Chapter 6

DETERMINATION OF SYNTHETIC MEDIA FOR THE PRODUCTION OF ACETIC ACID BY

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In most of the studies complex media are used for proper growth & function of yeast. But complex media have no definite composition, whereas a synthetic medium has clearly defined nutritional composition. Although the nutritional factors essential for yeast growth have been extensively studied, the yeast cell yields obtained on media of known composition have always been much lower than those attainable on a natural medium. In the present investigation yeast nutritional requirements studied under aeration conditions shown have been to be optimal. A synthetic medium has been found on which yields equal to those attainable on natural media can be obtained. The present work was undertaken to find a synthetic medium that would support yeast growth equivalent to that obtained on a natural medium (356). According to Bergman, synthetic defined (SD) medium, also known as minimal medium, supports the growth of yeast, which has no nutritional requirements (357). According to Saghbini et al., SD medium for yeast is also known as complete minimal and synthetic complete. It contains defined mixture of salts, vitamins, and a nitrogen source collectively known as yeast nitrogen base (358) to which a carbon source, usually dextrose, is added along with nutrient supplements consisting of various amino acids and nucleotide. Minimal medium is commonly used when testing the mating type of yeast cells using specific mating tester strains (of both mating types). Minimal medium is most often used as a basal medium to which mixtures of amino acids and nucleoside precursors are added. SD dropout medium lacks a single (or several) nutrient that allows selection for maintenance of particular plasmids or induction or repression of specific gene promoters. SD medium supports the vigorous growth of virtually all strains of *S. cerevisiae* with a doubling time of ~140 min during the exponential phase of growth (359,360). SD lacking nutrients is known as omission or dropout medium. It is used to select yeast containing vectors with specific nutritional markers. Fleming and Quinn (361) described a complicated chemically defined medium which supported the growth of *C. thermocellum.* According to Johnson et al., *C. thermocellum* requires

four vitamins (biotin, pyridoxamine, B12 and p-aminobenzoic acid), and these can replace the yeast extract of complex media to yield a minimal defined medium (362).

MATERIALS & METHODS:

Microorganism: The microorganism used in this study is alcohol, temperature & acetic acid resistant (2.5%) strain *Saccharomyces cerevisiae* Y_{Bmax} (same as Chapter 5).

Medium & Cultural Conditions: In this study we have compared acetic acid production between a complex & a synthetic medium. Table 3. indicates the composition of these two media & the cultural conditions maintained.

Estimation of Acetic Acid: Amount of acetic acid produced was measured by the procedure described in Chapter 4.

Assay of Alcohol Dehydrogenase: 0.1ml of supernatant was added to the mixture of 15mM β-NAD^+, 50mM sodium pyrophosphate buffer (pH 8.8) and 95% ethanol and incubated for 6min. at 25°C in a suitably thermostatted spectrophotometer. Absorbance was recorded at 340nm. Enzyme activity was expressed as mu/ml. One unit of enzyme activity represents the amount of enzyme which can convert 1.0µmole of ethanol to acetaldehyde per minute at pH 8.8 at 25°C (363).

Assay of Aldehyde Dehydrogenase: 0.1ml of supernatant was added to the mixture of 1(M) tris-HCl buffer (pH 8.0), 20mM β-NAD^+, 100mM acetaldehyde solution, 3(M) KCl and 1(M) 2-Mercaptoethanol and incubated for 5min. at 25°C in a suitably thermostatted spectrophotometer. Absorbance was recorded at 340nm. Enzyme activity was expressed as mu/ml. One unit of enzyme activity represents the amount of enzyme which can convert 1.0µmole of acetaldehyde to acetic acid per minute at pH 8.0 at 25°C in presence of β-NAD^+, potassium and thiols (364).

Table 3: Composition of the Complex & Synthetic Media used in Acetic acid production:

COMPLEX MEDIUM (BEFORE)	SYNTHETIC MEDIUM (AFTER)
Sucrose – 12%	Sucrose – 12%
$(NH_4)_2SO_4$ – 0.5%	$(NH_4)_2SO_4$ – 0.5%
KH_2PO_4 – 0.1%	KH_2PO_4 – 0.1%
$MgSO_4,7H_2O$ – 0.05%	$MgSO_4,7H_2O$ – 0.05%
Yeast extract-0.1%	pH-4.5
pH-4.5	Time of Fermentation- 72hours
Time of Fermentation- 72hours	

RESULTS & DISCUSSION:

The comparative study on the production of acetic acid & the activities of alcohol dehydrogenase & aldehyde dehydrogenase in both complex & synthetic media has been stated in Table 4.

Table 4: Comparison of Acetic acid production and activities of Alcohol dehydrogenase and Aldehyde dehydrogenase in Complex and Synthetic medium:

SYNTHETIC MEDIUM				COMPLEX MEDIUM			
Cell weight (gm/100ml)	Acetic acid (gm/100ml)	Alcohol dehydro genase (mu/ml)	Aldehyde dehydro genase (mu/ml)	Cell weight (gm/100ml)	Acetic acid (gm/100ml)	Alcohol dehydro genase (mu/ml)	Aldehyde dehydro genase (mu/ml)
0.1099 ± 0.003	0.2074 ± 0.006	53.70± 1.01	12.21 ± 0.2	0.1609 ± 0.005	0.2874 ± 0.008	60.77± 1.09	15.66±0.5

Production values are expressed as mean ± SD.

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

Here in Table 4, it is found that, in comparison to complex medium, the cell growth, acetic acid production and activities of both alcohol dehydrogenase and aldehyde dehydrogenase has decreased in synthetic medium. Acetic acid production decreases almost 27%, cell weight decreases 31%, activities of alcohol dehydrogenase and aldehyde dehydrogenase decreases 11% & 22% respectively. But as we know that, a complex medium has no definite composition of nutrients in comparison to a synthetic medium, in our following studies, it is our intention to increase the acetic acid production and activities of both alcohol dehydrogenase and aldehyde dehydrogenase using this synthetic medium to reach our desired result.