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티오시안산염 이용 균주인 Bacillus Brevis의 지방산 개요도

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Fatty Acid Profile Of Thiocyanate Utilizing *Bacillus Brevis*

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요 약. 화탄과정의 폐수에서 단리된 티오시안산염 이용 균주인 Bacillus brevis의 지방산 조성을 기체크로마토그라피로 분석하였다. 이 균주는 포화 및 불포화 지방산이외에 히드록시 지방산을 포함하고 있다. 히드록시 지방산은 일반적으로 흥미로운 박테리아의 화학적 계통분리학의 지표로 알려져 있다. 이 균주에는 시클로프로판 지방산이 전혀 없다. 이 균주와 Baciluus brevi B-33 및 B-34 균주의 지방산 조성을 비교하였을 때 이 균주들 사이에 차이가 있었다. 이러한 차이는 티오시안산염의 충격 효과 때문일 것으로 보인다. 이 결과는 지방산의 생합성이 환경에 크게 의존된다는 것을 지지하여 준다.

주제어: 지방산, 티오시안산염, Bacillus brevis, 크로마토그라피

ABSTRACT. The fatty acid composition of thiocyanate utilizing *Bacillus brevis* isolated from carbonization wastewater was determined by Gas Chromatography and the results were analyzed. In addition to the saturated and unsaturated straight chain fatty acids this *B. brevis* strain contained a hydroxy fatty acid. The hydroxy fatty acids in general are shown to be interesting chemotaxonomic markers of bacteria. Cyclopropane fatty acids are totally absent in this strain. A comparison of the fatty acid composition of this strain with B-33 and B-34 strains of *Bacillus brevis* shows that there are deviations among these strains. The deviation in *Bacillus brevis* could be due to the stress effect of thiocyanate. This result supports that fatty acid synthesis depends highly on the environment.

Keywords: Fatty acid, Thiocyanate, *Bacillus brevis,* Chemotaxonomy

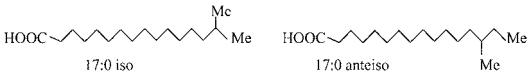
INTRODUCTION

Fatty acids occur in nearly all-living organisms as the important predominant constituent of lipids. The bacterial lipid composition is the characteristic of the bacterium and it also reflects the taxonomic status of the bacteria of different ranks in the taxonomic hierarchy. Bacterial lipid composition makes it possible not only to attribute the bacteria to certain genera or families but also to identify their strains.[1-3] Some of the commonly used methods for cellular fatty acids (CFA) profiling are based on the analysis of fatty-acid derivatives by gas chromatography/mass spectrometry (GC/MS). Most frequently, fatty acids isolated from bacterial cells are connected to methyl esters for desirable chromatographic properties. Until recently there was a wide spread opinion, based on a study of wild strains of *Escherichia Coli*, that alterations in fatty – acid composition of Gram-negative bacteria occurred under changes in environmental conditions, while their phospholipid (PL) composition remained

intact.[4-6] However, essential changes were described in PL composition of some bacterial species characterized by a large diversity of environmental conditions like pH, temperature, salinity, inorganic and organic nutrients, herbicides, antibiotics, and other chemicals.[7-10] Accumulating evidence also indicates that alterations in lipid composition have a direct effect on membrane function. Common bacterial fatty acids generally contain 12 to 20 C-atoms and are of the saturated or mono-unsaturated type. Bacteria characteristically contain odd chain and branched chain fatty acids as well as 2 or 3 – OH, and cyclopropyl derivatives, which are much less common in higher organisms.[11] Microbial lipids have economic importance in areas such as the food and pharmaceutical industries.

Branched-chain fatty acids are common constituents of the lipids of the bacteria and animals, although they are rarely found in the integral lipids of higher plants. Normally, the fatty acyl chain is saturated, and the branch is a methyl group. However, unsaturated branched – chain fatty acids are found in marine animals and microbial lipids.

The iso-methyl-branched fatty acids have the branch point on the penultimate C-atom (one from the end), while anteiso-methyl-branched fatty acid have the branch point on the ante-penultimate C-atom (second from the end) as illustrated by the examples shown below.



Thiocyanate is toxic to all classes of living cells and it is found in appreciable concentration in waste waters generated from coal gasification and coking facility, gas clean up system and coal pyrolysis operation. Several references are reported on bacterial degradation of thiocyanates.[12-16] In our laboratory we had carried out the degradation of thiocyanate by *Bacillus brevis* isolated from carbonization plant effluent. It is found that this bacterium degrades 95% of 200 ppm of thiocyanate in 20 hours.

Recently we reported the change in fatty acid profile of cyanide utilizing Yersinia species[17] and present work is to analyse the fatty acid composition of *Bacillus brevis* grown in thiocyanate and to compare its fatty acid profile with other B-33 & B-34 strains of *Bacillus brevis* species.

EXPERIMENTAL PART

Medium. Deionized distilled water was used throughout. All chemicals used were of AnalaR grade. The minimal medium used for the isolation of thiocyante – utilizing bacteria contained KH_2PO_4 (3.3 g); K_2HPO_4 (4.3 g), $MgCl_2$(0.3 g) in 1000 ml and it was amended with 0.5 ml of the trace element solution containing $MnCl_2$ (1.0 mg), $FeSO_4.7H_2O$ (0.8 mg), $CaCl_2.H_2O$ (2.4 mg) and $NaMoO_4.2H_2O$ (10.0 mg) in one liter. The pH was adjusted to 7.0 and the medium was autoclaved. The minimal medium plates were prepared by adding 15 g of agar to one liter of the medium.

Isolation of bacterium. The thiocyanate contaminated soil and waste water samples were collected from the carbonization plant effluent site. One ml of the wastewater (soil suspension) was inoculated into sterile test tubes containing 9 ml of the minimal medium supplemented with different concentrations of thiocyanates (60-400 mg/l). Streaks of the isolates were drawn from these tubes. The growth pattern of these streaks after 24 hours of incubation at 25 °C was observed. The results are given in *Table* 1. There is a significant growth up to 400 ppm of thiocyanate and above this concentration the growth was found to be scanty. Colonies that grew on the plates containing 400 ppm of thiocyanate were selected for identification.

Identification of Bacterium: The isolate was identified at the Microbial Type Culture Collection Centre and Gene Bank (MTCC) of the Institute of Microbial Technology (IMTECH) Chandigarh, India and named as *Bacillus brevis*-MTCC3136.

Bacterial fatty acid analysis. The culture was grown in the petri dishes containing Tripticase soy broth (30 g/l) and Bactoagar (15 g/l) medium incubated at 28 °C. After 24 hrs, the cells from the third

Table 1. Growth of culture at different concentrations of thiocyanate

Concentration of Thiocyanate (mg/L)	Growth pattern (After 24 Hours)
0	Spreading type
60	Spreading type
120	Intermediate
200	Intermediate
300	Intermediate
400	Rhizoid type

quadrant of the dishes were harvested and saponified with NaOH (3.7 N) in aq.CH_3OH (1:1). The saponified cells were methylated by. CH_3OH in 6N HCl (1:1), the methyl esters formed were extracted with hexane / t-BuOMe (1:1) and the aq.phase was removed. Finally mild base solution (2.4 N NaOH) was added to the sample to remove free fatty acids and residual reagents from organic extract. The top phase was then transferred into the GC vial and sealed. The sample (extract) was injected into the GC (Hewlett Packard 5890A). The different fatty acids of the sample were separated while passing through the capillary column loaded with 5% phenyl methyl silicone at a temperature range of 170-310 °C for over 23 min. The retention time of the sample was compared for the identification of various fatty acids. The system was initially calibrated for various fatty acids by the calibration standard supplied by the company.

RESULTS AND DISCUSSION

In microorganisms, fatty acids occur mostly in the form of phospholipids mainly located on cell membranes. The *Bacillus brevis* isolated from carbonization plant wastewater has been reported for its thiocyante degrading capacity.[16] Fatty acid composition of thiocyanate-utilizing bacteria isolated from carbonization wastewater soil is given in *Table* 2 and its chromatogram is shown in *Fig*. 1.

The results also match with the fatty acid composition of *B.brevis* given in the book bacteriological

Table 2. Fatty acid composition of thiocyanate utilizing bacillus brevis MTCC(3136)

Retention time (min)	Area	Area / Height	Name[@]	% of Fatty Acids
1.533	50696000	0.101	Solvent	-
2.974	19614	0.030	10:0	10.49
4.153	484	0.033	12:0 Iso	0.24
5.173	13418	0.035	13:0 Iso	6.21
5.271	1633	0.043	13:0 Anteiso	0.75
6.417	4796	0.038	14:0 Iso	2.12
6.921	5781	0.042	14:0	2.52
7.854	79531	0.042	15:0 Iso	33.97
7.989	7605	0.042	15:0 Anteiso	3.24
8.418	1072	0.042	15:0	0.45
9.048	1869	0.049	16:1ω7C alcohol	0.78
9.437	10386	0.047	16:0 Iso	4.31
9.654	1202	0.045	16:1ω11c	0.50
10.046	12210	0.046	16:0	5.03
10.414	837	0.044	15:0 2OH	0.34
10.706	6548	0.045	Iso 17:1 ω10c	2.68
10.832	9195	0.050	Iso 17:1 ω5c	3.76
10.968	942	0.043	17:1 Anteiso	0.38
11.118	29171	0.046	17:0 Iso	11.90
11.274	3890	0.046	17:0 Anteiso	1.59
13.482	759	0.049	18:0	0.31

[@]The figure in front of the colon indicates the number of carbon atoms in the chain and the figure after the colon indicates the number of double bonds; iso and anteiso indicates isomeric forms; ω - followed by number indicates position of double bond from methyl end; C – indicates cis-olefinic; 2-OH indicates hydroxyl group and the position.

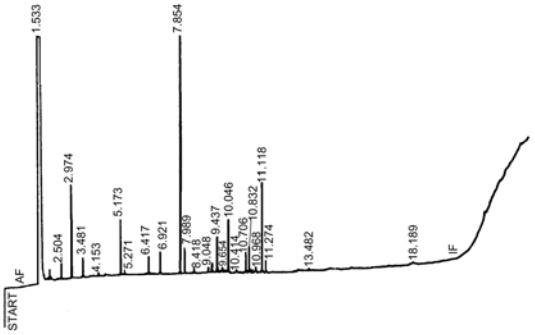


Fig. 1. Chromatogram of *Bacillus brevis* fatty acids.

reviews by Kaneda[18].

The fatty acid composition of the bacteria, *Bacillus brevis* is significantly different from that of higher organisms in having no polyunsaturated fatty acids. The predominance of terminally methyl branched iso and anteiso fatty acid with 12 to 17 carbons is a characteristic of all species of *Bacillus*.[19-20] The minor constituent of the normal fatty acids in the genus *Bacillus* is myristic acid.[21]

Lipids in *B.brevis* (MTCC 3136) contained a high concentration of 10:0, 13:0 Iso, 15:0 Iso, 16:0, 17:0 Iso and a low concentration of 12:0 Iso; 13:0 Anteiso, 15:0 – 2 – OH, 17:1 Anteiso, 17:0 Anteiso, 18:0 fatty acids. In addition to the saturated and unsaturated straight chain acids this *B.brevis* (MTCC 3136) contains a hydroxy fatty acid in 15:0 – 2-OH. The hydroxy fatty acids in general are shown to be interesting Chemotaxonomic markers of bacteria.[9] Cyclopropane fatty acids are totally absent in this *B.brevis* (MTCC 3136) strain.

A comparison of the fatty acid composition of the *B.brevis*, B-33 and B-34 strains shows that there are deviations among the 3 strains (*Table* 3). In the 15:0 Iso the total amount of fatty acids is high in the *B.brevis* (MTCC 3136) as well as in B-33 and B-34 strain. But on the other hand in 15:0 Anteiso the total amount is high in the 2 strains B-33 and B-34

Table 3. Comparison of fatty acid composition of thiocyanate utilizing *Bacillus brevis MTCC (3136) with other B-33 and B-34 strains of Bacillus brevis*

FATTY ACID[a]	*B. brevis*[b] STRAIN B-33	*B. brevis*[b] STRAIN B-34	*B. brevis*[c] MTCC 3136
10:0[#]	-	-	10.49
12:0 Iso[#]	-	-	0.24
13:0 Iso	0	0	6.21
13:0 Anteiso	0	0	0.75
14:0 Iso	0	3	2.12
14:0	4	1	2.52
15:0 Iso	25	28	33.97
15:0 Anteiso	36	43	3.24
15:0[#]	-	-	0.45
16:1ω7C alcohol[#]	-	-	0.78
16:0 Iso	5	9	4.31
16:1ω11C[#]	-	-	0.50
16:0	21	6	5.03
15:0 2OH[#]	-	-	0.34
Iso 17:1ω10C[#]	-	-	2.68
Iso 17:1ω5C[#]	-	-	3.76
17:1 Anteiso[#]	-	-	0.38
17:0 Iso	6	3	11.90
17:0 Anteiso	4	8	1.59
18:0[#]	-	-	0.31

a – Nomenclature as per *Table* 1

b – Percentage of Total Amount of Fatty Acids (from Ref. 18).

c – The Percentage of Fatty Acids in Thiocyanate Utilizing Bacterium.

- Not reported in Ref.18.

relative to *B.brevis* (MTCC 3136) indicating that synthesis of 15:0. Anteiso fatty acid is affected in MTCC 3136 strain. A similar trend is observed 17:0 Iso and 17:0 Anteiso forms. The thiocyanate affects the metabolism of the species. This may be due to the stress effect of thiocyanate on the organism (MTCC 3136). The stress effect on the fatty acid composition of various bacteria had been reported.[22,23] The deviations in fatty acid composition reveal that fatty acid synthesis depends upon the nature of the environment. The incorporation of branched chain fatty acids into the viral envelope can alter the structure of the envelope protein.

CONCLUSIONS

Analysis of the fatty acid composition of the bacterium confirms the identification of the bacterium as *B.brevis.* The change in the fatty acid composition of this identified bacterium when compared to B-33 and B-34 strains of *B.brevis* is found to be due to the stress effect of thiocyanate. This reveals that the bacterial fatty acid synthesis depends upon the environment.

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