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Ethanol Fermentation by the Yeast Saccharomyces Cerevisiae NCIM-2012 Exposed to Mutagen 4-Aminobiphenyl

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ABSTRACT

This article shows how generational definitions or assessments of mutation have responded to the technologies added to science and the experiments that abounded with the enquiries of each successive generation. These observations are combined with an exploration of how the nonscientific public has shifted its understanding and concern about mutations over the past 150 or more years.

1. Introduction

Glycolysis is the anaerobic degradation of six carbon glucose molecules to two three carbon lactate molecules in the absence of molecular oxygen. It is also called HOMOLACTIC FERMENTATION. Homolactic fermentation is common in many microorganisms and in the cells of most higher animals and plants. Another closely related pathway is alcoholic fermentation which is the characteristic of many yeasts. Here glucose molecule is broken down into two molecules of two carbon compound, ethanol and two molecules of Co_2. The sequence of reactions involved was discovered by parallel studios on the two pathways by Buchner and Halden and young in the early part of this century and later by Embden, Meyerboff, Neuberg, Warburg and Cori.

The pathway of glycolys is and alcoholic fermentation is shown in figure I. In the first stage different hexoses enter into glycolysis by getting converted to the common product, i.e., glyceraldehyde-3-phosphate. These are energy requiring steps were ATP molecules are expanded to phosphorylate hexoses. In the second stage all the hexoses follow the same path i.e., glyceraldehyde is converted into lactate. The steps here are energy releasing and produce ATP.

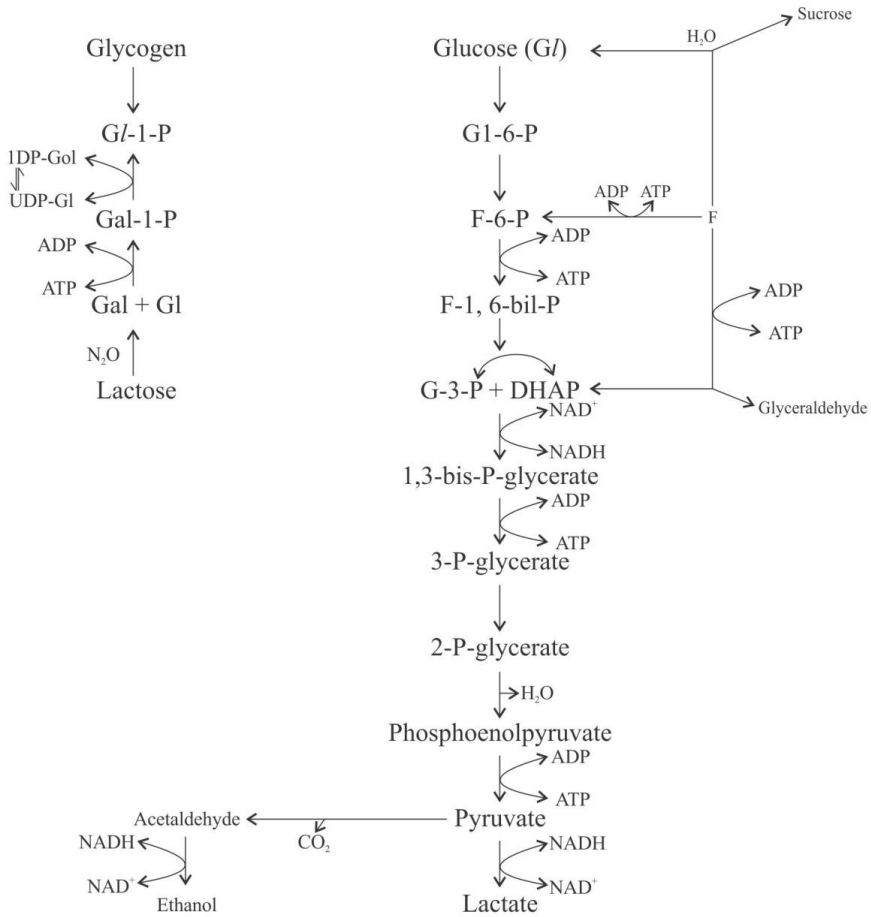


Figure I: Steps of glycolysis and alcoholic fermentation showing entry points for various sugars.

Abbreviations: Glucose, Gl; Fructose, F; Phosphate group, P; Phosphate ion, P; Glyceraldehyde-3-phosphate G-3-P; Dihydroxyacetonephosphate, DHAP; Galactose, Gel.

2. Mutation Breeding

"Mutation" is a description of a change in an individual gene and more precisely as some minute alteration of the DNA of that gene, especially a nucleotide substitution. But, the idea of mutation has changed considerably from the pre-mendelian concepts of Darwin's generation who viewed "fluctuating variations" as the raw material on which evolution acted, to today's up-to-the-minute genomic context of mutation.

After exploring Darwin's and Francis, Galton's concepts of mutation, Carls on shows how the 1900 rediscovery of Gregor Mendel's experiments let to a discontinuous model of evolution by mutation and how cytological investigations led to the chromosome theory of heredity of classical genetics in which there was random mutation in genes. Carison details how Mendelian and biometric approaches to heredity and evolution were closely related and how induction of mutations by radiation and chemical mutagens led to biochemical investigations of gene action, shifting attention to the chemistry of the gene. The interpretation of the gene as DNA and the deciphering of the genetic code then gave rise to molecular interpretation of mutation, views that also impacted evolutionary biology, population genetics, commercial development of plants and animals and human genetics.

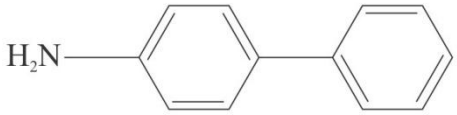
Mutation breeding is the process of exposing seeds to chemical or radiation in order to generate mutants with desirable traits to be breed with other cultivators. Plants created using mutagenesis are sometimes called mutagenic plants or mutagenic seeds. From 1930 to 2007 more than 2540 mutagenic plant varieties have been produced that have been derived either from direct mutation (70%) or from their progenic (30%) crop. Plants account for 75% of released mutagenic species with the remaining 25% ornamentals or decorative plants. However, it is unclear how many of these varieties are currently used in agricultural production around the world, as these seeds are not always identified or labelled as being mutagenic or having mutagenic provenance.

There are different kind of mutagenic breeding such as using chemical mutagens like EMS and DMS, radiation and transposons are used to generate mutants. Mutation breeding is commonly used to produce traits in crops such as larger seeds, new colours or sweater fruits that either cannot be found in nature or have been lost during evolution.

3. Ethanol Formation by Yeast when Exposed to Mutagen (4-Amino biphenyl)

Various chemical mutagens and some other mutagenic agents are used to produce mutants. If microbial population exposed to the effect of mutagens differs in cultural properties, then these mutations may be differentiated according to size, shape, structure and colour of the colonies. Biochemical properties of mutations are revealed by means of minimal media containing only salts and carbohydrates. Thus, it is concluded that a large number of mutagens have been employed to generate the mutants of different microbes but still there are some chemical mutagens whose influence on alcoholic fermentation by species of yeast saccharomyces cerevisiae have not been well studied and established.

Therefore, the present investigation the author has made an attempt to study the ethanol formation by the yeast Saccharomyces cerevisiae NCIM-2012 exposed to the chemical mutagen. 4-Aminobyphenyl (4-ABP).



4-ABP

4. Experimental:

The influence of 4-Aminobiphenyl on ethanol formation by yeast *Saccharomyces cerevisiae* NCIM-2012.

The composition of production medium for the ethanol formation by yeast *Saccharomyces cerevisiae* NCIM-2012 is prepared as follows:

Molasses	:	22%
Malt extract	:	1.75%
Yeast extract	:	1.75%
Peptone	:	1.75%
$(NH_4)_2 HPO_4$	:	1.25%
pH	:	5.0

Distilled water is used to make up the volume upto 100ml

The pH of the medium was adjusted to 5.0 by adding requisite amount of lactic acid. Now, the some production medium for ethanol formation by yeast *Saccharomyces cerevisiae* NCIM-2012 was prepared for 99 fermentor flask i.e. each containing 100ml of production medium. These fermentor-flasks were then arranged in 10 sets each comprising 9 fermentor-flasks. The remaining 9 fermentor-flasks out of 99 fermentor flasks. The remaining 9 feremento-flask out of 99 fermentor were kept as control and these were also rearranged in 3 subsets each consisting of 3 fermentor flasks.

Now, M/1000 solutions of 4-aminobiphenyl was prepared and 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 ml of this solution was added to the fermentor-flasks of first 10 sets respectively. The control fermentor-flask was made upto 100 ml by adding requisite amount of distilled water.

Thus, the concentration of 4-aminobiphenyl in first, second, third, fourth, fifth, sixth, seventh, eighth, ninth and tenth subsets were approximately as given below:

A × 10^{-5} M
1.0 × 10^{-5} M
2.0 × 10^{-5} M
3.0 × 10^{-5} M
4.0 × 10^{-5} M
5.0 × 10^{-5} M
6.0 × 10^{-5} M
7.0 × 10^{-5} M
8.0 × 10^{-5} M
9.0 × 10^{-5} M
10.0× 10^{-5} M

Where, A = amount of mutagens in ml, i.e.; from 1.0 ml to 10.0 ml
M=Molarity of solution

The fermentor-flasks were then steam sterilized, cooled, insulated, incubated at 25^{0}C and analysed colorimetrically after 50,52 and 54 hours for alcohol formed and molasses sugars left infermented as described in experiment.

5. Result & Discussion:

The result obtained in the study of the influence of chemical mutagen 4-Aminobiphenyl under trial on ethanol formation by yeast *Saccharomyces cerevisiae* NCIM – 2012 are tabulated in the table.

Concentration of mutagen used A×10–×M	Incubation Period of Time	Yield of ethanol* in ml/100 ml	Molasses sugars* left unfermented (g/100ml)	% Difference in yield of ethanol in comparison to control
Control (–) Mutagen	50	7.01	2.90869	-
	52	7.35	2.62685	-
	54	7.15	2.53412	-
1.0×10^{-5} M (+) Mutagen	50	7.03	2.88719	+0.28530
	52	7.39	2.60465	+0.54421
	54	7.18	2.51749	+0.41958
2.0×10^{-5} M (+) Mutagen	50	7.04	2.86680	+0.42796
	52	7.40	2.58531	+0.68027
	54	7.19	2.49700	+0.55944
3.0×10^{-5} M (+) Mutagen	50	7.05	2.84363	+0.57061
	52	7.42	2.53941	+0.95238
	54	7.20	2.47411	+0.69930
4.0×10^{-5} M (+) Mutagen	50	7.07	2.81763	+0.85592
	52	7.47	2.51181	+1.63265
	54	7.23	2.44301	+1.11888
5.0×10^{-5} M (+) Mutagen	50	7.14	2.79783	+1.85449
	52	7.56	2.41596	+2.85714
	54	7.31	2.39810	+2.23776
6.0×10^{-5} M (+) Mutagen	50	7.17	2.77893	+2.28245
	52	7.58	2.38596	+3.12925
	54	7.48	2.36361	+4.61538
7.0×10^{-5} M** (+) Mutagen	50	7.29	2.74053	+3.99429
	52	7.75***	2.37113	+5.44217
	54	7.52	2.32001	+5.17482
8.0×10^{-5} M (+) Mutagen	50	7.10	2.80113	+0.71326
	52	7.60	2.40390	+1.76870
	54	7.33	2.38113	+1.39860
9.0×10^{-5} M (+) Mutagen	50	7.06	2.83810	-
	52	7.48	2.42531	-
	54	7.25	2.40363	-
10.0×10^{-5} M (+) Mutagen	50	****	-	-
	52	****	-	-
	54	****	-	-

*Each value represents mean of three trials

**Optimum concentration of the chemical mutagen used

***Optimum yield of ethanol in 52 hours (+) values indicate % increase in the yield of ethanol in comparison to control.

Experimental deviation (±) 1.5-3%

****Insignificant value

The influence of 4-aminophenyl on ethanol formation by the yeast *Saccharomyces cerevisiae* NCIM – 2012.

The recorded in the above table shows that 4-aminobiphenyl also has stimulatory effect on ethanol formation by the yeast *Saccharomyces cerevisiae* NCIM – 2012.

The data reveals that the chemical mutagen 4-aminobiphenyl stimulates the ethanol formation by the yeast and enhances the yield of ethanol upto its (4-aminobiphenyl) concentration from 1.0×10^{-5} M to 9.0×10^{-5} M in two phases.

In the first phase, i.e. from 1.0×10^{-5} M to 7.0×10^{-5} M the effect of 4-aminobiphenyl on the productivity (yield) of ethanol was gradually in increasing order and attains its best function at 7.0×10^{-5} M where maximum yield of ethanol, i.e., 7.75 ml/100ml is fetched in 52 hours of optimum incubation period which is 5.44217% higher in comparison to control fermentor flasks (7.35 ml/100 ml).

In the second phase of mutagenic chemical effect the molar concentration i.e., from 8.0×10^{-5} M to 9.0×10^{-5} M the production of ethanol has been enhanced but the order of ethanol productivity is reversed in respect to increasing molar concentration of 4-aminobiphenyl used has been found stimulating and the yield of ethanol has been found greater than that obtained in the control fermentor flasks. In both the phases the order of productivity and % of ethanol formed after 52 hrs. is as under:

- Concentration of 4-aminobiphenyl from 1.0×10^{-5} M to 7.0×10^{-5}M

Productivity of ethanol: 0.54421%, 0.68027%, 0.95238%, 1.632659%, 2.85714%, 31.12925% and 5.44217%

- Concentration of 4-aminobiphenyl from 8.0×10^{-5} M to 9.0×10^{-5}M

Productivity of ethanol: 3.40136% and 1.76870%

- Concentration of 4-aminobiphenyl from 10.0×10^{-5} M

Productivity of ethanol: Insignificant Value.

The exposure on the yeast *Saccharomyces cerevisiae* NCIM – 2012 to 4-aminophenyl may produce a variety of effects. Depending upon the concentrator of 4-aminobiphenyl to which the yeast stain *Saccharomyces cerevisiae* NCIM – 2012 were exposed may influence disruption of cells. Precipitation of cell protein, inactivation of enzymes and leakage of amino acids from the cells. Although the special mode of action is not very clear, there is a consensus that the lethal effect is associated with physical damage of the membrane structure of the cell structure surface, which initiates further determination.

Thus, it is concluded that 4-aminophenyl at lower concentration is stimulatory and at higher concentrations is deterioratory for the ethanol formation by the yeast *Saccharomyces cerevisiae* NCIM – 2012

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