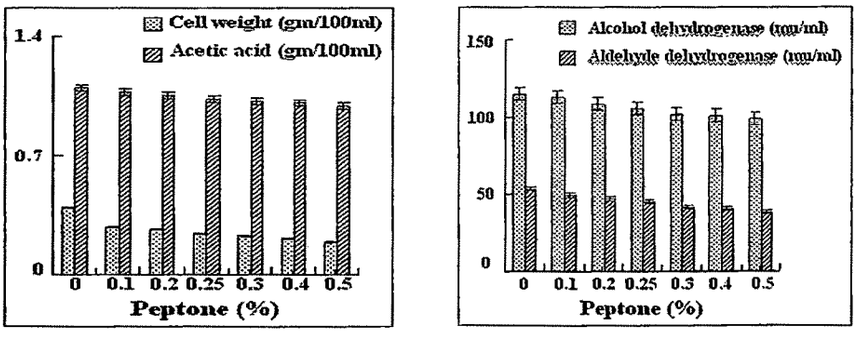

Fig.8a) Effect of different concentrations of Peptone on Acetic acid production and activities of Alcohol dehydrogenase and Aldehyde dehydrogenase

Fig.8a) shows that Peptone exerts an effective decline in cell growth, acetic acid production and activities of alcohol dehydrogenase and aldehyde dehydrogenase.

Effect of Soybean Meal on Acetic Acid production by an ethanol & temperature resistant *Saccharomyces cerevisiae* Y_{Bmax} :

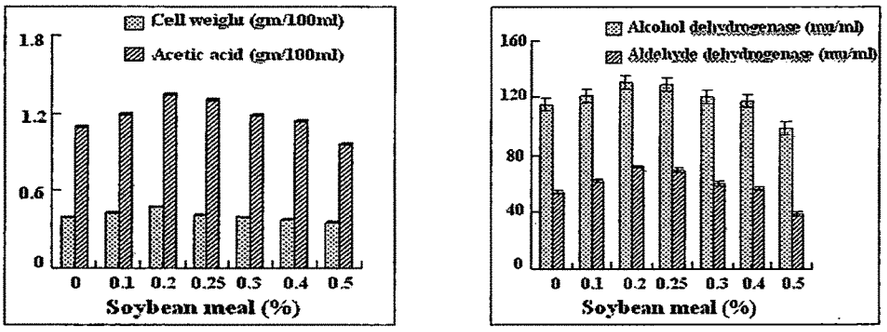

Fig.8b) Effect of different concentrations of Soybean Meal on Acetic acid production and activities of Alcohol dehydrogenase and Aldehyde dehydrogenase

Fig.8b) indicates that Soybean Meal has a significant positive effect on cell growth, acetic acid production and activities of alcohol dehydrogenase and aldehyde dehydrogenase.

Effect of Wheat Bran Extract on Acetic Acid production by an ethanol & temperature resistant *Saccharomyces cerevisiae* Y_{Bmax} :

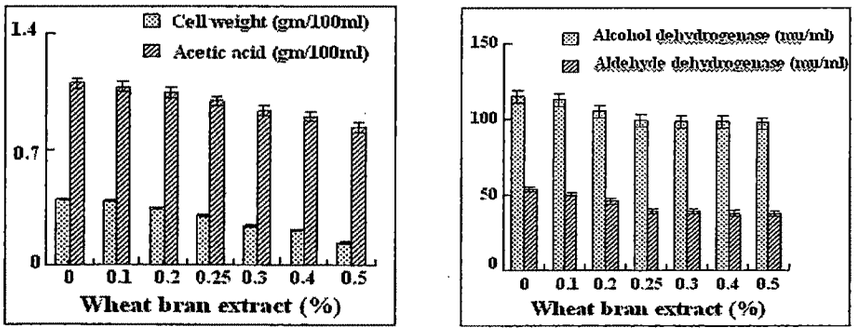

Fig.8c) Effect of different concentrations of Wheat Bran Extract on Acetic acid production and activities of Alcohol dehydrogenase and Aldehyde dehydrogenase

Fig.8c) depicted that Wheat bran extract has a negative influence on cell growth, acetic acid production and activities of alcohol dehydrogenase and aldehyde dehydrogenase.

Table 7: Effect of other complex nutrients on Acetic Acid production by an ethanol & temperature resistant *Saccharomyces cerevisiae* Y_{Bmax} :

Complex nutrients	Concentrations (%)	Cell weight (gm/100ml)	Acetic acid (gm/100ml)	Alc DH (mu/ml)	Ald DH (mu/ml)
Control	00	0.3953±0.003	1.0978±0.01	115.03±1.1	53.38±0.53
Yeast extract	0.10	0.3087±0.003	1.1184±0.01	117.73±1.1	55.62±0.43
	0.20	0.3692±0.003	1.1370±0.009	117.37±1.1	55.73±0.49
	0.25	0.4201±0.004	1.1544±0.01	117.59±1.1	56.70±0.51
	0.30	0.3642±0.003	1.1067±0.01	117.25±1.1	54.35±0.49
	0.40	0.3405±0.003	1.0927±0.009	114.53±1.1	52.87±0.45
	0.50	0.3014±0.002	1.0834±0.008	114.47±1.1	52.74±0.47
Meat extract	0.10	0.4124±0.004	1.1092±0.009	117.28±1.1	54.40±0.48
	0.20	0.4221±0.004	1.1132±0.01	117.70±1.1	55.54±0.51
	0.25	0.4407±0.004	1.1462±0.01	117.49±1.1	55.91±0.50
	0.30	0.4158±0.004	1.089±0.009	114.89±1.1	53.19±0.43
	0.40	0.3944±0.003	1.0694±0.008	109.99±0.8	49.99±0.41
	0.50	0.3576±0.003	1.0232±0.006	103.48±0.9	43.84±0.36
Malt extract	00	0.3953±0.003	1.0978±0.009	115.03±1.1	53.38±0.5
	0.10	0.3992±0.003	1.1081±0.01	117.21±0.8	54.35±0.52
	0.20	0.4137±0.004	1.1151±0.01	117.71±0.9	55.60±0.43
	0.25	0.4092±0.003	1.1068±0.01	117.15±1.1	54.30±0.51
	0.30	0.3973±0.003	1.0938±0.009	114.20±1.1	53.01±0.43
	0.40	0.3814±0.003	1.0822±0.006	114.15±1.1	52.90±0.42
	0.50	0.3721±0.003	1.0741±0.008	112.83±0.8	49.62±0.36
Beef extract	0.10	0.3991±0.003	1.0997±0.009	115.20±0.5	53.67±0.46
	0.20	0.4234±0.004	1.1229±0.01	117.87±1.1	55.78±0.52
	0.25	0.4420±0.004	1.1442±0.006	117.40±1.1	55.88±0.49
	0.30	0.4147±0.004	1.0989±0.008	115.24±1.1	53.42±0.50
	0.40	0.3923±0.003	1.0613±0.009	109.62±1.1	49.49±0.41
	0.50	0.3794±0.003	1.0470±0.007	107.87±0.9	47.54±0.41
Tryptone	0.10	0.4121±0.004	1.1035±0.01	114.15±0.7	45.27±0.4

Complex nutrients	Concentrations (%)	Cell weight (gm/100ml)	Acetic acid (gm/100ml)	Alc DH (mu/ml)	Ald DH (mu/ml)
	0.20	0.4237±0.004	1.1125±0.01	115.70±0.8	53.90±0.49
	0.25	0.4482±0.004	1.1303±0.008	117.31±1.1	55.69±0.5
	0.30	0.4501±0.004	1.1422±0.01	117.38±1.1	55.77±0.48
	0.40	0.3332±0.003	1.1207±0.01	117.31±1.1	55.73±0.47
	0.50	0.3046±0.003	1.1027±0.006	114.09±1.1	45.13±0.4
Paddy soak liquor	**0.10**	0.3721±0.003	1.0628±0.004	109.40±0.9	49.41±0.42
	0.20	0.3523±0.003	1.0158±0.01	101.50±0.8	41.56±0.39
	0.25	0.3020±0.003	1.002±0.006	100.67±0.9	40.76±0.4
	0.30	0.2891±0.002	0.9975±0.007	99.30±0.6	39.36±0.32
	0.40	0.2670±0.002	0.9715±0.008	98.60±0.6	38.21±0.33
	0.50	0.2347±0.002	0.9429±0.008	98.35±0.8	38.15±0.33
Corn steep liquor	**0.10**	0.4164±0.004	1.0636±0.01	109.76±0.9	49.67±0.41
	0.20	0.4258±0.004	1.0393±0.01	105.22±1.1	45.24±0.4
	0.25	0.4353±0.004	1.0134±0.01	101.55±0.9	41.55±0.31
	0.30	0.4427±0.004	1.0028±0.01	100.67±0.8	40.76±0.33
	0.40	0.4301±0.004	0.9803±0.009	98.67±0.7	38.25±0.35
	0.50	0.4141±0.004	0.9627±0.009	98.55±0.9	38.20±0.28
Rice bran extract	**0.10**	0.3882±0.003	1.0706±0.01	112.73±0.9	52.37±0.44
	0.20	0.367±0.003	1.0612±0.01	109.39±1.1	49.50±0.42
	0.25	0.3413±0.003	1.0423±0.01	107.62±0.9	47.26±0.4
	0.30	0.3313±0.003	1.0257±0.01	103.21±0.6	43.45±0.4
	0.40	0.3058±0.002	1.0028±0.01	100.67±0.4	40.76±0.36
	0.50	0.2741±0.002	0.9767±0.009	98.60±0.3	38.21±0.38

From Table 7, it is found that, these complex nutrients exert no significant effect on acetic acid production and activities of alcohol dehydrogenase and aldehyde dehydrogenase.

Production values are expressed as mean ± SD.

All values are biologically significant ($p<0.001$).

Results of cell growth, acetic acid production and enzyme activity in media containing different complex nutrients are presented in Fig 8a) to 8c) & in table 7 respectively. It was observed that, amongst the complex nutrients of plant origin, only soybean meal showed significant improvement in acetic acid production and growth rate, whereas four out of five complex nutrients of animal origin, showed no significant production and growth rate. The better yield was probably due to presence of certain nutrients in soybean meal. It is generally a rich source of nitrogen, water soluble vitamins, amino acids, minerals, other growth stimulants and approximately 4.6% solids.

It is observed that among the 3 complex nutrients from animal origin no promising acceleration in acetic acid production is found.

Among 6 complex nutrients from plant sources only Soybean meal is capable of stimulating the acid producing capacity of the organism used. Positive influence of a few non-commercial complex nutrients on filamentous fungi was observed by earlier workers (557). Soybean meal was also found to be a cheap and suitable nitrogen source (due to its high protein content) during feather degradation by bacteria.

Rice bran extract is reported to have growth inhibitory effect on *Aspergillus niger.*