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Studies on *in vitro* biotic production of H_3Cit exposed to ethanesufonyl chloride

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Abstract: The efficacy of ethanesufonyl chloride on biotic production of H_3Cit by fungal strains such as *Aspergillus fischeri* RS – 119*, Aspergillus foetidus* RS–215*, Aspergillus aculeatus* RS-313, *Aspergillus carbonarius* RS-418 and *Aspergillus wentii* RS- 519 has been assessed. It has been observed that the fungal strain *Aspergillus wentii* RS- 519 has been found most significant and effective for the citric acid fermentation process. It has been found that the compound, i.e., ethanesufonyl chloride has stimulatory effect on biotic production of H_3Cit by *Aspergillus wentii* RS- 519 and enhances the yield of citric acid to an extent of 12.008% higher in comparison to control fermenter flasks, i.e., 7.245g/100 ml under the optimized conditions.

(**Keywords:** Citric acid fermentation, ethanesufonyl chloride and *Aspergillus wentii* RS- 519)

Introduction

The study of chemical mutagenesis is sure to reveal even greater complexities than these, for there are several quite different classes of mutagenic agents. The influence of different chemical mutagens in different fermentations process has been investigated by a number of workers[1-20]. A large number of chemical mutagens are recorded as a good agent for different microbes and microbial processes[21-54]. Thus, from the above brief review it is evident that chemical mutagens are required for genetic manupulation and exploitation specially for citric acid fermentation and in view of this the author has studied the influence of ethanesufonyl chloride on biotic production of citric acid by *Aspergillus wentii* RS-519

Experimental

The influence of ethanesufonyl chloride on biotic production of H_3Cit by *Aspergillus wentii* RS- 519.

Aspergillus wentii

The composition of the production medium for biotic production of H_3Cit by *Aspergillus wentii* RS- 519 has been prepared as follows: Molasses:22%(w/v), NH_4NO_3: 0.66% , KH_2PO_4 : 0.66%, $MgSO_4.7H_2O$:0.66%, pH : 2.2 The pH of the production medium was adjusted to 2.2 by adding requisite amount of KCl-HCl buffer solution, and this pH was also ascertained by a pH meter. The above composition medium represents volume of a fermenter flask, i.e., "100ml" production of citric acid by *Aspergillus wentii* RS- 519. Now, the same production medium for biotic production of H_3Cit by *Aspergillus wentii* RS- 519 was prepared for 99-fermenter flask, i. e; each contained '100ml' of production medium.

The above 99-fermenter flasks were then arranged to 11-sets each comprising of 9-fermenter flasks. Each set was then rearranged in 3-subsets,

Table - 1
Studies on *in vitro* biotic production of H_3Cit exposed to ethanesufonyl chloride

Concentration of mutagen used A x 1/100000/ M	Incubation Period in days	Yield of citric acid* in g/100 ml	Molasses* left Unfermented in g/100 ml	% of citric acid increased after 11 days
Control	11	7.245	4.112	–
0.1 × 1/100000M	11	7.259	2.965	+ 0.193%
0.2 × 1/100000M	11	7.267	2.957	+ 0.303%
0.3 × 1/100000M	11	7.274	2.950	+ 0.400%
0.4 × 1/100000M	11	7.282	2.942	+ 0.510%
0.5 × 1/100000M	11	7.289	2.935	+ 0.660%
0.6 × 1/100000M	11	7.295	2.929	+ 0.690%
0.7 × 1/100000M	11	7.304	2.921	+ 0.814%
0.8 × 1/100000M**	11	7.309	2.916	+ 0.883%
0.9 × 1/100000M	11	7.315	2.910	+0.966%
1.0×1/100000M	11	7.348	2.877	+1.421%
1.1×1/100000M	11	7.352	2.873	+ 1.476%
1.2×1/100000M	11	7.369	2.856	+ 1.711%
1.3×1/100000M	11	7.383	2.842	+ 1.904%
1.4×1/100000M	11	7.399	2.826	+ 2.125%
1.5×1/100000M	11	7.445	2.781	+ 2.760%
1.6×1/100000M	11	7.451	2.775	+ 2.843%
1.7×1/100000M	11	7.459	2.767	+ 2.953%
1.8×1/100000M	11	7.468	2.758	+ 3.077%
1.9×1/100000M	11	7.478	2.748	+ 3.216%
2.0×1/100000M	11	7.483	2.743	+ 3.285%
2.1×1/100000M	11	7.489	2.737	+ 3.367%
2.2×1/100000M	11	7.494	2.732	+ 3.436%
2.3×1/100000M	11	7.506	2.720	+ 3.602%
2.4×1/100000M	11	7.511	2.716	+ 3.671%
2.5×1/100000M	11	7.517	2.710	+ 3.754%
2.6×1/100000M	11	7.521	2.706	+ 3.809%
2.7×1/100000M	11	7.528	2.699	+ 3.906%
2.8×1/100000M	11	7.532	2.695	+ 3.961%
2.9×1/100000M	11	7.538	2.689	+4.044%
3.0×1/100000M	11	7.545	2.682	+ 4.140%

Contd..

Concentration of mutagen used A x 1/100000/ M	Incubation Period in days	Yield of citric acid* in g/100 ml	Molasses* left Unfermented in g/100 ml	% of citric acid increased after 11 days
3.1×1/1000M	11	7.551	2.676	+4.223%
3.2×1/1000M	11	7.555	2.673	+4.278%
3.3×1/1000M	11	7.559	2.669	+4.334%
3.4×1/1000M	11	7.563	2.665	+4.389%
3.5×1/1000M	11	7.567	2.661	+4.444%
3.6×1/1000M	11	7.572	2.656	+4.513%
3.7×1/1000M	11	7.579	2.649	+4.610%
3.8×1/1000M	11	7.583	2.645	+4.665%
3.9×1/1000M	11	7.588	2.641	+4.734%
4.0×1/1000M	11	7.592	2.637	+4.789%
4.1×1/1000M	11	7.596	2.633	+4.844%
4.2×1/1000M	11	7.603	2.625	+4.941%
4.3×1/1000M	11	7.609	2.620	+5.024%
4.4×1/1000M	11	7.614	2.615	+5.093%
4.5×1/1000M	11	7.619	2.610	+5.162%
4.6×1/1000M	11	7.622	2.607	+5.203%
4.7×1/1000M	11	7.629	2.601	+5.300%
4.8×1/1000M	11	7.633	2.597	+5.355%
4.9×1/1000M	11	7.639	2.591	+5.438%
5.0×1/1000M	11	7.644	2.586	+5.507%
5.1×1/1000M	11	7.648	2.582	+5.562%
5.2×1/1000M	11	7.652	2.578	+5.617%
5.3×1/1000M	11	7.658	2.572	+5.700%
5.4×1/1000M	11	5.652	2.568	+5.755%
5.5×1/1000M	11	5.668	2.562	+5.538%
5.6×1/1000M	11	7.672	2.559	+5.893%
5.7×1/1000M	11	5.676	2.555	+5.948%
5.8×1/1000M	11	7.680	2.551	+6.004%
5.9×1/1000M	11	7.686	2.545	+6.086%
6.0×1/1000M	11	7.691	2.540	+6.155%
6.1×1/1000M	11	7.694	2.537	+6.197%
6.2×1/1000M	11	7.698	2.533	+6.252%
6.3×1/1000M	11	7.706	2.525	+6.363%
6.4×1/1000M	11	7.710	2.522	+6.418%
6.5×1/1000M	11	7.715	2.517	+6.487%
6.6×1/1000M	11	7.718	2.514	+6.528%
6.7×1/1000M	11	7.726	2.506	+6.639%
6.8×1/1000M	11	7.761	2.502	+6.708%
6.9×1/1000M	11	7.737	2.495	+6.790%
7.0×1/1000M	11	7.745	2.487	+6.901%

Contd..

Concentration of mutagen used A x 1/100000/ M	Incubation Period in days	Yield of citric acid* in g/100 ml	Molasses* left Unfermented in g/100 ml	% of citric acid increased after 11 days
7.1×1/100000M	11	7.755	2.477	+7.039%
7.2×1/100000M	11	7.765	2.463	+7.177%
7.3×1/100000M	11	7.785	2.443	+7.453%
7.4×1/100000M	11	7.890	2.339	+8.902%
7.5×1/100000M	11	7.996	2.233	+10.365%
7.6×1/100000M	11	8.006	2.225	+10.503%
7.7×1/100000M**	11	8.115***	2.113	+12.008%
7.8×1/100000M	11	7.969	2.259	+9.993%
7.9×1/100000M	11	7.862	2.366	+8.516%
8.0×1/100000M	11	7.758	2.475	+7.080%
8.1×1/100000M	11	7.701	2.532	+6.293%
8.2×1/100000M	11	7.655	2.578	+5.659%
8.3×1/100000M	11	7.602	2.631	+4.927%
8.4×1/100000M	11	7.550	2.683	-
8.5×1/100000M	11	7.505	2.728	+3.588%
8.6×1/100000M	11	7.301	2.932	+0.772%
8.7×1/100000M	11	7.299	2.934	+0.745%
8.8×1/100000M	11	7.295	2.939	+0.690%
8.9×1/100000M	11	7.289	2.945	+0.607%
9.0×1/100000M	11	7.284	2.950	+0.538%
9.1×1/100000M	11	7.279	2.955	+0.469%
9.2×1/100000M	11	7.274	2.960	+0.400%
9.3×1/100000M	11	7.269	2.965	+0.331%
9.4×1/100000M	11	7.262	2.972	+0.234%
9.5×1/100000M	11	7.259	2.976	+0.193%
9.6×1/100000M	11	7.254	2.981	+0.124%
9.7×1/100000M	11	7.249	2.986	+0.055%
9.8×1/100000M	11	7.248	2.989	+0.041%
9.9×1/100000M	11	7.247	1.480	+0.027%
10.0×1/100000M	11	7.246	2.991	+0.013%

* Each value represents mean of three trials ** Optimum concentration of mutagen used
*** Optimum yield of citric acid (+) values indicate % increase in the yield of citric acid after 11 days. Experimental deviation (±) 1.5-3%

each consisting of 3-fermenter flasks. The remaining 9-fermenter flasks out of 99-fermenter flasks were kept as control and these were also rearranged in 3-subsets each consisting of 3-fermenter flasks.

After preparing the above sets of fermentor flasks M/1000 solution 0.1 ml to 10 ml of ethanesufonyl chloride was prepared and from the above mutagen solution was added to the fermentation flasks of above 1st to 10th sets respectively. The control fermentor flasks contained no mutagen.

Now, the total volume in each fermentor flasks was made upto 100 ml by adding requisite amount of distilled water. Thus, the molar concentration of ethanesufonyl chloride in 1st,2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th subsets were approximately as given below :
A x 1/1000M
1.0x1/1000M to 10.0×1/1000M respectively.
Where : A = amount of mutagen
in ml, i.e. 0.01ml ..... to 10 ml.
1/1000M =Molarity of the mutagen solution

The above fermentor flasks were then sterilized, cooled inoculated, incubated at 32^0C and analysed after 9, 11 and 13 days for citric acid[55] formed and molasses[56] left unfermented.

Results and Discussion

The influence of ethanesufonyl chloride on biotic production of citric acid by *Aspergillus wentii* RS-519

The data recorded in the table-1 shows that the chemical mutagen, i.e; ethanesufonyl chloride has also been found stimulatory for the biotic production of citric acid by *Aspergillus wentii* RS- 519 at all its molar concentrations, i.e; from 0.1 × 1/1000 M to 10.0 x 1/1000 M in two phases. In the first phase the molar concentration of chemical mutagens i.e; ethanesufonyl chloride from 0.1 × 1/1000 M to 7.7 x 1/1000 M is stimulatory and the biotic production of citric acid gradually increases till a maximum value is reached at 7.7 x 1/1000 M, i.e; 8.115g/100ml in 11 days of optimum incubation period which is 12.008% higher in comparison to control, i.e; 7.245g/100ml.

In the second phase of chemical mutagenic effect, i.e., at 7.8 x 1/1000 M and onwards the molar concentration of ethanesufonyl chloride on biotic production of citric acid by *Aspergillus wentii* RS- 519 has been found increased but in lesser quantum in the presence of ethanesufonyl chloride So, the gradual addition of ethanesufonyl chloride after the optimum concentration of 7.7 x 1/1000M gradually slows down the biotic production of citric acid by *Aspergillus wentii* RS-519 and the part of % increase at 7.8 x 1/1000M to 10.0 x 1/1000 M molar concentration of ethanesufonyl chloride is in decreasing order.

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