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Production of Succinic Acid Through Anaerobic Screening of Related Microbial Strain

[1]*M. Kamran Khan,* [2]*Pankaj Kishor Mishra,* [2]*Umesh Kumar,*
[3]*Meenakhsi Mishra,* [1]*Taru Sharma and* [1]*Vipin Permar*

[1]Department of Tissue Culture,
[2]Department of Biochemistry and Biochemical Technology,
[3]Department of Biological Sciences,
Allahabad Agricultural Institute-Deemed University, Allahabad-211007, India

Abstract: Fermentation derived succinic acid is an economic process for supplying the existing succinic acid especially in chemical market. Production of succinic acid by fermentation can generate significant new markets for agricultural carbohydrates. The present work was undertaken with the objective to investigate a novel and simple fermentation process of succinic acid production at low cost from the renewable sources. Out of one hundred and two DBRL; isolates which are used in the study, only five strains gives positive results under both aerobic and anaerobic condition for succinic acid production. When these one hundred and two isolates were tested in indicator medium without $CaCO_3$ only nineteen strains gives positive results under anaerobic condition. However, only eighteen strains showed positive results with $CaCO_3$ under same condition and only five strains showed positive results with and without $CaCO_3$ under anaerobic condition. From the present study it is concluded that the strains tested positive for succinic acid because the biologically produced succinic acid is provided a new route to the chemical industries without pollution and biofriendly in nature.

Key words: Anaerobic screening · $CaCO_3$ · Fermentation · Succinic acid

INTRODUCTION

Succinic acid is a four carbon dicarboxylic acid has the potential to become a large commodity chemical that would from the basis for supplying many important intermediates and specific chemicals for the consumer product industries. Succinic acid derived from fermentation of agricultural carbohydrates has a specialty chemical market in industries producing food and pharmaceutical products, surfactants and detergents, green solvents, biodegradable plastics and ingredients to stimulate animal and plant growth as a carbon intermediate chemical. Hydrogen gas shown to increase the succinic acid production during glucose fermentation by a succinogenes [1].

In the presence of glucose and hydrogen, *Actinobacillus succinogenes* can generate low redox-potential electrons by its hydrogenase activity; therefore, more phosphoenol pyruvate (PEP) flows to succinate than to pyruvate. The PEP carboxykinase pathway used for succinic acid production by a *Succiniciproducens* and *A. succinogenes* is regulated by CO_2 levels [2]. Succinic acid is mostly produce by physico-chemical processes with other side secondary metabolites. Recently fermentative products succinic acid from renewable biomass by anaerobic bacteria has generated great interest. The existence of different enzymes indicates different pathways fermentation in *E. coli* and the studies of also confirm multiple routes for succinate formation [3]. The anaerobic bacterium *Anaerobiospirillum succiniciproducens* has been considered as one of the best succinic acid producers because it can produce significant amount of succinic acid from glucose. The present study was conducted to investigate a novel and simple fermentation process of succinic acid production at low cost from the renewable sources.

Corresponding Author: Umesh Kumar c /o Dr. Veeru Prakash, Department of Biochemistry and Biochemical Technology, Allahabad Agricultural Institute- Deemed University Allahabad, Uttar Pradesh- 211007, India.

MATERIALS AND METHODS

One hundred and two microbial culture of RRL Jammu (the cultures isolated at RRL Jammu) named as DBRL strains were used for succinic acid production by fermentation process.

For the preparation of seed, one loopful culture of DBRL strains was inoculated in 20 ml autoclaved MK-1 media in 100 ml conical flask. The inoculated flask were incubated at 30°C for 24 h growth, the culture which are used frequently referred to as stock culture, stock culture were transferred aseptically grown at 28-30°C and preserved in nutrient agar slants at 4°C for short term storage. These stored cultures were used for making the seed culture in liquid culture media. With the help of syringes and needles the overnight grown seed culture was transferred to flask 100 ml volume 1 ml and vials 0.5 ml (6.0 ml vol.) containing 20 ml and 30 ml sterilized MK-1 media respectively. Carbon dioxide gas passed through both flask and vials for 1 minute containing $Na_2S.9H_2O$ final concentration 1 mg l^{-1} or cysteine HCl 1 mg l^{-1} was added to make anaerobic conditions. The flask and vials are incubated at 37°C to test for acid (succinic acid), MK-3 indicator media for acid testing was prepared. The media plated on two plates, one plate contains bromocresol green solution with $CaCO_3$ and the other plate contains only dye. The plate containing only dye (bromocresol green solution) gave yellow color in the wells as end point indicating acid formation. At least four wells about five mm in diameter were made in each plate by the help of well borer.

For checking acid production under aerobic condition, a known volume of the 1 ml culture was taken from the aerobically grown fermented broth in eppendrof tube and centrifuged at 1500 rpm for five minutes. The supernatant (30 µl) was pipetted out in to the wells of the plate containing indicator media. Inoculated plates were incubated at 30°C for 24 h, after incubation formation of zone was considered as positive.

For checking acid production under anaerobic conditions a known volume of the 1 ml culture was taken from the anaerobically grown fermented broth in eppendorf tube and centrifuged. The supernatant (30 µl) was pipette out in the wells of the plate containing indicated media. Inoculated plates were incubated at 30°C for 24 h, after incubation fermentation of zone was considered as positive.

RESULTS AND DISCUSSION

One hundred and two microbial strains from Jammu hills designated as DBRL were screened for succinic acid production under both aerobic and anaerobic conditions. The results on their growth and acid production are given in Table 1. Out of one hundred and two organisms tested for growth, ninety five organisms showed anaerobic growth while seven organisms reported to be obligate aerobes.

Ninety-five organisms grown in anaerobic conditions were tested for production of acid using indicator medium with and without $CaCO_3$. Thirty one organisms were found to be positive for acid production. The five organisms showed acid production in indicator medium both with and without $CaCO_3$. However, in nineteen organisms acid production was detected when indicator medium used without $CaCO_3$. Acid production by DBRL strains are showed in Table 1.

The findings were reported production of succinic acid by *E. coli* strain (ATCC 202021) from inexpensive source of a carbohydrate, corn sugar [4]. These results are comparable to observation obtained in the present study. Anaerobic production of succinic acid was studied by using glycerol as a carbon source [5]. They found that succinic acid producing microorganisms normally produce acetic acid, lactic acid and formic acid simultaneously.

It has been observed that under same culture condition *E.coli* switches from ethanol to succinate production [6]. It was found that the genes involved in metabolic pathway of succinate production and these results should contribute to our fundamental understanding of genetic regulation of anaerobic production of succinic acid. Succinic acid has wide application in industry and therefore deeper understanding of genetics involved in succinic acid production will help in the genetic engineering of more efficient producer organisms. With the respect of above finding we examined during our study that twenty eight organisms were positive for acid production.

Fermentation derived succinic acid is an economic process for supplying the existing succinic acid specially chemical markets. Production of succinic acid by fermentation can generate significant new markets for agricultural carbohydrates. One of the hundred and two DBRL isolates which is used in the study, only three strains gives positive results under both aerobic and anaerobic condition for succinic acid production.

Table 1: Succinic acid production by DBRL microbial strains in anaerobic condition

SL. No.	DBRL Culture No.	Growth		Acid Production	
		Aerobic	Anaerobic	With CO_3	Without CO_3
1	31	++	++	-	-
2	33	++	++	-	-
3	34	++	++	-	-
4	36	++	++	-	-
5	37	++	++	-	-
6	40(a)	++	++	-	-
7	40(b)	++	++	-	-
8	62	++	++	-	-
9	65	++	++	-	-
10	67	++	++	-	-
11	69#β	++	++	-	+
12	71	++	++	-	-
13	84	++	++	-	-
14	87*	++	-	-	-
15	88	++	++	-	-
16	89*#β	++	-	-	+
17	90	++	++	-	-
18	92	++	++	-	-
19	93	++	++	-	-
20	94	++	++	-	-
21	97	++	++	-	-
22	100	++	++	-	-
23	125	++	++	-	-
24	127	++	++	-	-
25	129	++	++	-	-
26	131	++	++	-	-
27	132	++	++	-	-
28	135	++	++	-	-
29	136	++	++	-	-
30	138	++	++	-	-
31	143	++	++	-	-
32 `	146	++	++	-	-
33	153	++	++	-	-
34	155*	++	-	-	-
35	156	++	++	-	-
36	164	++	++	-	-
37	167	++	++	-	-
38	168	++	++	-	-
39	169*	++	-	-	-
40	170	++	++	-	-
41	171	++	++	-	-
42	172	++	++	-	-
43	173	++	++	-	-
44	174	++	++	-	-
45	175	++	++	-	-
46	179	++	++	-	-
47	187	++	++	-	-
48	194	++	++	-	-
49	203#β	++	++	-	+
50	206	++	++	-	-
51	210	++	++	-	-
52	215	++	++	-	-
53	216#	++	++	-	+
54	220#ß	++	++	-	+
55	221	++	++	-	-
56	224	++	++	-	-
57	226	++	++	-	-
58	282	++	++	-	-
59	286#ß	++	++	-	+
60	287#ß	++	++	-	+
61	290#ß	++	++	-	+
62	292	++	++	-	-
63	296#ß	++	++	-	+
64	298#ß	++	++	-	+
65	299	++	++	-	-
66	300	++	++	-	-
67	302*	++	-	-	-
68	303*	++	-	-	-
69	306*	++	-	-	-
70	308	++	++	-	-
71	309	++	++	-	-
72	311	++	++	-	-
73	312	++	++	-	-
74	314	++	++	-	-
75	**385#α	++	++	+	+
76	386#ß	++	++	-	+
77	387#ß	++	++	-	+
78	**388#α	++	++	+	+
79	389#	++	++	+	-
80	390#ß	++	++	-	+
81	391#α	++	++	+	-
82	392#α	++	++	+	-
83	393#α	++	++	+	-
84	394#α	++	++	+	-
85	395#α	++	++	+	-
86	396#α	++	++	+	-
87	397#α	++	++	+	-
88	398#α	++	++	+	-
89	**392#αß	++	++	+	+
90	400#α	++	++	+	-
91	401	++	++	-	-
92	403#α	++	++	+	-
93	404#α	++	++	+	-
94	405#α	++	++	+	-
95	418	++	++	-	-
96	419#ß	++	++	-	+
97	420	++	++	-	-
98	422	++	++	-	-
99	426	++	++	-	-
100	427	++	++	-	-
101	**2951#αß	++	++	+	+
102	**2051#αß	++	++	+	+

Organisms positive for acid production

* Organism with obligate aerobic growth

** Acid production both with and without $CaCO_3$

α Acid production with $CaCO_3$

β Acid production without $CaCO_3$

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