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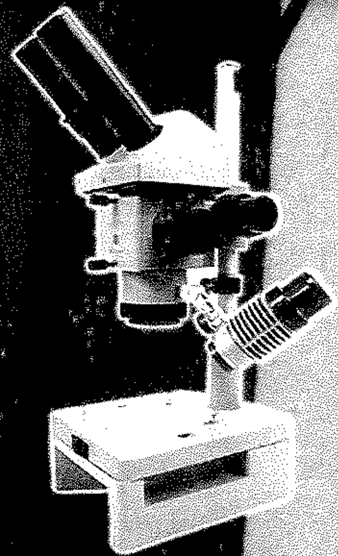

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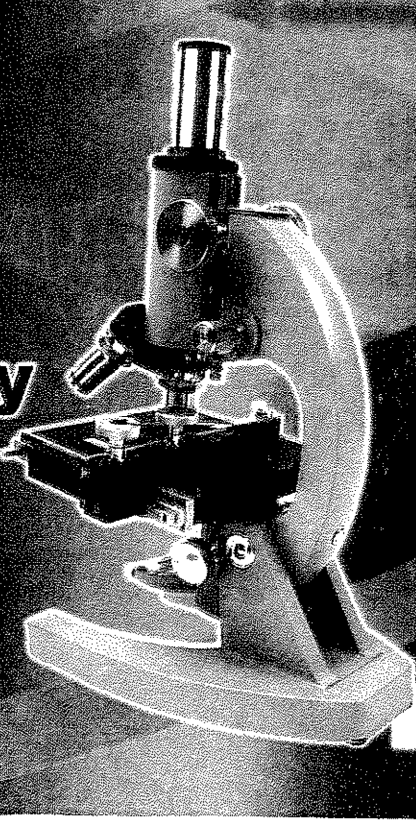

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EFFECT OF GROWTH TEMPERATURE AND TEMPERATURE TRANSITION ON STEREOSPECIFIC DISTRIBUTION OF FATTY ACIDS IN *APHANIZOMENON SP.* GLYCEROLIPIDS

By

Hana M. Gashlan [1], Khalid O. Abulnaja[1], Terence J, Walton[2]

From

[1] *Department of Biochemistry, Faculty of Science, King Abdul-Aziz University*

[2] *Biochemistry Research Group, School of Biological Science, University of Wales Swansea.*

ABSTRACT

In this study of *Aphanizomenon sp.* culture grown at either 28°C or 15°C or during temperature transition from 28°C to 15°C were investigated for the stereospecific distribution of fatty acid in the major glycerolipid classes. The results indicated that the positional distribution of fatty acid in all lipid classes from culture grown at 28°C and 15°C were characterized by the predominance of C-18 fatty acid at the *sn*-1 position and C-16 fatty acid at the sn-2 position, in a pattern consistent with that described for other group 2 cyanobacteria. Cells growth at 15°C were characterized by high proportion of unsaturation fatty acids compared to growth at 28°C. The increase in the degree of unsaturation was induced essentially by increasing C18:3 at *sn*-1 position in MGDG and DGDG and increasing C16:3 at *sn*-2 in PG. The positional distribution was not altered except that in the PG fraction, the C16:1 was esterified in *sn*-1 position instead of *sn*-2 position in cell grown at 15°C. In changing the growth temperature from 28°C to 15°C (24h and 48h) the Sterospecific distribution of fatty acid at *sn*-1 and *sn*-2 glycerolipids was largely conserved with C-16 fatty acid dominating at *sn*.2 and C-18 fatty acid dominating at *sn*-1. The most prominent change induced by shift to lower temperature was decrease in C18:1 and C18:3 levels at *sn*-1 accompanied by an increase in C16:3 at both *sn*-1 and *sn*-2 for MGDG.

INTRODUCTION

Temperature is one of the most important environmental factors that influence the fatty acid composition of membrane lipids (**Sumner *et al.*, 1969; Hazel and Prosser, 1974**). Poikilotherm has to adapt their lipid composition during sudden changes in environmental temperature in order to preserve lipid bilayer phase fluidity and membrane function (**Manson and Kates, 1984**). For example compositional studies of cyanobacteria have demonstrated that growth at low temperature causes one or more changes in membrane lipid composition, including increases in fatty acid unsaturation, shortening in acyl chain length, changes in the proportions of lipid classes and changes in lipid; protein ratio (**Holton *et al.*, 1964; Sato *et al.*, 1979; Suutari *et al.*, 1990, 1997 and Cossins, 1994**).

The glycerolipids of *A. nidulans*, a group 1 cyanobacterium, contain fatty acids with 14, 16 and 18 carbon atoms; these fatty acids are distributed on the glycerol backbone of glycerolipids with daturated and monosaturated fatty acids that estrified mainly at the *sn*-1 and *sn*-2 position respectively. Lowering the growth temperature led to, a decrease in chain length of saturated fatty acid at *sn*-1 of all lipid classes, and at the same time, to an increase desaturation of 16:0 to 16:1 at *sn*-2 of monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) (**Sato *et al.*, 1979**). In contrast in *A. variabilis*, which contains polyunsaturated fatty acids of 18 and 16 carbon atoms, localized respectively at *sn*-1 and *sn*-2 positions, growth at lower temperature indicated that only desaturation of C-18 acids was dependent on the growth temperature in all four major lipid classes (MGDG, DGDG, PG, and SL) (**Sato *et al.*, 1979**).

In the Baltic, *Aphanizomenon sp.* and other cyanobacteria are exposed during the summer to a wide range of temperature from a maximum of 30°C whilst floating at the surface under calm conditions to a minimum of 10°C when they sink to the thermocline following a wind induced mixing event.

In our previous studies (under press) we established that *Aphanizomenon sp.* modulates the fatty acid composition of its membrane glycerolipids in response to changes in growth temperature. This work describes further studies into the detail of these changes by an analysis of the stereospecific distribution of fatty acids within each of the major glycerolipid classes from *Aphanizomenon sp.* cultures grown either isothermally at 28°C or at 15°C and during temperature transition from 28°C to 15°C.

MATERIALS AND METHODS

Growth Conditions:

A cultures of *Aphanizomenon sp.* (obtained from the biochemistry research group, university of Wales, Swansea) were grown photoautophically up to 25 days in modified ASM-1 liquid medium lacking a fixed nitrogen source, and isothermally at two different temperature either "high temperature" (28°C) or "low temperature" (15°C). Another culture of *Aphanizomenon sp.* was grown in the same medium for 10 days, and then the incubator temperature was then reduced to 15°C where the growth continued for 3 days.

Extraction of Total Lipid Fraction

Cells of *Aphanizomenon sp.* culture grown at either 28°C or 15°C or during temperature transition were harvested by centrifugation, normally at 3,000xg essentially according to the method of **Bligh and Dyer (1959)** as modified by **Sato and Murata (1981)**.

Separation of Glycerolipid Classes

The major glycerolipid classes were separated by preparative silica gel-G TLC. Total lipid extracts were applied as a short band to silica gel-G TLC plates together with authentic samples of MGDG, DGDG, PG and SL. Glycerolipid classes were developed in chloroform-methanol-acetic acid-water, 170: 30: 20:7 (by vol.). Plates were sprayed with 0.05% (w/v) primulin in acetone/water (4: 1. v/v), and glycerolipid were detected under UV light. Visualization also and used for lipase hydrolysis. The MGDG fraction isolated as described above was subjected to further preparative TLC in a solvent of chloroform – methanol – acetic acid – water 85: 15: 5: 1 (by vol.).

The Stereospecific Distribution of Fatty Acids

The distribution of fatty acids at the *sn*-1 and *sn*-2 position in each glycerolipid fraction recovered from preparative silica gel-G T was established by lipase hydrolysis as described by **Sato and Murata (1988)** which was developed from the procedures described by **Fischer *et al.*, (1973)**. The lysoglycerolipid containing the *sn*-2 fatty acid was recovered by double development TLC. Samples of each lysoglycerolipid and the original glycerolipid from which the sample was derived were individually transesterified, and the resulting fatty acid methyl aster FAME fractions were subjected to GLC analysis.

The fatty acid composition at *sn*-2 was determined directly from the lysoglycerolipid samples and the composition at *sn*-1 was determined by difference between the original glycerolipid and the corresponding lysoglycerolipid, according to the following expression,

sn-1 fatty acid =[2x glycerolipid overall fatty acid composition]– [glycerolipid *sn*-2 fatty acid composition]

RESULTS AND DISCUSSION

Effect of Growth Temperature on the Stereospecific Distribution of Fatty Acids.

Figure 1 and 2 show the fatty acid composition of MGDG, DGDG, PG and SL fractions together with the positional distribution of the fatty acids at *sn*-1 and *sn*-2 for culture grown at 28°C and 15°C respectively. The positional distribution of fatty acids in all lipid classes from culture grown at 28°C and 15°C was characterized by the predominance of C-18 fatty acids at the *sn*-1 position and C-16 fatty acids at the *sn*-2 position, in a pattern broadly consistent with that described for other group 2 cyanobacteria (**Sato and Murata, *et al.*, 1979**). When *Aphanizomenon sp.* cells grown at low temperature (15°C) compared with cells grown at high temperature (28°C) (figures 1 and 2), several differences in fatty acid composition could be detected. Low growth temperature led to an increase in the degree of unsaturation which accompanied by increase in the proportion of unsaturation fatty acids and decrease in the proportion of saturated fatty acid in all classes, in MGDG and DGDG fractions, the relative proportion of C16:0 at the *sn*-2 position in cells grown at 28°C was 68.8% and 86.5% respectively. Whilst it was reduced to 55.3% and 72.6% at 15°C in MGDG and DGDG. This reduvtion in c16:0 was accompanied by a sharp increase in the proportion of C16:3 at *sn*-2 of the MGDG and DGDG fractions in cell grown at 15°C. At the lower temperature an approximate doubling in the relative proportion of C18:3 fatty acid at *sn*-1 position in MGDG and DGDG was also observed, whilst the relative proportions of C18: and C18:2 at *sn*-1 were decreased. The C16:0 fatty acid in PG fraction was present mainly at the *sn*-2 position in cell grown at 28°C, where it represented 48.2% of the fatty acid, whilst it represented 51.3% in cells grown at 15°C (Fig. 2). At *sn*-2 C16:1 represented 24.1% of fatty acid, but could not detected at 15°C, whilst C16:3 which was absent at 28°C and constituted 37.3% of the PG *sn*-2 fatty acid at 15°C. In the SL fraction C16:0 was the only C-16 fatty acid at the *sn*-2 position at either high temperature (28°C) or low temperature (15°C), and there was little difference in the relative proportion at the two growth temperatures C-18 acids were located almost entirely at the *sn*-1 position in SL from cells grown at 28°C and 15°C, and an increase in the relative proportion of C18: at *sn*-1 was seen in cells grown at 15°C, and this increase was accompanied by a marked decrease in the relative proportion of C18:1 and C18:2 observed at 28°C. In spite of all these changes in fatty acid relative proportion, the positional distribution was not altered except that in the PG fraction, the C16:1 fatty acid was estrified in *sn*-1 position instead of *sn*-2 position in cells grown at 15°C.

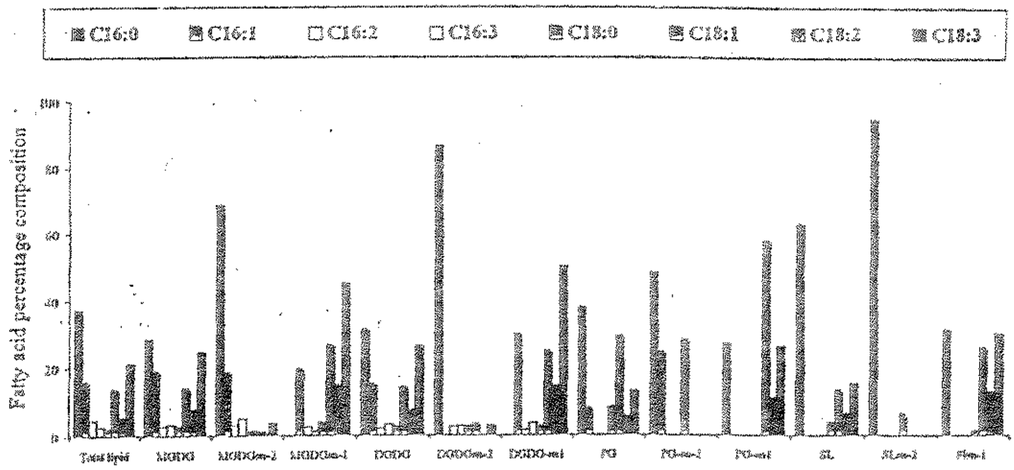


Figure 1. The positional distribution of fatty acid in Total lipid, MGDG, DGDG, PG, and SL from *Aphanizomenon sp.* culture at 28°C

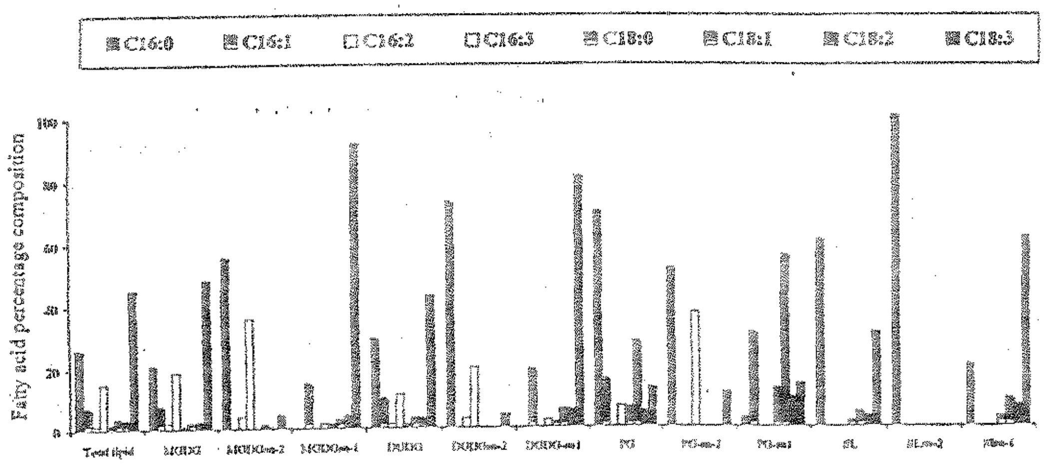


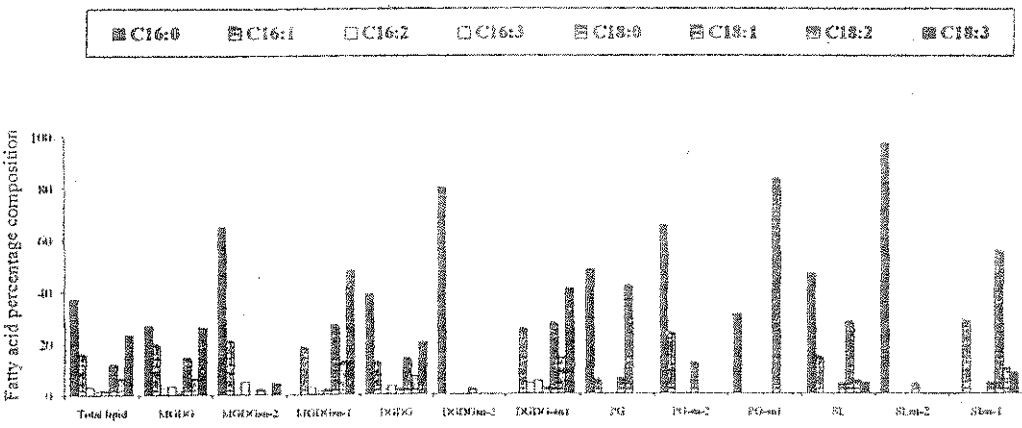
Figure 2. The positional distribution of fatty acid in Total lipid, MGDG, DGDG, PG and SL from *Aphanizomenon sp.* culture at 15°C.

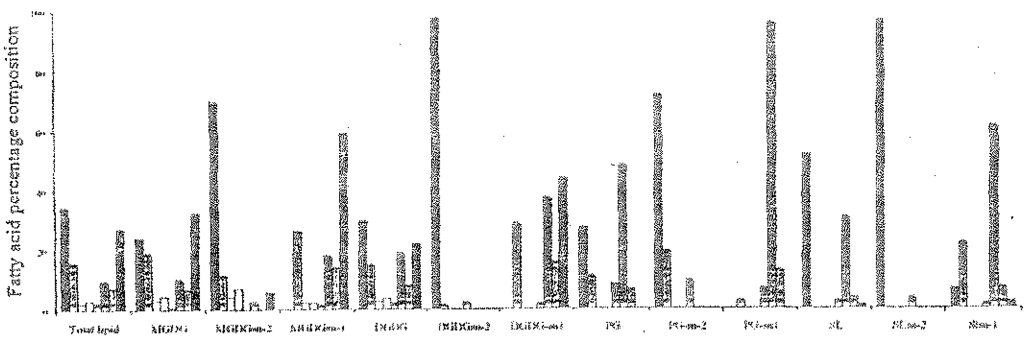
This finding is in contrast to that of **Sato *et al.*, (1979)**. They found that in *Anabaena variabilis* the C-16 and C-18 acids were located almost exclusively at *sn*-1 and *sn*-2 position respectively in all the lipid classes. It thus appears that in group 2 cyanobacterium *Aphanizomenon sp.* as in *A. viriabilis*, adaptation of fatty acid composition is different from that in group 1 cyanobacteria e.g. *Anacystis nidulans*. In this organism **Sato *et al.*, (1979)**, found that in growth at reduced temperature the change in chain length was found at *sn*-1 position; the monounsaturated acids were dominant at *sn*-1 and saturated once at *sn*-2. When the growth temperature was changed, the chain length of monounsaturated fatty acids was varied at *sn*-1, and the desaturation of C-16 acids in galactolipids was influenced at *sn*-2. In the acidic lipids (SL and PG), only palmitic acid eas estrified to *sn*-2, and no change with growth temperature was observed at this position.

Effect of Temperature Transition on the Stereospecific Distribution of Fatty Acids

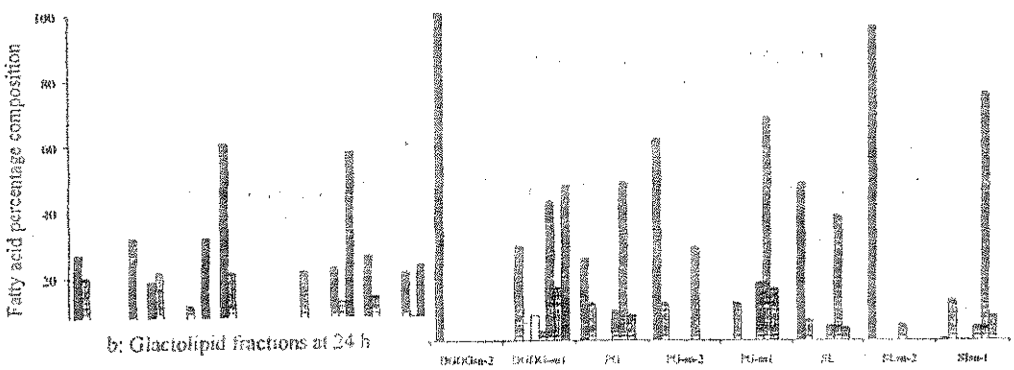
During adaptation to low temperature (15°C. 24h and 48h), the stereo specific distribution of fatty acid at *sn*-1 and *sn*-2 of the glycerolipid found during growth at 28°C was largely conserved with C-16 fatty acids predominant at *sn*-2 and C-18 fatty acids dominating at *sn*-1 (Fig. 3). The most prominent change brought about by the shift to lower temperature in the galactolipid fractions was a decrease in C18:1 and C18:3 levels at *sn*-1 accompanied by an increase in C16:3 at both *sn*-1 and *sn*-2 of MGDG. The observed changes in the C-18 fatty acids at sn-1 are consistent with direct desaturation of C-18 acids occurring at *sn*-1 of pre-existing MGDG species to yield C18:3 MGDG. No significant increase in C18:3 in either the PG or SL fractions was apparent, but an increase in the proportion of C18:1 in the SL fraction was observed, suggesting that the accelerated synthesis of C18:1 and replacement of C16:1 and C18:3 with the newly synthesized C-18 monoenoic fatty acid is an effect specifi to this lipid class.

In contrast, in *viriabilis*, during the first 10^{th} following the downward temperature shift, there was a substantial decrease in the C16:0 in the MGDG fraction whilst there was a concomitant increase in C16:1 levels consistent with the direct desaturation of C16:0- MGDG to C16:1- MGDG (**Sato and Murata, 1980**). With further incubation at low temperature however, the relative contents of C16:0 and C16:1 fatty acids were almost restored to original level. In *Aphanizomenon sp.* C18:0 and C18:1 level were decreased and C18:3 was increased in a similar way to *A. variabilis* (**Sato and Murata, 1980**), consistent with direct desaturation of lipid-linked C18:0 and C18:1. These changes in the C-16 fatty acids at *sn*-2 and the C-18 fatty acids at *sn*-1 of the pre-existing MGDG species in *Aphanizomenon* to yield C18:3/C16:1- and C18:3/C16:3 – MGDG which accompanied by an increase in the degree of unsaturation would be expected to bring about an increase in the membrane fluidity allowing the organism to adapt to the lower temperature.





a: Glactolipid fractions at 0 h



b: Glactolipid fractions at 24 h

c: Glactolipid fractions at 48 h

Figure 3: Stereos specific analysis of fatty acid distribution in Galactolipids fraction from *Aphanizomenon sp.* culture following a temperature shift from 28 °C to 15 °C.

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تأثير درجات الحرارة على نمو الأفانيزومينون وتأثير تغيير درجات الحرارة على مكان توزيع الأحماض الدهنية الموجودة فى الجليسروليبيدات

فى هذه الدراسة ، تم تنمية خلايا الأفانيزومينون فى درجة حرارة ٢٨ وكذلك درجة حرارة ١٥ كلا على حده ، وكذلك خلال تنمية خلايا الأفانيزومينون فى درجة حرارة ٢٨ ثم تنخفض درجة الحرارة الى ١٥ لدراسة أماكن توزيع الأحماض الدهنية فى الليبيدات الأساسية الموجودة فى البكتريا ،

النتائج أكدت أن الأحماض الدهنية من نوع C-18 كان توزيعها ومكانها فى *sn*-1 ووجد أن الأحماض الدهنية من النوع C-16 أن توزيعها ومكانها فى *sn*-2 فى وضع مماثل لذلك الموضح لأنواع السيانوبكتيريا فى المجموعة ٢ ، الخلايا التى نمت عند درجة حرارة ١٥ تميزت بوجود نسبة كبيرة من الأحماض الدهنية الغير مشبعة مقارنة بالخلايا التى نمت عند درجة حرارة ٢٨.

وأوضحت الدراسة أن الزيادة فى نسبة الأحماض الدهنية الغير مشبعة من النوع C18:3 فى أحادى الجلاكتوزيل ثنائى أسيل جليسرول (MGDG) فى ثنائى الجلاكتوزيل ثنائى أسيل جليسرول (DGDG) وموجودة فى مكان *sn*-1 وكذلك زيادة C16:3 الموجودة فى الفوسفاتيديل جليسرول (PG) فى مكان *sn*-2 ،

وقد لوحظ أن توزيع الأحماض الدهنية لم يتأثر فى أنواع الجليسروليبيدات الأساسية الموجودة فى البكتريا فى عدا ذلك الذى فى جزء الفوسفاتيديل جليسرول (PG) وقد ظهر C16:1 فى *sn*-1 بدلا من *sn*-2 وذلك عند تنمية الخلايا فى درجة حرارة ١٥ ،

وفى خلال (٢٤ ــ ٤٨ ساعة) من تغيير درجة الحرارة من ٢٨ الى ١٥ وجد أن توزيع الأحماض الدهنية من نوع C-18 بقى كما هو فى *sn*-1 والتى هى من النوع C-16 بقيت فى *sn*-2 ،

وقد وجد أن التغيير الأبرز فى عملية تغيير درجة الحرارة من الأعلى إلى الأقل هو نقص مستوى C18:1 و C18:3 الموجودة فى *sn*-2 وزيادة مستوى C16:3 فى المكانين *sn*-1 و *sn*-2 فى الدهون من نوع أحادى الجلاكتوزيل ثنائى أسيل جليسرول (MGDG) ،