Antimicrobial activity of essential oil extracted from *Thymus vulgaris* on *Bacillus cereus* cells

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Introduction

- ***B. cereus*: gram-positive, rod-shaped, motile, beta hemolytic bacterium**
- **Isolated: cooked rice, dairy and meat products**
- **Known as foodborne pathogen**
- **Causing problems to foodstuff industry both**
- ✓ **by deteriorating the products**
- ✓ **by endangering people's health upon consuming contaminated foods**
- ***B. cereus* produce enterotoxins that cause food poisoning**
- **Danger posed by this organism: increased by its ability to adapt to chemical**

Schlegelova *et al*., 2003; Edward, 2010

Cont…

- **Resistant: β-lactam antimicrobial agents**
  - ✓ **Ampicillin**
  - ✓ **cephalothin**
  - ✓ **oxacillin.**

- **Production of β-lactamase enzyme: potential virulence factor that makes producing strain resistant even to 3rd generation of cephalosporins**

- **Literature review: Pathogenic specie, that is found in serious brain infections, gastrointestinal tract infections, post-operation and post-traumatic infections of wounds**

Fenselau *et al*., 2008; Kong *et al*., 2011

Cont…

- Serious concern especially in South Africa: little work has been done on resistant food-borne pathogens
- Threat : people with poor immune systems
- Causing severe health problems
- POTENTIAL PROBLEM !

Hoffmaster *et al*., 2006; Iwamoto *et a*l., 2010

Aims of Study

To investigate the cellular effects of *Thymus vulgaris* essential oil on antibiotic resistance *Bacillus cereus*

To quantify the mode of action of thyme oil on *B. cereus*

To assess morphological and chemical changes of the cell membrane

Materials and methods

SAMPLE COLLECTION

Samples obtained from South African hospices

↓

Sub-culture to agar plates

↓

Incubation at 37°C for bacteria

SAMPLE PREPARATION

Bio-assay preparation
Agar diffusion assay (Kock *et al.*, 2009)

Inhibitory effect of thyme oil
MICs were determined using microdilution assay, as previously described (Ismaili *et al.*, 2004)

Gram stain preparation
Gram stain was performed according to the method of El-Garnal *et al.* (2009)

Identification of fatty acids
Fatty acids were extracted using fatty acid extraction kit (Sigma-Aldrich, SA) and analyzed using GC-FID

Sample preparation for SEM and TEM

Fixation
Primary fixative - 3% gluterdialdehyde added to cells for 3h
Secondary fixative – 1% Osmium tetroxide added to cells for 1h

Dehydration
Samples dried through graded ethanol series (SEM) of acetone (TEM only) (50%, 70%, 95% & two changes of 100%)
Additional steps for TEM: impregnation, polymerization and making sections are performed

Drying
Samples dried using a critical point dryer

Coating
Samples coated using 200nm gold

ANALYSIS

SEM

TEM

BioMerieux, 2002; Venter *et al.*, 2004; Breakwell *et al.*, 2012

Results and Discussion

Inhibitory effect of thyme oil:

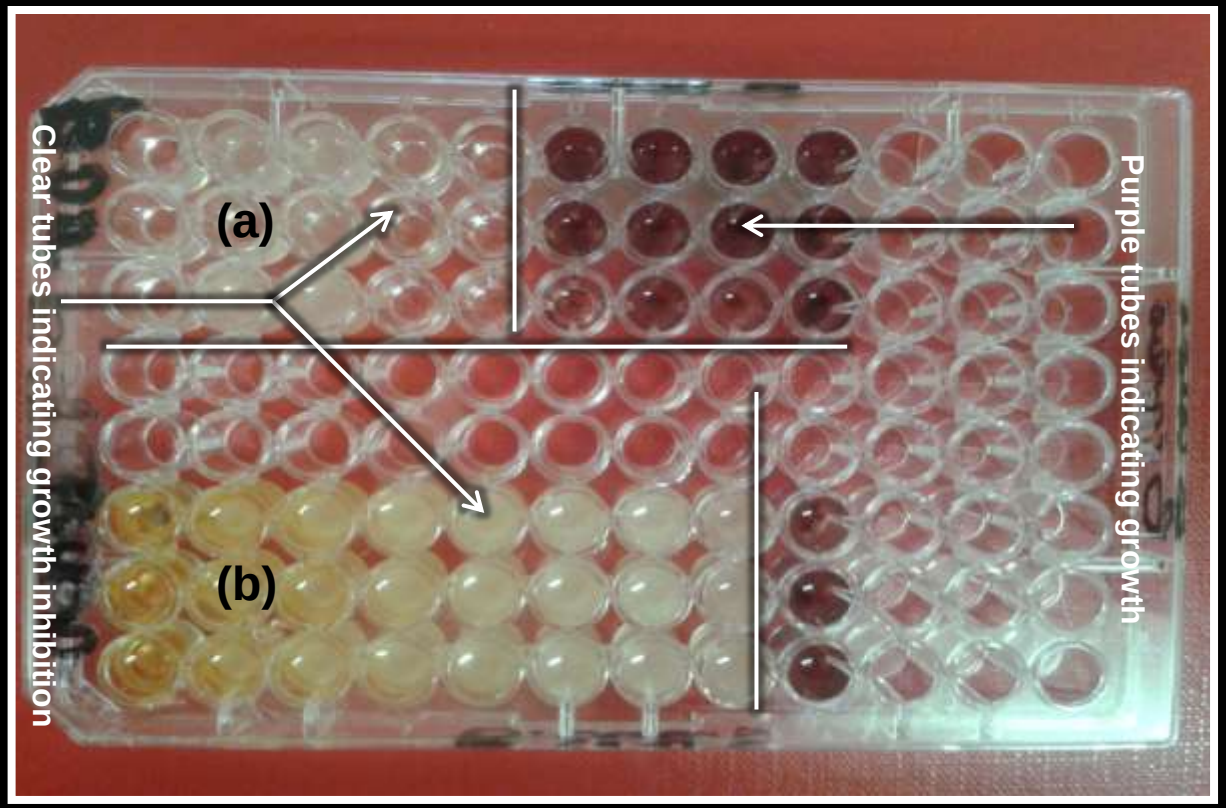


Figure 1: Microtiter plate assay for *B. cereus* isolate: (a) diluted thyme oil (2 ml of essential oil into 100 ml of ethanol) with 1.6 mcg/ml MIC (b) undiluted thyme oil with no MIC.

Bioassay:

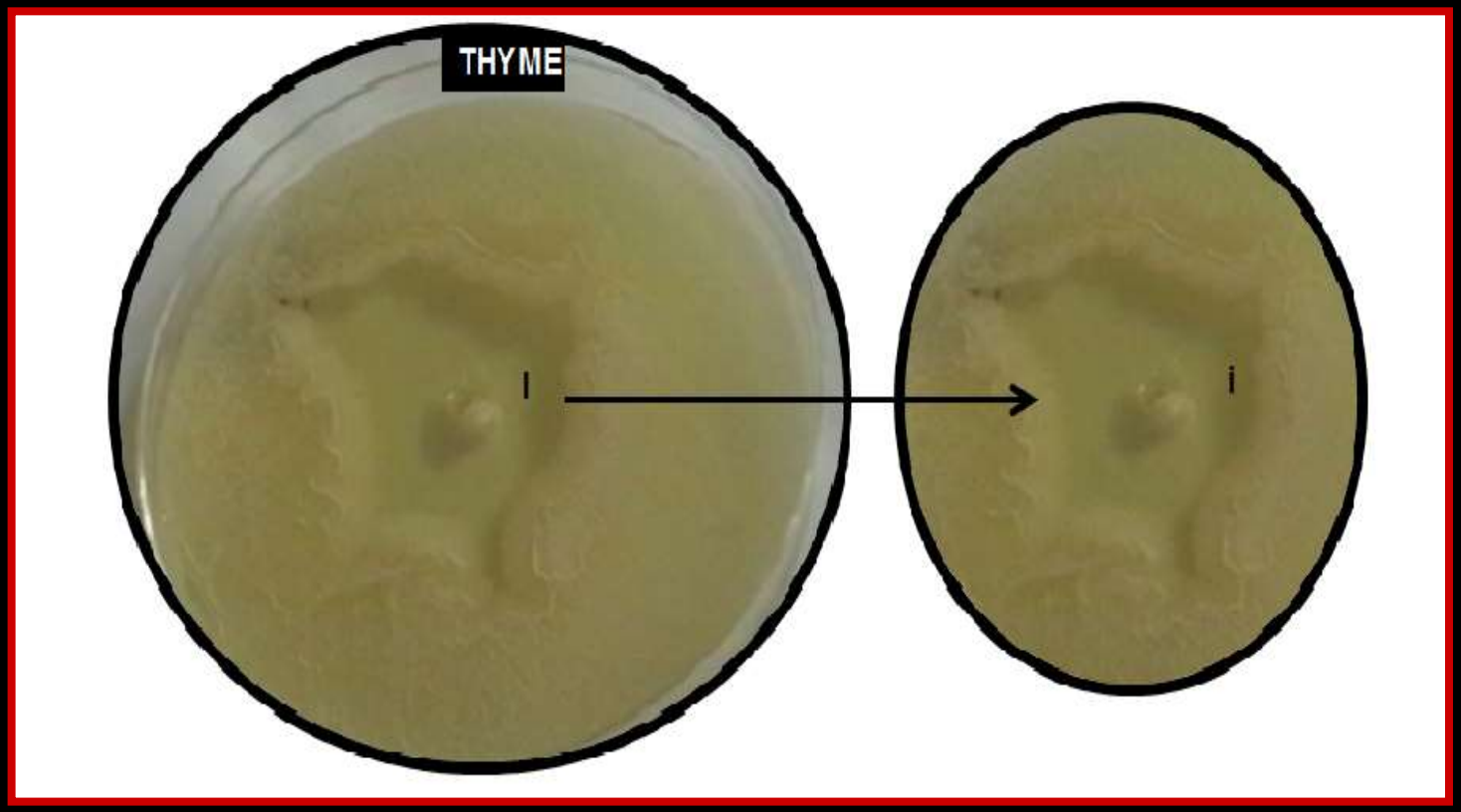
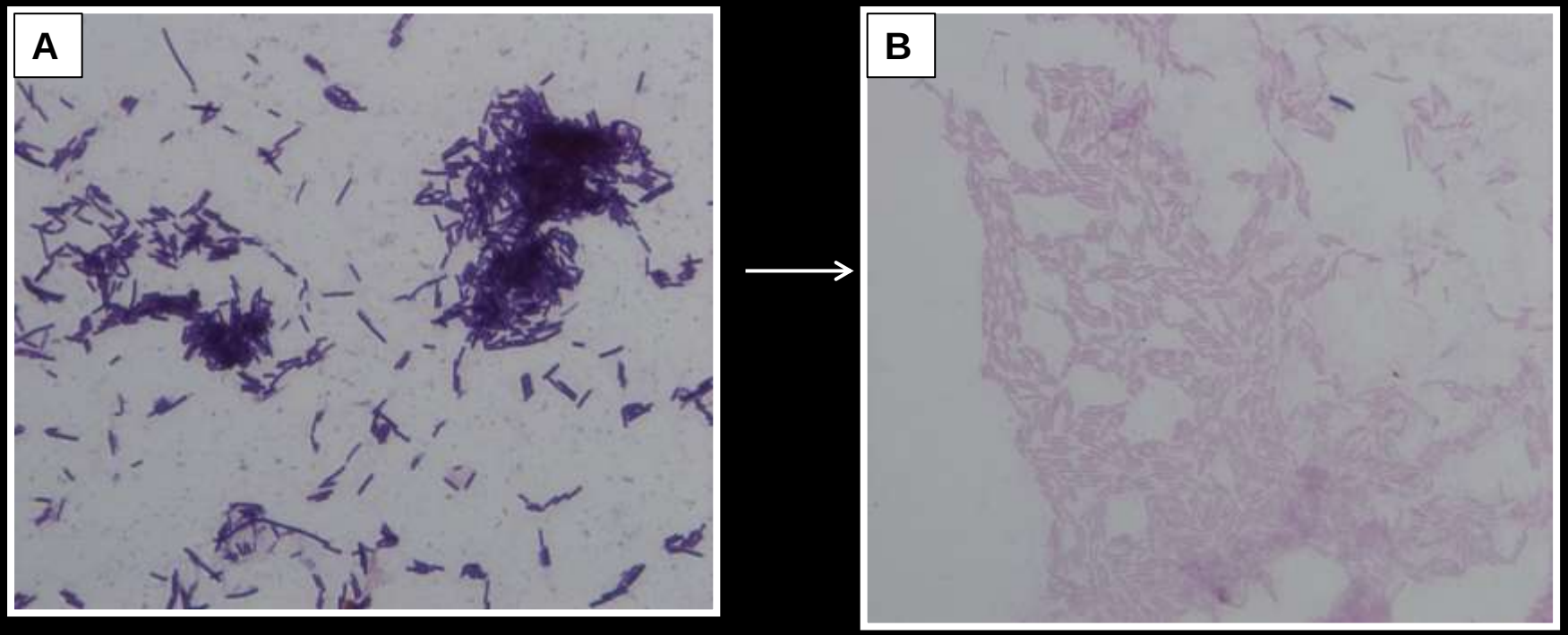


Figure 2: A bioassay showing antimicrobial activity of thyme oil against *B. cereus* on a agar plate. I – designates zone of inhibition.

Assessment of cellular damage produced by the oil on the bacterium :



B. cereus **untreated**

B. cereus **treated with thyme oil**

Figure 3: Gram stain light micrographs showing (A) untreated (control) *B. cereus* cells staining purple and (B) treated *B. cereus* cells staining pink indicating the negative effect of thyme essential oil on the bacteria.

Identification of changes in cell morphology : SEM

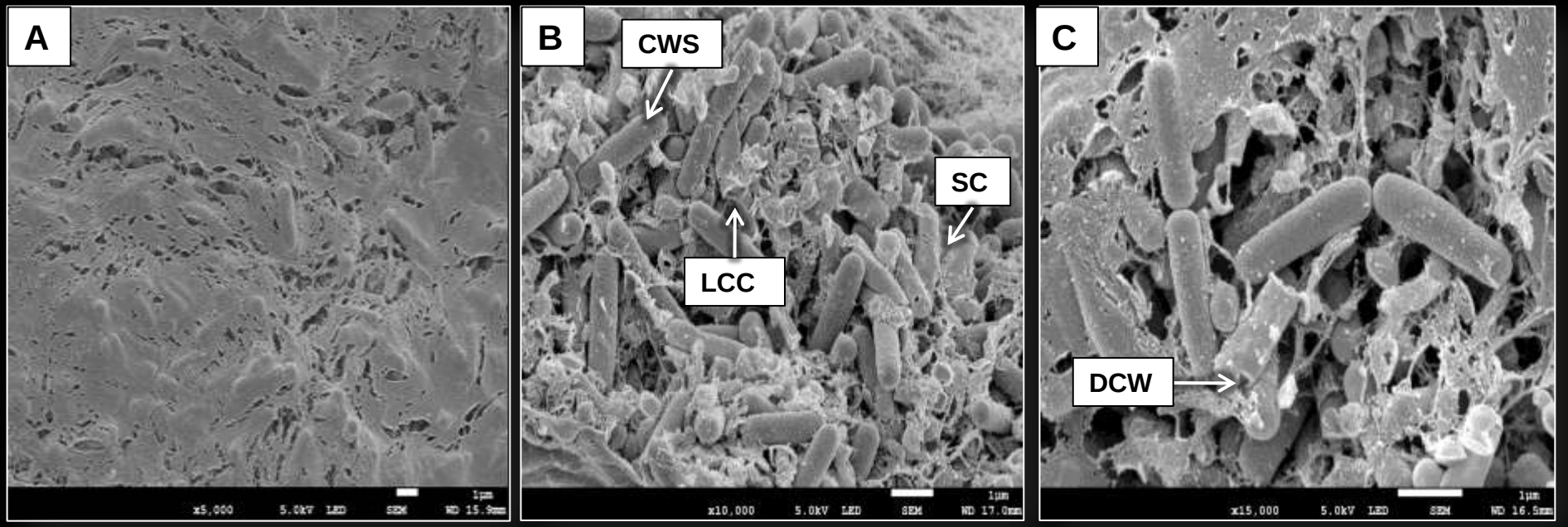


Figure 4: (A) control cells and (B-C) different types of injuries induced by thyme oil on the bacterial cell wall and membrane structure. DCW - Damaged cell wall with formation of holes on the cell surface; LCC - Loss of cellular contents; CWS -. Cell wall completely swollen; SC - shrinkage of the cell.

Identification of changes in cell morphology : TEM

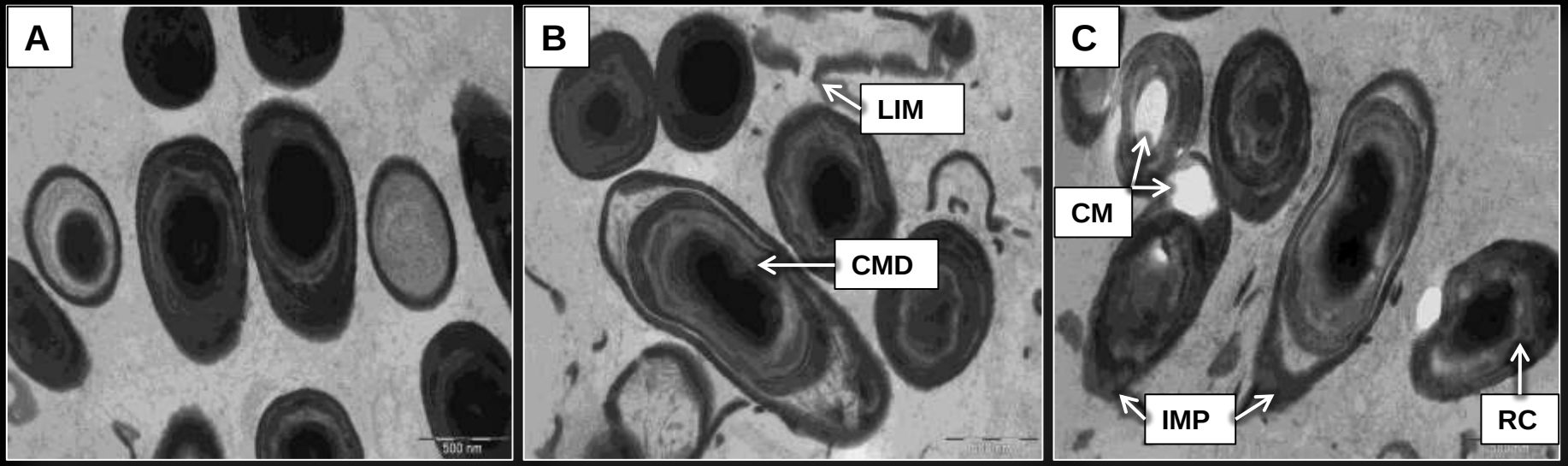


Figure 5: (A) control cells and (B-C) morphological changes of *B. cereus* cells after exposure to thyme oil and its major components. IMP - Increased membrane permeability that results on shrinkage of the protoplasm or cell wall deformation; LIM - Loss of intracellular material; RC -The slight roughness of the cell; CMD - The presence of cytoplasmic membrane damage; CM- Coagulated material

Identification of fatty acids: GC-FID

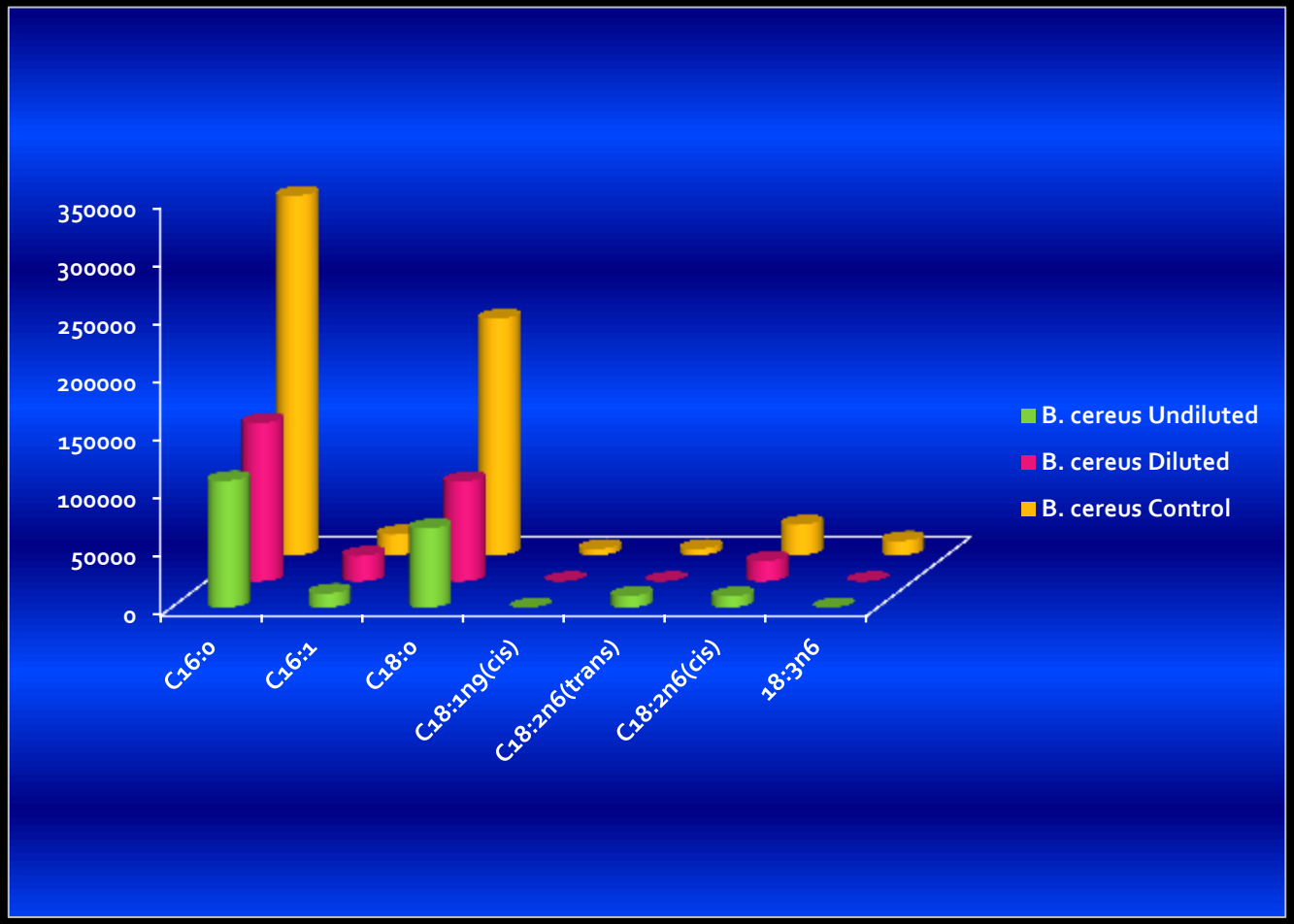


Figure 6: Fatty acid profile of *B. cereus* cells affected by *Thymus vulgaris* essential oil

Discussion

- In general, *Thymus vulgaris* essential oil showed strong antibacterial activity against *B. cereus.*

- Gram stain, SEM and TEM showed that thyme oil degrades the cell wall and membrane integrity of *B. cereus.*

- For example, loss of cellular contents, irregular cytoplasmic membrane, swollen cells, shrinkage of the cell, and the presence coagulated material, as indicated by SEM and TEM.

Discussion

- Decrease in the amount of saturated fatty acids (SFAs) when compared to untreated cells results in:
  - ✓ gain of membrane fluidity
  - ✓ and as a consequent decrease in membrane rigidity as noticeable by SEM examination

- Activity of thyme oil not attributed to single event: acts on the outer membrane, leads to dispersion of desaturase enzyme, no production of SFAs

- Similar results were obtained by Nazzaro *et al*. (2013)

- Results from the current and previous studies indicate the significant role played by thyme oil as potential antimicrobial agent for the fight against resistant *B. cereus*.

Conclusion

- **The current findings demonstrate that thyme oil damage the cellular membrane of antibiotic resistant *B. cereus*, which leads to cell death.**

- **As a result, this shows that thyme oil has the capability to target the bacterial sites of *B. cereus* that antibiotics such ampicillin, cephalothin and oxacillin failed to target.**

- **Thyme essential oil is therefore considered a potential antimicrobial agent.**

- **Moreover, from adequate scientific evidence provided in this study it can be concluded that, thyme essential oil might enhance the chances of developing new conventional and natural antimicrobial agents (drugs as well as food preservatives) and be good alternatives to synthetic chemicals.**

Thank you!!!