EFFECT OF SANDOZ 9785 ON GAMMA-LINOLENIC ACID (GLA) IN SPIRULINA Z19/2

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Abstract

Spirulina Z19/2, the herbicide Sandoz 9785 resistant strain, could grow in Zarrouk's medium containing 150 ppm SAN 9785 whereas growth of the wild type strain (BP) was completely inhibited. Spirulina Z19/2 grown in Zarrouk's medium containing 150 ppm SAN 9785 showed the reduction in GLA content (36.8% reduction as of total fatty acid and 39.6% reduction as of D.W.). The alteration of fatty acid composition resumed to normal after 24 h when cells were transferred to normal Zarrouk's medium.

Results shown that strains resistant to SAN 9785 have relatively higher GLA content when transferred to normal Zarrouk's medium compare with the wild type strains.

Introduction

The interest in fatty acids of the ω6 family derives from the fact that they are precursors for prostaglandins, thromboxanes, and leukotrienes (Holman, 1981). GLA (18:3 ω6) was also shown to alleviate the symptoms of the premenstrual syndrome (Horrobin, 1983). The cyanobacterium Spirulina has found worldwide recognition as health food due to its high content of this rare fatty acid, GLA. Cohen et al. (1987) reported the increase in GLA to 30.5% of total fatty acid and 1.3% of the dry weight by varying growth conditions.

Other possible method of increasing the GLA is by using desaturase inhibitor. It is possible that strains that are resistant to inhibitor are the over producers of GLA. In this study, the herbicide SAN 9785, a substituted pyridazinone which is effective for inhibition of Δ15 desaturation was used. The effects of SAN 9785 on the production of fatty acids were investigated.

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Materials and Methods

Cell cultivation

Spirulina Z19/2 were cultivated in Zarrouk's medium in erlenmeyer flasks placed in a transparent water bath illuminated from below with four cool-white fluorescent lamps providing 100 $\mu E.m^{-2}.s^{-1}$ at the surface of the bath. Mixing was achieved by bubbling air using aquarium air pumps. Study on the effect of SAN 9785 on growth and fatty acids composition of Spirulina was done by adding SAN 9785 at different phase of growth to the desired final concentrations.

Cell were filtered through whatman GF/C filter papers and freeze dried for furthur analyses of the fatty acids.

Lipid extraction and transmethylation

Freeze - dried sample of Spirulina (100 mg) were treated with 2 ml of MeOH-AcCl according to reference (Lepage,G.,1984) . C17:0 was added as an internal standard and the mixture sealed in a vial under Ar atmosphere and heated to 80°C for 1 h. The vial was then cooled, its contents diluted with 1 ml H_2O and the mixture extracted with 1 ml hexane. The hexane layer was dried ($Na_2 SO_4$), evaporated to dryness and redissolved in hexane.

Fatty acid analysis

G.C. analysis was performed on a 3 m x 3 mm. i.d. stainless column packed with 15% DEGS on uniport B 80/100 mesh at 190°C FID injector and FID detector temperature 230°C, attenuation range, 2^4 x10^2 mV.V. Fatty acid Me esters were identified by cochromatography with authentic standards (Sigma Co.). Fatty acid contents were determined by comparing their integrated peak areas with that of the internal standard.

Results and Discussion

Study on the effect of SAN 9785 on growth and fatty acid compositions have shown that SAN 9785 inhibited growth and GLA production. The inhibition of GLA is more pronounced if added at the early stage of growth. When cells were older the effect on GLA production was also more pronouced (when SAN 9785 was added on the first day). The accumulation of C 18:1 was observed. It could be noticed that SAN 9785 may inhibit the desaturation of C 18:1 to C 18:2 and C18:3. The similar effect could also be seen in the case of C16:0 to C16:1 (Table 1). Cohen et al. (1990) also reported the same finding in Spirulina.

Table 1 Fatty acid content of Spirulina Z19/2 grown in normal Zarrouk's medium and in Zarrouk's medium with added SAN 9785 in d1 and d4.

A. Normal Zarrouk's medium

	Fatty acid composition (% TFA)						% D.W.	
Day	16:0	16:1	18:0	18:1	18:2	18:3	TFA	GLA
d3	46.82	3.67	0.71	4.75	17.20	21.67	4.61	1.41
d4	48.87	3.94	0.84	5.08	15.90	23.29	5.03	1.17
d5	47.77	4.17	0.95	6.66	15.66	22.80	5.04	1.15
d6	48.36	4.16	0.83	6.94	14.89	22.71	5.61	1.27
d7	49.85	4.17	0.82	7.99	13.94	20.91	5.82	1.22
d8	47.86	3.57	0.99	7.76	15.33	22.02	4.67	1.02

B. Zarrouk's medium + SAN 9785 (d1)

	Fatty acid composition (% TFA)						% D.W.	
Day	16:0	16:1	18:0	18:1	18:2	18:3	TFA	GLA
d3	50.9	2.03	1.54	9.72	13.67	20.47	4.48	0.92
d4	42.18	2.17	1.39	12.68	15.82	21.15	6.02	1.27
d5	50.44	1.99	1.65	14.03	11.99	18.52	5.38	0.99
d6	51.08	1.26	1.31	15.72	11.78	17.98	5.19	0.93
d7	50.52	1.73	2.25	17.29	10.74	16.16	3.77	0.61
d8	50.92	1.39	2.55	17.41	9.96	16.57	5.34	0.88

C. Zarrouk's medium + SAN 9785 (d4)

	Fatty acid composition (%TFA)						% D.W.	
Day	16:0	16:1	18:0	18:1	18:2	18:3	TFA	GLA
d6	50.62	2.69	1.86	9.64	13.05	20.38	5.13	1.04
d7	48.25	3.09	1.68	12.97	11.96	20.33	4.48	0.98
d8	48.56	2.57	2.08	17.58	10.02	17.52	6.34	1.11

The effect on the inhibition of growth and GLA production were studied when Spirulina were grown in Zarrouk's medium plus SAN 9785 at different concentration. The higher the SAN 9785 concentration, the more effect on the inhibition of growth and GLA production detected (Fig.1 and Table 2).

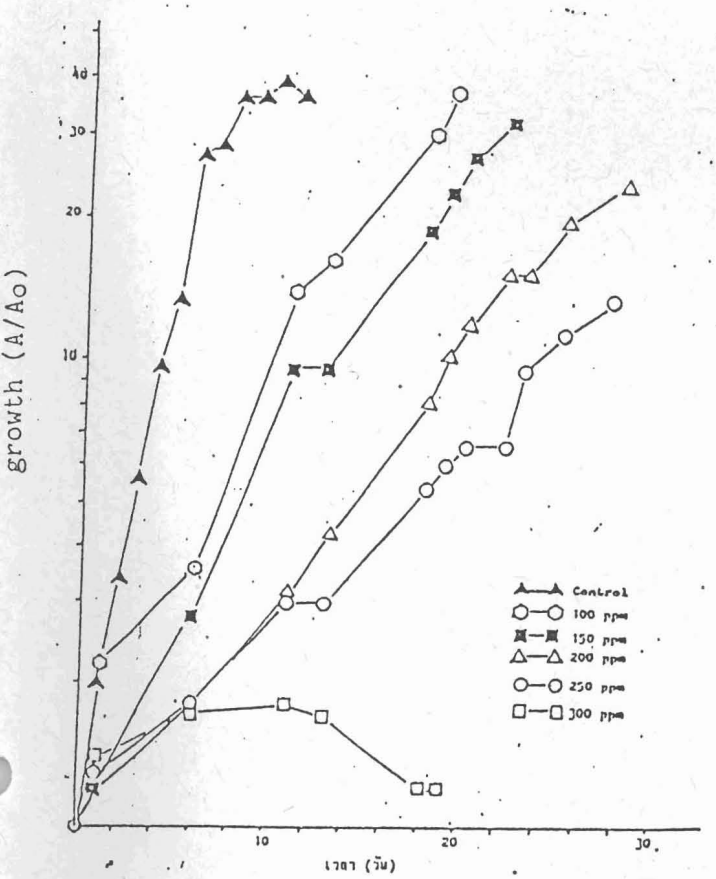

Fig 1. Growth of Spirulina in Zarrouk's medium with different concentrations of SAN 9785.

Table 2 Fatty acid composition of Spirulina Z19/2 in Zarrouk's medium with different concentration of SAN 9785 and the changes in composition after tranferring cells to normal Zarrouk's medium after 24 h.

Conc. of San 9785 (ppm)	Fatty acid Composition (%TFA)						% D.W.	
	16.0	16.1	18.0	18.1	18.2	18.3	TFA	GLA
0	40.9	6.1	1.3	7.6	12.3	24.7	5.5	1.34
100	37.2	0.8	4.5	27.6	10.8	17.2	5.4	0.9
150	49.0	0.9	2.8	19.8	11.5	14.1	6.7	0.9
200	48.6	0.6	4.6	22.9	9.1	11.7	5.8	0.7
250	48.8	1.0	3.9	24.7	9.6	10.5	6.1	0.64
150 (15 day)	49.0	1.2	2.6	19.4	10.9	15.6	5.2	0.81
150--->0 (24 h.)	46.6	1.8	1.3	14.6	13.3	20.4	5.6	10.14

Moreover, cells grown in Zarrouk's with 150 ppm SAN 9785 for 15 days then transferred to normal Zarrouk's medium showed an increase in GLA from 15.6% to 20.4% of TFA after 24 h. Growth rate resumed to normal after transferring back to normal medium.

By selecting strains resistant to SAN 9785, we could obtain strains of higher GLA content than the wild type (Table 3 and Table 4.)

Table 3 Fatty acid composition of some selected strains obtained by SAN 9785

Strain	Fatty acid compcsition (%TFA)						% D.W.	
	16:0	16:1	18:0	18:1	18:2	18:3	TFA	GLA
Z12C	46.5	3.3	0.8	4.7	18.2	24.9	6.9	1.7
Z5B	40.6	4.4	1.7	9.4	16.0	26.3	6.4	1.7
Z3	42.3	3.9	1.3	7.7	16.3	27.2	6.6	1.8
Z16	41.4	4.8	0.8	8.6	20.4	29.1	6.8	2.0
Z12	46.2	3.7	1.2	5.6	18.3	23.9	7.1	1.7
Z14	42.6	6.5	0.4	1.4	19.8	28.1	6.8	1.9
Z1A	46.0	3.7	0.4	2.1	18.1	28.9	7.3	2.1
Z19	41.5	4.2	0.5	2.4	19.7	31.3	7.5	2.3
Z14A	42.7	3.2	2.9	9.1	17.0	23.7	7.5	1.8
Z6	48.6	3.5	0.7	2.3	17.1	24.5	7.5	1.9
Z12A	44.6	3.2	1.3	5.2	17.9	27.7	6.7	1.9
Z11	46.2	3.3	0.6	7.9	15.4	25.5	7.1	1.8
Z12B	41.5	4.3	1.2	5.2	17.4	27.1	8.2	2.2
Z12D	48.3	3.8	0.5	1.3	17.9	24.8	7.9	2.0
Z1B	37.3	3.1	0.9	3.1	19.2	32.1	6.6	2.1

Table 4 Fatty acid composition* in Spirulina strains** (Cohen et al.,1987)

	Fatty acid compostion (%TFA)							
Strain	16:0	16:1	18:0	18:1	18:2	GLA	Total#	GLA#
SB	44.6	4.4	0.5	6.4	17.1	27.0	5.2	1.4
Mad	47.0	0.5	0.7	9.3	10.8	31.7	4.2	1.3
Cat	47.6	2.5	1.0	8.0	15.3	25.6	5.1	1.3
Art.B	46.1	1.0	1.6	10.9	13.6	26.8	4.7	1.3
1928	47.3	2.0	1.0	2.9	18.1	28.7	4.3	1.2
L1	45.0	1.4	1.0	15.5	16.4	20.7	5.6	1.2
AR	49.1	2.2	1.0	6.4	15.7	25.6	4.3	1.1
B4	49.6	2.1	0.7	5.0	16.5	26.1	3.9	1.0
B2	47.3	3.4	0.8	5.8	20.7	20.7	3.8	1.0
G	49.2	2.9	0.9	8.0	15.7	23.3	4.0	0.9
PC	52.5	2.4	0.8	7.2	14.0	23.2	4.0	0.9
B3	52.9	2.2	1.1	7.6	13.7	22.5	4.1	0.9
Art.A	48.5	2.4	1.3	6.0	15.8	26.0	3.4	0.9
Eth	54.1	2.6	1.0	7.7	13.5	21.3	4.1	0.9
L2	50.7	1.1	0.8	7.3	14.3	25.8	3.0	0.8
Minor	46.8	1.2	1.5	12.0	18.4	20.1	3.6	0.7
2342	47.5	1.6	0.5	9.3	21.8	19.3	3.8	0.7
2340	49.3	2.2	1.2	8.6	30.7	8.0	3.2	0.3
Subsalsa	49.2	35.0	1.7	1.0	13.1	-	1.6	-

\# Weight percent of total fatty acids
** Cultures were grown at 35°C
* Weight as percentage of biomass (ash-free dry wt-AFDW)

Spirulina of strians resistant to SAN 9785 would be the method of obaining high GLA production strains after transferring to normal Zarrouk's mdeium.

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