Scholars Journal of Applied Medical Sciences (SJAMS)
Abbreviated Key Title: Sch. J. App. Med. Sci.
ISSN 2320-6691 (Online)
ISSN 2347-954X (Print)

A Unit of Scholars Academic and Scientific Society, India
www.saspublishers.com

Laboratory Medicine

Biosynthesis of Ant carcinogenic Conjugated Linoleic Acid by Probiotics

Rajiv Kumar Chawla[1]*, Disha. S. Goyal[2]
[1]Department of Laboratory Medicine, Consultant Microbiologist Delhi Heart Institute & Multispeciality Hospital, Bhatinda-151001, Punjab, India
[2]Department of Laboratory Medicine, Consultant Pathologist Delhi Heart Institute & Multispeciality Hospital, Bhatinda-151001, Punjab, India

Original Research Article

***Corresponding author**
Rajiv Kumar

Article History
Received: 28.01.2018
Accepted: 10.02.2018
Published: 15.02.2018

DOI:
10.36347/sjams.2018.v06i02.008

Abstract: Conjugated linoleic acid (CLA) is a fatty acid with many health effects, which includes its anti-carcinogenic activity. On the other hand Probiotics have been shown to convert linoleic acid (LA) into conjugated linoleic acid. As dietary agents are more consumer acceptable and the research showed that probiotics acts as immunopotentiaters. But probiotics cannot be used as immunocompromised host. Conjugated linoleic acid (CLA) can ameliorate the immune responses as well as acts as ant cancerous hence present study was conducted to Biosynthesis of ant carcinogenic conjugated linoleic acid by microorganism *L. acidophilus* MTCC 447 in vitro.

Keywords: Anti-carcinogenic, CLA- Conjugated linoleic acid, LA- linoleic acid, Lactobacillus acidophilus.

INTRODUCTION

Conjugated linoleic acid (CLA) is a term that refers to a mixture of positional and geometric isomers of linoleic acid (LA) in which double bonds are conjugated. CLA has

Received great attention for their beneficial health properties. It has been reported to prevent carcinogenesis [1-3], atherosclerosis [9] and reduce body fat [5, 6] and modulates immune response [7]. It has been found to possess ant oxidative effect which is helpful in preventing many chronic diseases by inhibiting free radicals [8] .

CLA is a term for specific isomers (forms) of linoleic acid with conjugated double bonds (double bonds adjacent to each other C=C-C=C). Of the 20 different isomers of CLA that have been identified, the cis 9-trans 11 form (commonly called "rumenic acid") is believed to be the most common natural form of CLA that is found in diet food and dairy substances. The production of CLA by micro-organism has been reported to convert linoleic acid to Conjugated linoleic acid. Jiang [9] reported the formation of CLA from linoleic acid by *Propionibacterium*, Lin [1], Ogawa [10] Alonso [1] and John [11] reported the formation of CLA from linoleic acid by using *Lactobacillus acidophilus* bacteria. B. Yang, H. Chen, Z. Gu, [12] Synthesis of conjugated linoleic acid by the linoleate isomerase complex in food-derived lactobacilli**.** Kim J.H [13] Conjugated Linoleic Acid: Potential Health Benefits as a Functional Food Ingredient keeping above in view the present study was designed to Biosynthesis of anti-carcinogenic conjugated linoleic acid by probiotics.

MATERIALS AND METHODS

Microorganisms

Lactobacillus acidophilus MTCC 447 was procured from Institute of Microbial and Technology (IMTECH), Chandigarh as freeze dried ampoule and revived on specified media and then in MRS medium at 37ºC for 24 h and checked for specificity by routine microbiological methods.

Linoleic acid and Conjugated Linoleic acid

Linoleic acid and conjugated linoleic acid were purchased from Sigma-Aldrich private limited.

Biosynthesis of CLA

The Biosynthesis of CLA (BCLA) was done by the method of Alonso *et al.* [1] by using Linoleic acid. *Lactobacillus acidophilus* was incubated at 37ºc for 24 hours and 48 hours respectively in the presence of Linoleic acid. Thereafter flasks were centrifuged and supernatant was collected. The supernatant was analyzed by TLC (Thin layer chromatography). The supernatant was mixed with internal standard Heptadecanoic acid and again centrifuged. The whole

sample was collected in round bottom flask and dried under Nitrogen gas at 65ºC temperature in LV- rotary evaporator. The methylation of mixture was done by adding 14% of Boron- Difluoride in methanol. The organic layer was recovered and analyzed for CLA production by using Gas-chromatography (GC) from National institute of Pharmaceutical Education and Research (NIPER), MOHALI (PUNJAB) CLA peaks were identified with Retention time (RT) and were compared with commercial available CLA (CCLA) from Sigma-Aldrich.

ACKNOWLEDGMENTS

The Support to the Department is provided by Delhi Heart Institute & Multispecialty Hospital under instruments were purchased by the Department of Laboratory Medicine is fully acknowledge.

RESULTS

Results of Thin layer chromatography (TLC)

The supernatant of the *lactobacillus acidophilus* culture was collected after centrifugation at 2300xg and was subjected to thin layer chromatography (TLC) to confirm the production of Bioconverted conjugated linoleic acid (BCLA) and to compare it with commercially available conjugated linoleic acid (CCLA). The results are given below:

Suitable solvent systems

Hexane: Ethyl acetate
80: 20

Derivitizing reagent = Iodine

Number of spots = 2

R_f values:

CCLA Rf = 0.5

BCLA Rf = 0.42

RESULTS OF GAS CHROMATOGRAPHY

It was observed from the chromatogram that the production of BCLA was affected by the time period as it was observed that the higher amount of CLA (80 %) was produced from the culture incubated for 24-hrs. The CLA produced by the culture after 48 hrs reduced to 55.2%. (Given in FIGURES 1,2,3 respectively)

Quantification of BCLA

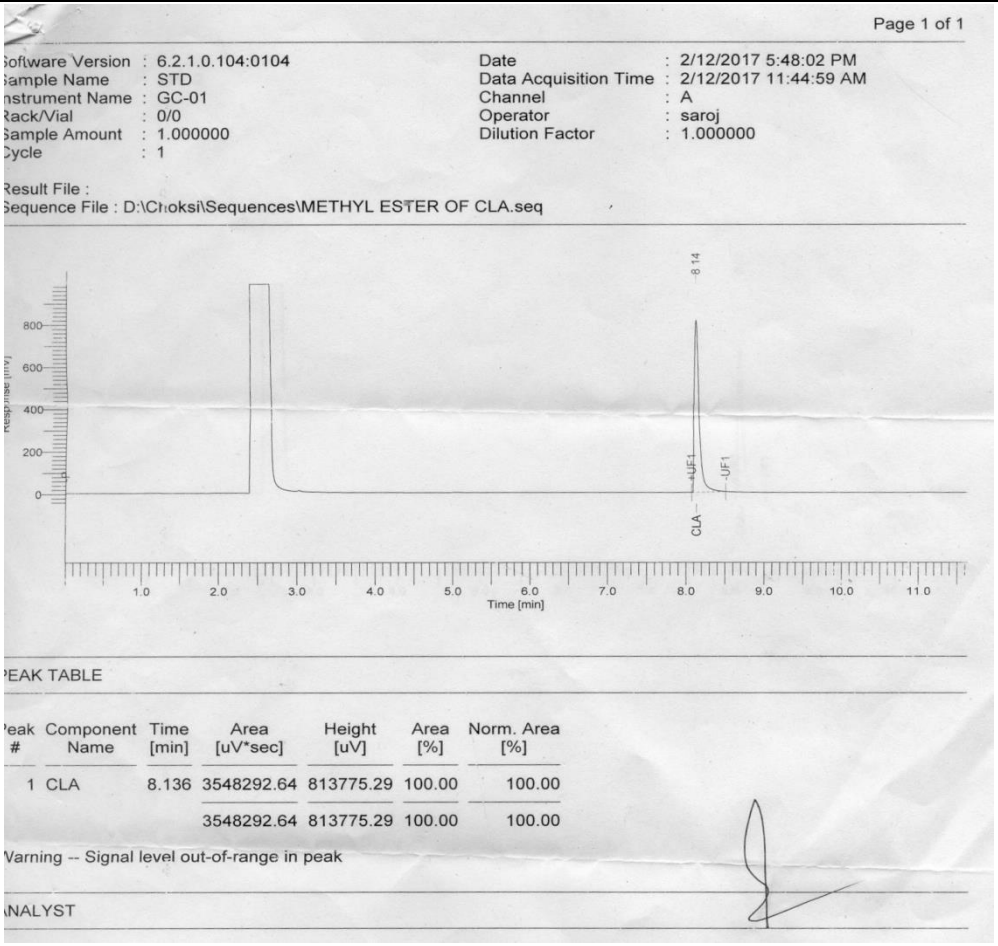
$$\% \text{ CLA produced} = \frac{\text{Peak Area produced by the Sample}}{\text{Peak Area produced by the standard}} \textbf{ X 100}$$

$$\% \text{ CLA produced (24 hrs)} = \frac{2840913}{3548292} \text{ X } 100$$

$$= 80 \%$$

$$\% \text{ CLA produced (48 hrs)} = \frac{1958795}{3548292} \textbf{ X 100}$$

$$= 55.2\%$$



Page 1 of 1

oftware Version : 6.2.1.0.104:0104
ample Name : STD
nstrument Name : GC-01
ack/Vial : 0/0
ample Amount : 1.000000
ycle : 1

Date : 2/12/2017 5:48:02 PM
Data Acquisition Time : 2/12/2017 11:44:59 AM
Channel : A
Operator : saroj
Dilution Factor : 1.000000

esult File :
equence File : D:\Chromatography\Sequences\METHYL ESTER OF CLA.seq

EAK TABLE

eak #	Component Name	Time [min]	Area [uV*sec]	Height [uV]	Area [%]	Norm. Area [%]
1	CLA	8.136	3548292.64	813775.29	100.00	100.00
			3548292.64	813775.29	100.00	100.00

Varning -- Signal level out-of-range in peak

NALYST

Fig-1: Chromatogram of the commercially available conjugated linoleic acid

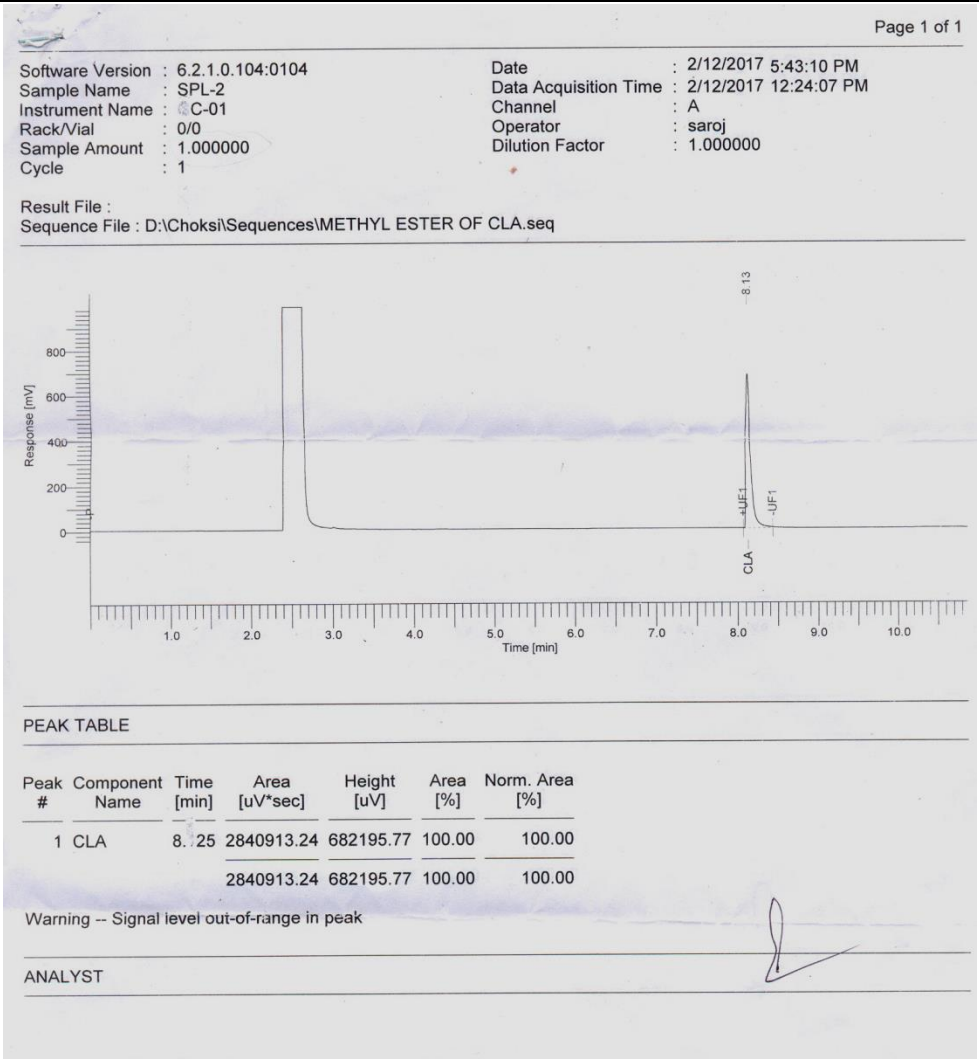


Fig-2: Chromatogram of the Bioconverted conjugated linoleic acid after the incubation of 24 hours

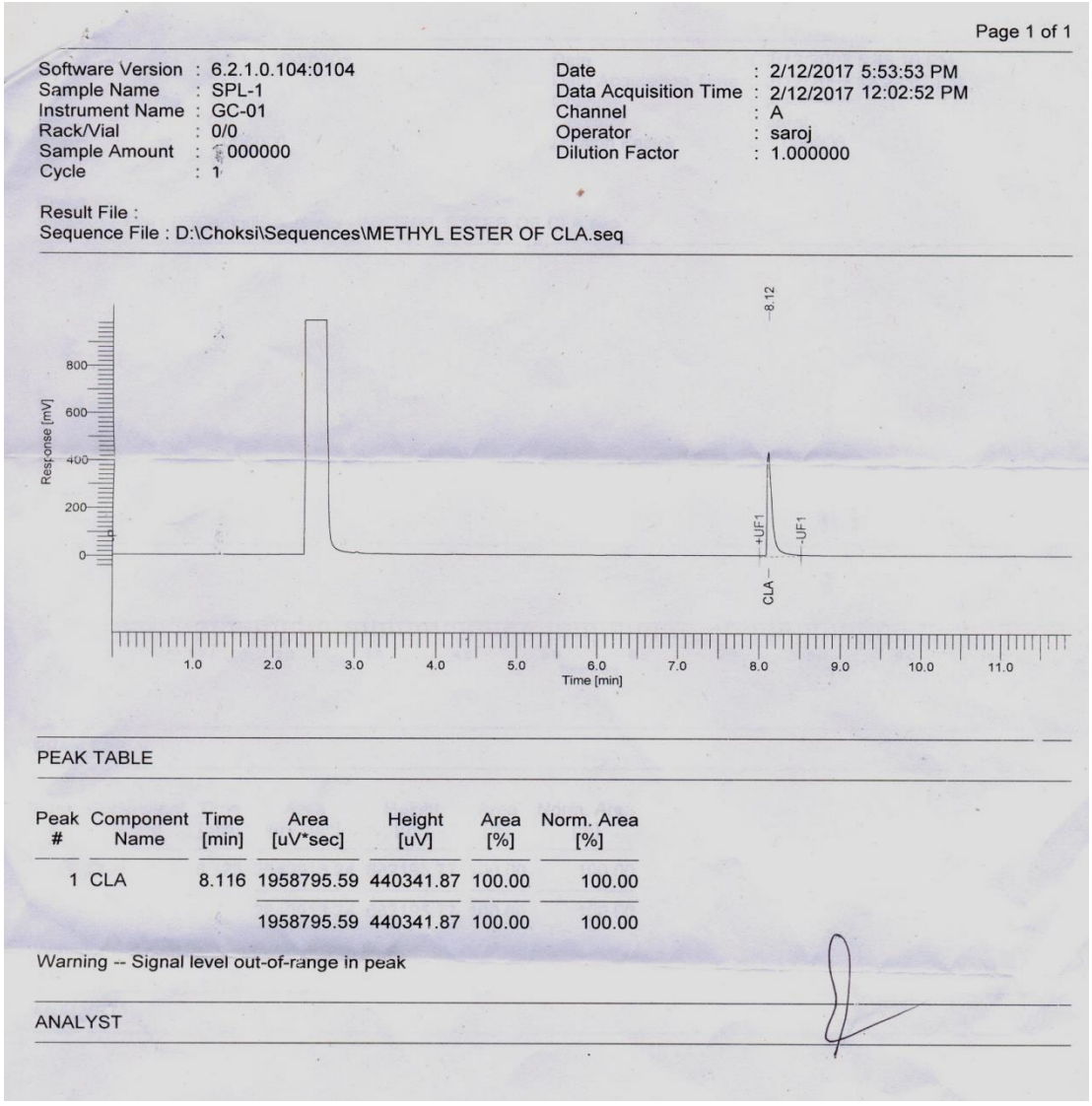


Fig-3: Chromatogram of the Bioconverted conjugated linoleic acid after the incubation of 48 hours

DISCUSSION

In the present work CLA was produced by bioconversion of linoleic acid in vitro to conjugated linoleic acid by *Lactobacillus acidophilus.* On the other hand linoleic acid has been reported in many food articles. Hence it is concluded that microorganism may be employed to produce conjugated linoleic acid from linoleic acid and further BCLA can be applied as an immunotherapeutic agent in many disease which are related to immune response of the individual. The CLA can be employed as safe immunomodulatory even in immunosuppressed host.

Moreover the presence of linoleic acid in many food items increase the application of CLA as food items can be used for production of CLA *in vitro* by *Lactobacillus acidophilus* and collected CLA may be applied to prepare nutraceuticals or *Lactobacillus acidophilus* or probiotics can be given along with foods and linoleic acid so that bioconversion occur in body itself. CLA can be applied as an alternative source of immunomodulatory or immunotherapeutic agent in various infected or non-infected immune related diseases.

REFERENCES

1. Pariza MW, Park Y, Cook ME. Conjugated linoleic acid and the control of cancer and obesity. Toxicological sciences: an official journal of the Society of Toxicology. 1999 Dec 1;52(suppl_1):107-10.
2. Wang Y, Jones PJ. Dietary conjugated linoleic acid and body composition. The American journal of clinical nutrition. 2004 Jun 1;79(6):1153S-8S.
3. Ip C, Lisk DJ, Scimeca JA. Potential of food modification in cancer prevention. Cancer research. 1994 Apr 1;54(7 Supplement):1957s-9s.
4. Nicolosi RJ, Rogers EJ, Kritchevsky D, Scimeca JA, Huth PJ. Dietary conjugated linoleic acid reduces plasma lipoproteins and early aortic atherosclerosis in hypercholesterolemic hamsters. Artery. 1997;22(5):266-77.
5. Terpstra AH. Effect of conjugated linoleic acid on body composition and plasma lipids in humans: an

overview of the literature. The American journal of clinical nutrition. 2004 Mar 1;79(3):352-61.

6. Sophie D, Benoit L. Metabolic syndrome and effect of conjugated linoleic acid on obesity and lipoprotein disorders. Am. J. Clin. Nutr. 2004;79:1149S-52S.
7. 14. Klein, A., Friedrich, U., Vogelsang, H. and Jahreis, G.(2008) *Lactobacillus acidophilus* 74-2 modulate unspecfic cellular immune response in healthy adults. Eur. J. Clin. Nutr., 62(5) : 583-593.
8. Liangli Yu. (2001)Conjugated linoleic acid isomer differs in their Free radicalscavenging Properties *J. Agri. Food. Chem.*, 51: 4135-4140.
9. Jiang J, Björck L, Fonden R. Production of conjugated linoleic acid by dairy starter cultures. Journal of Applied Microbiology. 1998 Jul;85(1):95-102.
10. Ogawa J, Matsumura K, Kishino S, Omura Y, Shimizu S. Conjugated linoleic acid accumulation via 10-hydroxy-12-octadecaenoic acid during microaerobic transformation of linoleic acid by Lactobacillus acidophilus. Applied and environmental microbiology. 2001 Mar 1;67(3):1246-52.
11. Wallace RJ, McKain N, Shingfield KJ, Devillard E. Isomers of conjugated linoleic acids are synthesized via different mechanisms in ruminal digesta and bacteria. Journal of lipid research. 2007 Oct 1;48(10):2247-54.
12. Yang B, Chen H, Gu Z, Tian F, Ross RP, Stanton C, Chen YQ, Chen W, Zhang H. Synthesis of conjugated linoleic acid by the linoleate isomerase complex in food-derived lactobacilli. Journal of applied microbiology. 2014 Aug 1;117(2):430-9.
13. Kim JH, Kim Y, Kim YJ, Park Y. Conjugated linoleic acid: potential health benefits as a functional food ingredient. Annual review of food science and technology. 2016 Feb 28;7:221-44.
14. Alonso L, Cuesta EP, Gilliland SE. Production of free conjugated linoleic acid by lactobacillus acidophilus and lactobacillus casei of human intestinal origin1. Journal of Dairy Science. 2003 Jun 1;86(6):1941-6.