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The preservative effect of Trehalose on *Staphylococcus aureus* and *Escherichia coli*

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By

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Summary

Two isolates were used in this study, *Staphylococcus aureus* and *Escherichia coli* were identified according to morphological, physiological and biochemical characteristics.

The two bacteria were treated with 100mM of Trehalose to study the effect of this sugar on the preservation of the bacteria from several factors

The results showed that Trehalose preserved the *S.aureus* from dehydration (drying) for one month in room temperature and the Trehalose keep the bacteria more viable in comparison with the control. While no effect was observed in the Trehalose treated *E.coli*.

Trehalose showed it's preservative effect when the bacteria were stored in freeze condition (-20°C) about 4 weeks the percentages of Viable cells of *S.aureus* treated with Trehalose after 4 weeks was 67% compared with the control 30%, while the results on *E.coli* was 66% and the control 32%.

Results indicated that Trehalose protect the lyophilized bacteria even in the unsuitable environment such as storing in room temperature for four months, the percentage of the viable cells of bacteria treated with Trehalose after 4 months was 27% while the control 2% .

Result also showed the protective effects of Trehalose towered the UV irradiation and Electroporated bacteria.

While Trehalose had no effect on the protection from antimicrobial substance (antagonism activity), in contrast it showed a considerable effect of protection from antibiotics by increasing the resistance to the antibiotics, result showed that *S.aureus* treated with trehalose become resist to, Tetracycline, Gentamicin, Erythromycin and Norfloxacin, while the control stilled sensitive to these antibiotics, and no effect were showed on the *E.coli* in protection from antibiotics.

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List of Abbreviations

Abbreviation	Mean
TRE	Trehalose
BHI	Brain Heart Infusion
C	Chloramphenicol
TE	Tetracycline
CZC	Cestazidime
Cn	Gentamicin
TPS	Trehalose-6-phosphate synthesis
D.W.	Distilled Water
TPP	Trehalose-6-phosphate phoaphatase
RT-PCR	Reveares Transcriptase Polymer Chain Reaction
NOR	Norfloxacillin
hrs	Hours
MIC	Minimum Inhibitory Concentration
P	Pencillin
V	Vancomycin
S	Streptomycin
NA	Nalidixic acid
PB	Phosphate Buffer
NCCL	National Committee for Clinical Laboratory Standard
E	Erythromycin
PY	Carbencillin
µM	Micrometer

AK	Amikacin
mm	Millimeter
HXK	Hexokinase
NMR	nuclear magnetic resonance
RACE PCR	rapid amplification of 5′ and 3′ cDNA ends

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة النهرين
كلية العلوم

التأثيرات الحافضة للتريهالوز على المكورات العنقودية الذهبية و أشيريشيا القولون.

رسالة

مقدمة الى كلية العلوم جامعة النهرين

وهي جزء من متطلبات نيل درجة ماجستير علوم في التقانة الاحيائية

من قبل

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بكلوريوس تقانة احيائية جامعة النهرين

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Conclusions

1. Trehalose preserve the bacteria from dehydration.
2. Trehalose protect and preserve the bacteria from freezing.
3. Trehalose increase the protection of the bacteria against UV light irradiation.
4. Trehalose preserved the bacteria after freeze-drying.
5. Trehalose protect the bacteria after electroporation.
6. Trehalose increase the antibiotic resistance of bacteria.
7. Trehalose had no effect on protection from antagonism activity.

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Dedication

To my eyes, my Father and Mother

To my Dearest friend, my Brother

To my soul, the sweet angel my Sister

1.1 Introduction

α–α-Trehalose, a naturally occurring, non reducing disaccharide consist of two glucose units, is commonly found in bacteria (Koch and Koch, 1998), yeasts (Zahringer *et al.*, 1998; Fernandez *et al*., 1998), algae, insects, plants (Ring and Danks 1998), and animals or human organs (Schick *et al.*, 1991).

Trehalose is known to help certain animals and plants and microorganisms to survive desiccation, high osmolarity, and damage by both freezing and heat. It is used to preserve biological materials (Schick *et al.*, 1991 and Kizawa *et al.*, 1995) and as a stabilizer for unstable proteins, including dehydrated and frozen enzymes, diagnostic reagents, pharmaceuticals, and cosmetics (Yoshida *et al*., 1995).

When bacterial cells are freeze dried with 100 mM trehalose as the lyoprotectant (lyophilized protectant), the viability of microorganisms improves four fold and eight fold. The improved viability may be the result of the ability of Trehalose to lower the temperature of the dry membrane phase transition and to maintain general protein structures in a dry state (Leslie *et al.*, 1995).

These findings suggest that Trehalose added to the growth medium and to organelle membranes.

Due to its particular physical features, Trehalose is able to protect the integrity of the cell against a variety of environmental injuries and nutritional limitations. In addition, data available on several species of bacteria and yeast suggest specific functions for Trehalose in these organisms. Some bacteria can use exogenous Trehalose as the sole source of

carbon and energy as well as synthesize enormous amounts of the disaccharide as compatible solute .

This ability to accumulate trehalose is the result of an elaborate genetic system, which is regulated by osmolarity. Some mycobacteria contain sterified trehalose as a structural component of the cell wall, whereas yeast cells are largely unable to grow on trehalose as carbon source. In these lower eukaryotes, trehalose appears to play a dual function: as a reserve compound, mainly stored in vegetative resting cells and reproductive structures, and as a stress metabolite. Recent findings also point to important biotechnological applications for Trehalose.

It has been shown that trehalose can protect proteins and cellular membranes from inactivation or denaturation caused by a variety of stress conditions, including desiccation, dehydration, heat, cold, and oxidation. Finally, in *Mycobacteria* and *Corynebacteria*, Trehalose is an integral component of various glycolipids that are important cell wall structures (Richard *et al*., 2002).

1.2 Aim of the study

1. Identifying the bacterial isolate *Staphylococcus* sp.
2. Study the effect of Trehalose on *S.aureus* and *E.coli* in preservation in dry state at room temperature.
3. Study the effect of Trehalose on protection of bacteria against UV light.
4. Study the effect of Trehalose on protection of bacteria after lyophilization.

5. Study the effect of Trehalose on protection of bacteria against freezing.
6. Study the effect of Trehalose on protection of bacteria against antibiotics.
7. Study the effect of Trehalose on protection of bacteria against antagonism activity.

1.3: Trehalose

Trehalose is a non reducing disaccharide in which the two glucose units are linked in an α,α-1, 1-glycosidic linkage (Qiaofang Chen and Haddad 2004).

In 1974, the current view on the role of trehalose was that it served as a storehouse of glucose for energy and/or for synthesis of cellular components (Elbein, 1974).

Since that time, our knowledge on the various functions of this simple disaccharide has greatly expanded; it is now clear that trehalose is much more than simply a storage compound. Certainly it's function in that capacity in some organisms, but in others it has a structural or transport role (Takayama and Armstrong, 1976), whereas in still others it may be involved in signaling or regulation, or functions to protect membranes and proteins against the adverse effects of stresses, such as heat, cold, desiccation, and anoxia (Crowe *et al*., 1984).

As shown in Fig.1-1 the reducing end of a glucosyl residue is connected with the other, trehalose has no reducing power. Trehalose is widely distributed in nature. It is known to be one of the sources of energy in most living organisms and can be found in many organisms, including bacteria, fungi, insects, plants, and invertebrates. Mushrooms contain up to 10–25 % trehalose by dry weight. Furthermore, trehalose protects organisms against various stresses, such as dryness, freezing, and osmopressure. In the case of resurrection plants, which can live in a dry state, when the water dries up, the plants dry up too. However, they can successfully revive when placed in water. The anhydrobitic organisms are able to tolerate the lack of water owing to their ability to synthesize large quantities of trehalose, and the trehalose plays a key role in stabilizing membranes and other

macromolecular assemblies under extreme environmental conditions (Higashiyama, 2002).

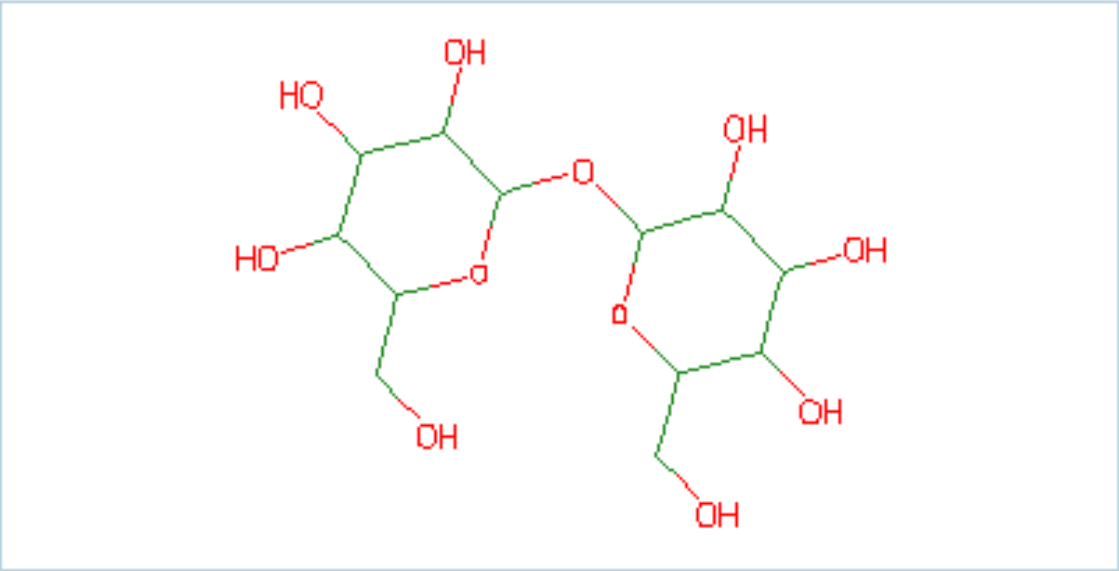


Figure (1-1) Structure of Trehalose

1.4: Natural distribution of Trehalose

Trehalose is found in over 80 species of organisms representing plants, algae, fungi, yeasts, bacteria, insects and invertebrates (Richards *et al*., 2002).

Trehalose highly distributed in nature, it's found in the plants cell, serum of the blood, in bacteria (*Mycobacterium*),yeast and many species of fungi, algae and some higher plants (Brich, 1972).

The high percentage content of trehalose in the yeast could reach 15% or exceed to such a limit of 23% of the dry weight of the cell depending on growth condition. The explain why the trehalose founded in few content in

the bread (1.2-1.5g/Kg) from the dry weight, in the honey (0.1-2.3g/100g) (Elbein , 1974).

Also the trehalose found in many species of Insect, Invertebrates including Nematoda, Trehalose is the main sugar in blood of many Insects in a concentration around (1-2%) in all the development stages of the Insect but in different concentrations. In flying Insect trehalose use as energy source and flying continuity. Also could be play as anti freezing in some Insects.

Trehalose found in high concentrations (7%) in materials named (Manna) used by bidwen peoples in north of Iraq desert as coffee sweeting, The Manna could be one of secretions of some Insects (Elbin, 1974).

Trehalose is also found in a number of different bacteria, including *Streptomyces hygroscopicus* and other of *Streptomyces* spp. (Martin *et al*., 1986).Different mycobacteria including *Mycobacterium smegmatis* and *Mycobacterium tuberculosis* (Elbein and Mitchell, 1973) and *Corynebacteria* (Shimakata and Minatagawa, 2000).

In *Mycobacterium* and *Corynebacteria*, this disaccharide plays a structural role as a cell wall component, but it may also serve other functions in these organisms. It is also present in *Escherichia coli* (Kaasen *et al*., 1994) and a number of other bacteria, such as *Rhizobium* spp. *Sulfdolobus acidocaldarius, Pimelobacter* spp. R48 (Nishimoto *et al*., 1995 and Maruta *et al*., 1996). *Arthrobacter* sp. Q36 and so on. In many of these organisms, the function of trehalose is still not clear. Several of the organisms listed appear to have rather unusual biosynthetic pathways for synthesizing trehalose (Elbein, 2003)**.**

1.5: Trehalose Metabolism

The biosynthesis of trehalose has been best studied in. *E.coli* and *Saccharomyces cerevisiae* and involves a two-step process catalysed by trehalose-6-phosphate synthesis (TPS; EC 2.4.1.15) and trehalose-6-phosphate phosphatase (TPP; EC 3.1.3.12). Trehalose-6-phosphate is formed from glucose-6-phosphate and uridine-5-diphosphoglucose by TPS and is then dephosphorylated to trehalose by TPP (Fig.1-2).

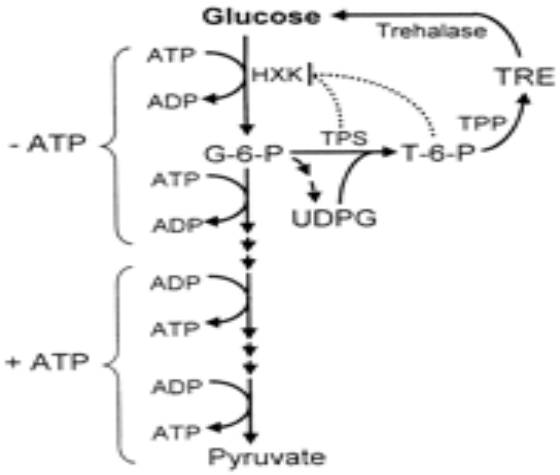


Fig. (1-2) A schematic diagram of the pathway of trehalose metabolism and its relationship to glycolysis in *Saccharomyces cerevisiae*. G-6-P is glucose-6-phosphate, UDPG is uridine-5-diphosphoglucose, T-6-P is trehalose-6-phosphate, TRE is trehalose, TPS is trehalose-6-phosphate synthesis, TPP is trehalose-6-phosphate phosphatase, and HXK is hexokinase (Eastmond, 2003).

There are now at least three different pathways described for the biosynthesis of trehalose. The best known and most widely distributed pathway involves the transfer of glucose from UDP-glucose (or GDP-

glucose in some cases) to glucose 6-phosphate to form trehalose-6-phosphate and UDP. This reaction is catalyzed by the trehalose-P synthesis (TPS, or OtsA in *E.coli*. Organisms that use this pathway usually also have a trehalose-P phosphatase (TPP, or OtsB in *E. coli)* that converts the trehalose-P to free trehalose. A second pathway that has been reported in a few unusual bacteria involves the intramolecular rearrangement of maltose (glucosyl-α1, 4-glucopyranoside) to convert the 1, 4-linkage to the 1, 1-bond of trehalose. This reaction is catalyzed by the enzyme called trehalose synthesis and gives rise to free trehalose as the initial product. A third pathway involves several different enzymes, the first of which rearranges the glucose at the reducing end of a glycogen chain to convert the α1,4-linkage to an α,α1,1-bond. A second enzyme then releases the trehalose disaccharide from the reducing end of the glycogen molecule. Finally, in mushrooms there is a trehalose phosphorylase that catalyzes the phosphorolysis of trehalose to produce glucose-1-phosphate and glucose. This reaction is reversible *in vitro* and could theoretically give rise to trehalose from glucose-1-P and glucose. Another important enzyme in trehalose metabolism is trehalase (T), which may be involved in energy metabolism and also have a regulatory role in controlling the levels of trehalose in cells. This enzyme may be important in lowering trehalose concentrations once the stress is alleviated. Recent studies in yeast indicate that the enzymes involved in trehalose synthesis (TPS, TPP) exist together in a complex that is highly regulated at the activity level as well as at the genetic level (Elbein, 2003).

In brewer's yeast, the biosynthesis of trehalose is catalysed by enzymes that facilitate the reaction of uridine diphosphate-D-glucose with D-glucose 6-phosphate, resulting in uridine diphosphate and a.a-trehalose 6-phosphate. The phosphate is enzymatically removed leaving a trehalose molecule.

Several other organisms produce trehalose by similar mechanisms. Degradation of trehalose is accomplished by a highly specific enzyme, trehalase. Trehalase has been identified in many organisms shown to contain trehalose but is not found in mammals (Richards *et al*., 2002).

Maltose and trehalose assimilating pathways in *Lactococcus lactis*. The maltose phosphorylase (MP) and beta-phosphoglucomutase (b-PGM) of *L. lactic* were characterized and shown to constitute the major maltose-degrading pathway in this bacterium. Furthermore, MP and b-PGM were shown to be present in many other strains of LAB belonging to the low G+C content LAB of the clostridial sub-branch of Gram-positive bacteria. The MP-encoding gene, *Mal P*, was localized in an operon distinctive from that of the gene encoding b-PGM, *Pgm B*. In addition, *Mal R*, encoding the maltose operon regulator *Mal R*, was localized downstream of *Mal P*. The presence of *Mal R* was shown to be crucial for the synthesis of an ATP-dependent maltose translocation system. However, MP and b-PGM activity were not affected by a disruption of the *Mal R*-encoding gene. Instead, synthesis of b-PGM has been shown to be exposed to carbon catabolite repression, which was also shown to be the case for MP. The *Pgm B* is located in the putative trehalose operon including the genes presumed to code for the trehalose-specific components of the phosphotransferase system transporting trehalose into the cells. Furthermore, directly upstream of *Pgm B*, *Trep P* was localized. This gene was shown to encode a novel phosphorylase, trehalose 6-phosphate phosphorylase *Trep* p, catalysing the reversible Pi-dependent phosphorolysis of trehalose r6-phosphate to beta-glucose 1-phosphate and glucose 6-phosphate. The *Trep p* was biochemical characterized and shown to be present in a few other species, mainly *Enterococcus faecalis*, of low G+C content LAB. The role of b-PGM in

trehalose metabolism and in polysaccharide synthesis was assessed by disrupting its encoding gene. B-PGM was shown to be crucial for trehalose assimilation in *L. lactic*, while the b-PGM-deficient strain continued to grow with a tenfold decreased growth rate on maltose, compared to the wild-type strain. The b-PGM-deficient strain showed an enhanced production of polysaccharide, composed of alpha-1, 4-linked glucose units when cultivated on maltose. It was suggested that this polysaccharide was a result from another metabolic pathway, resembling the maltodextrin system in *E.coli***.** (Andersson, 2002)**.**

Axenically grown *Arabidopsis thaliana* plants were analyzed for the occurrence of trehalose. Using gas chromatography–mass spectrometry (GC–MS) analysis, trehalose was unambiguously identified in extracts from *Arabidopsis spp*. inflorescences. In a variety of organisms, the synthesis of trehalose is catalysed by trehalose-6-phosphate synthesis (TPS) and trehalose-6-phosphate phosphatase (TPP). Based on EST (expressed sequence tag) sequences, three full-length *Arabidopsis* cDNAs whose predicted protein sequences show extensive homologies to known TPS and TPP proteins were amplified by rapid amplification of 5′ and 3′ cDNA ends (RACE–PCR). The expression of the corresponding genes, *Attpsa*, *Attpsb* and *Attpsc*, and of the previously described TPS gene, *Attps1*, was analysed by quantitative Reverse Transcriptase (RT–PCR). All of the genes were expressed in the rosette leaves, stems and flowers of *Arabidopsis* plants and, to a lower extent, in the roots. To study the role of the *Arabidopsis* genes, the *Attpsa* and *Attps1* cDNAs were expressed in *Saccharomyces cerevisiae* mutants deficient in trehalose synthesis. In contrast to *Attps1*, expression of *Attpsca* and *Attpsc* in the *Tps 1* mutant lacking TPS activity did not complement trehalose formation after heat shock or growth on glucose. In

addition, no TPP function could be identified for *Attpsa* and *Attpsc* in complementation studies with the *S. cerevisiae Tps 2* mutant lacking TPP activity. The results indicate that while *Attps1* is involved in the formation of trehalose in *Arabidopsis,* some of the *Arabidopsis* genes with homologies to known TPS/TPP genes encode proteins lacking catalytic activity in trehalose synthesis (Vogel *et al.,* 2001)**.**

1.6: Physiological roles of Trehalose in bacteria and yeasts:

The disaccharide trehalose is widely distributed in nature and can be found in many organisms, including bacteria, fungi, plants, invertebrates and mammals. Due to its particular physical features, trehalose is able to protect the integrity of the cell against a variety of environmental stress and nutritional limitations. In addition, data available on several species of bacteria and yeast suggest specific functions for trehalose in these organisms. Bacteria can use exogenous trehalose as the sole source of carbon and energy as well as synthesize enormous amounts of the disaccharide as compatible solute .Cells of many organisms accumulate certain small organic molecules-called compatible and counteracting solutes, compensatory solutes, or chemical chaperones--in response to certain physical stresses. These solutes include certain carbohydrates, amino acids, methylamine and methylsulphonium zwitterions, and urea. In osmotic dehydrating stress, these solutes serve as cellular osmolytes. Unlike common salt ions and urea (which inhibit proteins), some organic osmolytes are compatible, This ability to accumulate trehalose is the result of an elaborate genetic system, which is regulated by osmolarity. Some *Mycobacterium* contains sterified trehalose as a structural component of the cell wall,

whereas yeast cells are largely unable to grow on trehalose as carbon source. In these lower eukaryotes, trehalose appears to play a dual function: as a reserve compound, mainly stored in vegetative resting cells and reproductive structures, and as a stress metabolite. Recent findings also point to important biotechnological applications for trehalose (Arguelles, 2000).

1.7: Physical-chemical properties:

Trehalose (α,α-Trehalose) is a disaccharide formed by a 1, 1 linkage of two D-glucose molecules. It is a non-reducing sugar that is not easily hydrolyzed by acid, and the glycosidic bond is not cleaved by the enzyme a-glucosidase. The molecular formula and weight are $C_{12}H_{22}O_{11}$ and 342.31, respectively. When purified it is usually found in a dihydrate form, which is the typical commercial product.

Trehalose can impart some beneficial properties to food products. Compared to most sugars, trehalose is more stable to wide ranges of pH and heat and it does not easily interact with proteinaceous molecules.

Trehalose has a low hygroscopic profile which is a main advantage compared to other sugars. It appears that trehalose could be of benefit compared with other sugars in dry blending operations in which low hygroscopicity is desired. The water content of trehalose dehydrate remains stable (9.54%) up to a relative humidity of approximately 92%. (Richards *et al*., 2002).

Physical properties that make trehalose unique are its high degree of optical rotation ($[a]^{2}$ D + 178°) and its melting behavior. Trehalose first melts at 97 ° C. Additional heat drives off the water of crystallization until

the material re-solidifies at 130 ° C, and then the anhydrous trehalose melts at 203 ° C. The combination of the molecular structure and the physical-chemical properties of trehalose results in a very stable disaccharide. Trehalose has a solubility and osmotic profile similar to maltose. Above 80 ° C Trehalose becomes more soluble in water relative to other sugars.

Trehalose possesses several unique properties, including high hydrophilicity, chemical stability, non hygroscopic glass formation and no internal hydrogen bond formation. The combination of these features explains the principal role of trehalose as a stress metabolite (Richards *et al*., 2002).

The properties of trehalose are shown in (table 1.1); its relative sweetness is 45 % of sucrose. Trehalose has high thermostability and a wide pH-stability range. Therefore, it is one of the most stable saccharides. When 4 % trehalose solutions with (3.5 - 10) pH were heated at 100 °C for 24 hrs, no degradation of trehalose was observed in any case. Because of no reducing sugar, this saccharide does not show Maillard reaction with amino compounds such as amino acids or proteins.

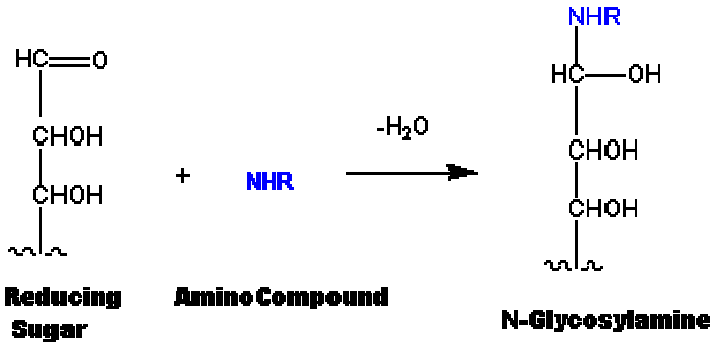


Figure (1-3) Maillard reaction is a type of non-enzymatic browning which involves the reaction of simple sugars (carbonyl groups) and amino acids (free amino groups). They begin to occur at lower temperatures and at higher dilutions than caramelization.

Its particular physical features make it an extremely attractive substance for industrial applications. Furthermore, this saccharide shows good sweetness like sucrose, and in the food industry, this saccharide is used as a sweetener (Higashiyama, 2002).

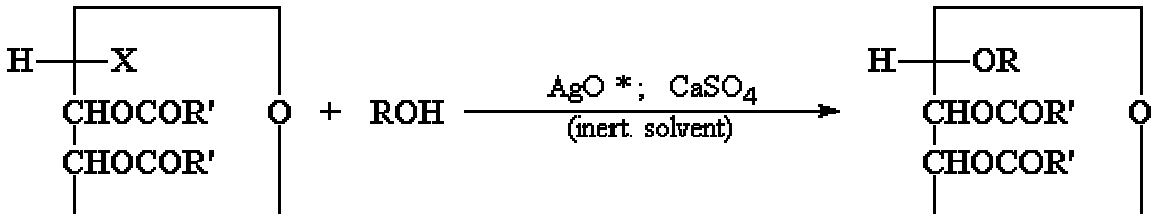
Table (1- 1) Properties of Trehalose (Higashiyama, 2002).

Melting point	dihydrate	97.0 °C
	Anhydride	210.5 °C
Heat of fusion	dihydrate	57.8 kJ mol–1
	Anhydride	53.4 kJ mol–1
Solubility	68.9 g/100 g H_2O at 20 °C	
Relative sweetness	45 % of sucrose	
Digestibility	digested and absorbed by the small intestine	
pH stability of solution	>99 % (pH 3.5–10, at 100 °C for 24 h)	
Heat stability of solution	>99 % (at 120 °C for 90 min)	

1.8: Production of Trehalose:

α,α-Trehalose has been synthesized chemically using the ethylene oxide addition reaction between 2, 3, 4, 5-tetra- O-acetyl-D-glucose and 3, 4, 6-tri-O-acetyl-1, 2-anhydro-D- glucose (Lemieux and Bauer, 1954). This same series of reactions also gives rise to one of the other trehalose anomers, specifically, α,ß-trehalose, also referred to as neotrehalose. Neotrehalose has also been synthesized using the Koenigs-Knorr reaction (Helferich and Weis, 1956). On the other hand, this anomer has not been isolated from any living Organisms, although it was identified in koji extract (Matsuda, 1956).

The other anomer of trehalose, ß,ß-trehalose, or isotrehalose, also has not been isolated from any living organisms, but it was found in starch hydrolysis's (Sato and Aso, 1957); it also has been synthesized chemically using the Koenigs-Knorr reaction, as well as by a dehydration reaction (Bredereck *et al*., 1953).



$X = Cl, Br \qquad * Ag_2CO_3$

Figure (1-4) Koenigs-Knorr reaction Synthesis of acylated *O*-alkylglucosides from alpha-acyl halides and alcohols or phenols in the presence of silver oxide.

Trehalose also can be produced chemically by an acid reversion of glucose (Thompson *et al*., 1954). On the other hand, α,α-trehalose is the only anomer of trehalose that has been shown to be biosynthesized in many different

types of organisms, although its usefulness was recognized, trehalose was not produced on an industrial scale until 1994 (Elbein, 2003).

The conventional method for production, for example, extraction from yeast, had too low yield and too high cost to be used. In order to implement industrial production of trehalose, they have researched new enzyme systems and have succeeded in isolating a novel enzyme system from a bacterial strain belonging to the genus *Arthrobacter* sp. Q36 that was obtained from soil. The system has been found to consist of two novel enzymes: malto-oligosyltrehalose synthesis (or MTSase) and malto-oligosyltrehalose trehalohydorolase (or MTHase). In the first step, MTSase catalyzes the intramolecular transglycosylation of glucose residue at the reducing end of malto-oligosaccharide from α-1, 4 bonds to α-1, 1 bond, then malto-oligosyltrehalose is produced. This contains trehalose residue at the end of the saccharide chain. Next, trehalose is liberated from malto-oligosyltrehalose by MTHase. This pathway needs no high-energy sugar derivative such as sugar phosphate or sugar nucleotide. And those enzymes can repeatedly act on α-1, 4 glucan to produce trehalose up to 80 % yield (Higashiyama, 2002).

1.9: History of human consumption:

In general, the fate of ingested or parenterally administered trehalose corresponds to that of glucose since trehalose is rapidly hydrolyzed to glucose by the enzyme trehalase. Trehalase is found in humans and most animals at the brush border of the intestinal mucosa, as well as in the kidney, liver, and blood plasma (Hore & Messer, 1968; Demelier *et al*., 1975; Labat-

Robert, 1982; Niederst & Dauça, 1985; Eze, 1989; Riby *et al.*, 1990; Yoshida, 1993). Trehalase activity has been found in the small intestine of humans, mice, rats, guinea-pigs, rabbits, pigs, and baboons (Hietanen, 1973; Ruppin *et al.*, 1974; Maestracci, 1976; Garland, 1989). No trehalase activity is found in the small intestine of cats (Hore & Messer, 1968; Hietanen, 1973; Garland, 1989).

A very few individuals have trehalase deficiency, which may be hereditary or acquired. However, in Greenland, the prevalence of trehalase deficiency has been reported to be 8%, which is considerably higher than that seen elsewhere (Dahlqvist, 1974; Gudmand-Høyer *et al*., 1988). The incidence of trehalase deficiency is lower than that of lactase deficiency, which in the United Kingdom is 3.2–6% (Gudmand-Høyer & Skovbjerg, 1996).

When trehalose is ingested by such individuals, it is either incompletely digested or undigested, and a small fraction (approximately 0.5%) may be absorbed by passive diffusion, as shown for other disaccharides (Van Elburg *et al.*, 1995).

The absorbed trehalose may then be metabolized to glucose in the liver or kidney or be excreted unchanged in the urine (Demelier *et al.*, 1975). Unabsorbed trehalose is likely to be fermented by the intestinal microflora to short-chain fatty acids such as acetate, propionate, and butyrate (Abbott, 2002).

Modem food sources may contain substantial quantities of trehalose. Some of these include honey (0.1 - 1.9%), mirin (1.3 - 2.2%), Sherries (< 10 - 391 mg/1), brewer's yeast (0.01 -5.0%) and baker's yeast (15 - 20%), and therefore most items made using yeast. Commercially grown mushrooms may contain 8-17 % (w/w) trehalose. It also occurs in lobsters (2.5 mg/100

ml blood), crab (1.5 mg/100 ml blood) and prawns (0.5% dry weight). Trehalose is not presently a significant part of the modem diet but has been a consistent part of the human diet for thousands of years (Richards *et al*, 2002).

Trehalose does not produce as great a sensation of sweetness as dose sucrose. Trehalose is believed to have only one glucose molecule occupying the binding site on the sweet taste receptor. Trehalose in aqueous solution (at concentrations from about 10-34% anhydrous trehalose) has a sweetness of about 40-45% relative to that of sucrose. The concentration at which a solution of trehalose is perceived as sweet, is about two-times higher than that of sucrose and the sweetness persists longer than sucrose (Richards *et al*., 2002).

1.10: Interaction of Trehalose with Phospholipid Bilayer:

Nature has developed numerous strategies for the long-term survival of organisms. Among the most intriguing are the biological mechanisms that preserve living organisms exposed to damaging conditions like extreme cold, dryness, or heat, or the absence of oxygen (Feofilova, 2003). This phenomenon, named cryptobiosis, involves a reversible suspension of the metabolism and an effective isolation from the environmental changes (Keilin, 1959; Clegg, 2001). Cryptobiosis is widespread in the animal and plant kingdoms, and occurs for example in tardigrades, nematodes, cysts of crustaceans, yeasts, bacteria, fungi, mosses, pollens seeds, and even in entire higher plants (Crowe *et al*., 1992; Guppy and Withers 1999; Feofilova, 2003).

A common feature of cryptobiotic processes is the production of large amounts of saccharides triggered by the detection of stressful conditions. However, the mechanisms whereby sugars may stabilize living systems during freeze-thaw, heat-cooling, or dehydration-rehydration cycles remain a matter of debate (Crowe *et al*., 2002; Crowe, 2002).

In the specific case of anhydrobiosis, three main hypotheses have been put forward to explain the protective effect of sugars. The water-replacement hypothesis suggests that during drying, sugars can substitute water molecules (in particular by forming hydrogen bonds) around the polar and charged groups present in phospholipids membranes and proteins, thereby stabilizing their native structure in the absence of water (Crowe *et al*., 1984,1992,1997, 1998b; Carpenter *et al*., 1994).

The water entrapment hypothesis, in contrast, proposes that sugars concentrate residual water molecules close to the biostructure, thereby preserving to a large extent its salvation and native properties (Belton and Gil, 1994; Cottone *et al*., 2002; Lins *et al.*, 2004). This hypothesis has been formulated in the context of proteins, based on thermodynamic data in solution showing that sugars are excluded from the bimolecular surface by water (Timasheff, 1982; Xie and Timasheff, 1997).

Finally, the vitrification hypothesis suggests that sugars found in anhydrobiotic systems, known to be good vitrifying agents, protect biostructures through the formation of amorphous glasses, thereby reducing structural fluctuations and preventing denaturation or mechanical disruption (Williams and Leopold, 1989; Sun *et al*., 1994, 1996; Sun and Leopold, 1994, 1997).

In the last few years, a consensus has emerged that these mechanisms are not necessarily mutually exclusive (Carpenter *et al*., 1994; Sun and Leopold, 1997; Crowe *et al.,* 1998, 2002; Clegg, 2001; Crowe, 2002).

Vitrification may occur simultaneously with a direct interaction of the biostructure with the sugar, or with an entrapment of residual water at its surface, depending on the type of the protected biostructure and on the nature of the environmental stress imposed on the organism. Additionally, some experimental investigations have suggested that other solutes (e.g., polyfructoses, arbutin, abcissic acid, and several families of stress proteins) may play a critical role in the protection of cells, which in some cases could be interconnected with the action of the saccharides (Singer and Lindquist, 1998; Oliver *et al.,* 2001; Clegg, 2001; Crowe *et al*., 2002).

Among the sugars related with the mechanisms of cryptobiosis, trehalose is one of the most effective Protestants and has therefore been extensively studied (Crowe *et al*., 2001; Richards *et al.,* 2002; Elbein *et al*., 2003).

Trehalose is a non-reducing disaccharide consisting of two glucopyranose units in α-(1→1) linkage. This naturally occurring compound is the principal carbohydrate component (~80–90%) in the hemolymph of insects (Wyatt and Kalf, 1957) and is also commonly found in yeasts and mushrooms (Koch andKoch, 1998; Birch, 1972).

Studies on the metabolism of tardigrades (Westh and Ramlov, 1991; Somme, 1996), nematodes (Madin and Crowe, 1975; Behm, 1997), yeasts (Singer and Lindquist, 1998; Damore *et al.,* 1991) resurrection plants (Scott, 2000; Wingler, 2002) and *Artemia* species (Clegg, 1965, 1997; Clegg and Jackson,

1992) have shown that these organisms accumulate Trehalose in large concentrations during anhydrobiosis, heat shock, or osmotic stress. Several studies have established that Trehalose stabilizes biostructures such as membranes and proteins under unfavorable conditions (Crowe *et al*., 1984, 1987, 2003; Nakagaki *et al.,* 1992; Hoekstra *et al*., 1997).

In numerous situations, Trehalose has been found superior to other common mono- and disaccharide in terms of its protecting ability (Crowe *et al.,* 1987).

The high efficiency of Trehalose as a bioprotector has been the subject of interesting discussions (Crowe *et al.,* 1987, 2001). Different possible reasons have been put forward to explain the superiority of Trehalose, including a high glass-transition temperature, a large hydration radius, or peculiarities in the phase diagram of Trehalose-water mixtures. Several experimental (Branca *et al.,* 1999, 2003; Sussich et al., 2001; Ballone *et al.,* 2000) and theoretical (Liu *et al.,* 1997; Conrad and de Pablo, 1999; Engelsen and Perez, 2000) Studies have investigated the properties of Trehalose in solution and in the solid state, attempting to provide a better understanding of the connection between these so-called anomalous properties and the outstanding bioprotective ability of Trehalose. However, a consensus opinion has not yet emerged. It has been clearly evidenced experimentally that Trehalose is able to stabilize biomembranes and model lipid bilayers exposed to damaging conditions (Crowe *et al*., 1984, 1987, 2001).

In particular, Trehalose is able to inhibit fusion between bilayers, leakage, lateral phase separations, and the formation of nonbilayer phases

(Crowe *et al*., 1987, 2001; Hoekstra *et al*., 1997). It has been suggested that Trehalose reduces the mechanical stress imposed on the membrane upon dehydration (lipid chains brought into close contact) by maintaining the spacing between the head groups and, consequently, keeping the membrane in the fluid phase. Direct interaction between lipid and Trehalose molecules through the formation of hydrogen bonds has been demonstrated by several experimental techniques including infrared spectroscopy, differential scanning calorimetry, nuclear magnetic resonance (NMR), and x-ray diffraction (Crowe *et al.*, 1984; Lee *et al.*, 1986; Nakagaki *et al.*, 1992; Tsvetkova *et al*., 1998; Nagase *et al.*, 1999; Luzardo *et al*., 2000; Lambruschini *et al*., 2000; Ricker *et al.*, 2003).

This indicates that Trehalose molecules are located close to the lipid headgroups, possibly mimicking the solvation by water molecules. Several authors attribute the stabilizing effect of Trehalose to this direct interaction with the membrane, i.e., in terms of the previously mentioned water-replacement hypothesis (Crowe *et al*., 1987, 1992, 1997, 1998b).

Other authors, after the theory proposed by Bryant and Wolfe for the stabilization of membranes by solutes (Bryant and Wolfe, 1992; Wolfe and Bryant, 1999), attribute the stabilization by Trehalose to a purely mechanical effect. This corresponds to the vitrification hypothesis, involving the formation of a Trehalose glassy matrix that mechanically hinders phase changes and conformational fluctuations in the lipid bilayer (Koster *et al*., 1994, 2000; Zhang and Steponkus, 1996).

This hypothesis is able to provide an explanation for the differences in protecting abilities among different vitrifying agents and for the higher

efficiency of Trehalose as a stabilizer. The theory does not exclude the possibility of specific hydrogen bonds between lipid and sugar molecules. However, such interactions are not viewed as a determinant factor in the stabilization process (Koster *et al*., 2000; Wolfe and Bryant, 1999).

In the few last decades, numerous experimental studies have addressed the problem of Trehalose-membrane interactions. However, only two theoretical investigations relying on modeling methods (energy minimization protocols) have been reported (Chandrasekhar and Gaber, 1988; Rudolph *et al*., 1990). These two investigations showed that, in the absence of Trehalose is able to form energetically stable conformations bridging a number of lipid molecules (Chandrasekhar and Gaber, 1988). However, an extension of the method to sucrose and glucose did not succeed in reproducing the experimental order for the stabilizing efficiency of these sugars (Rudolph *et al*., 1990).

Although Trehalose-protein interactions have been studied by atomistic simulations (Cottone *et al*., 2001, 2002; Lins *et al*., 2003), no simulations of Trehalose-membrane systems have been reported to date. Therefore a detailed picture for the molecular mechanism responsible for membrane stabilization is still lacking (Pereira *et al.*, 2004).

1.11: Functions and Applications of trehalose

Trehalose levels may vary greatly in certain cells depending on the stage of growth, the nutritional state of the organism or cell, and the environmental conditions prevailing at the time of measurement. In insects,

trehalose is a major sugar in the hemolymph and thorax muscles and is consumed during flight (Becker *et al*., 1996).

Trehalose is also an important component in fungal spores, where trehalose hydrolysis is a major event during early germination and presumably serves as a source of carbon for synthesis and glucose for energy (Rosseau *et al*., 1972; Thevelein, 1984).

1.11.1: As a stabilizer and protectant of proteins and membranes (Protection from dehydration).

The particular properties of Trehalose have given rise to a surprisingly wide range of applications of this disaccharide in technology including the stabilization of proteins, membranes, liposome, and vaccines (Crowe *et al*., 2001).The hypothermic storage of mammalian cells and organs (Crowe *et al*., 2003; Fukuse *et al*., 1999; Eroglu *et al*., 2002), and its use in cosmetics (Norcia, 2000) and food products (Richards *et al*., 2002).

Trehalose also appears to be efficient in the treatment of dry eye syndrome, for which it is currently being tested in human clinical trials (Matsuo, 2001; Matsuo *et al*., 2002).

Although water is obviously necessary for life, some organisms are able to survive almost complete dehydration, for instance, even when 99% of their water content is removed. This includes common organisms such as plants, yeast cells, and fungal spores, but also microscopic animals such as nematodes, rotifers, and the cysts of brine shrimp (Leopold, 1986).

Some of these dried organisms may remain in this state, known as anhydrobiosis, for decades under unfavorable conditions until water becomes available. When that happens, these organisms swell and resume the active state. Recent studies on these organisms have demonstrated mechanisms that allow them to survive dehydration; understanding some of these mechanisms have enabled researchers to develop new methods for the preservation of biological materials that are normally sensitive to drying (Crowe *et al*., 1992).

Anhydrobiotic organisms generally contain high concentrations of trehalose (and sometimes other disaccharides and oligosaccharides). Thus it was shown that when the nematode *Aphelenchus avenae* was slowly dehydrated, it converted as much as 20% of its dry weight into trehalose (Madin and Crowe, 1975).

The ability of this organism and others to survive in the absence of water has shown a strong correlation with the synthesis of trehalose (Madin and Crowe, 1975). Log-phase cultures of yeast have low concentrations of trehalose and are quite susceptible to dehydration, but as they enter the stationary phase of growth the levels of trehalose increase, along with their ability to survive dehydration (Gadd *et al*., 1987).

This ability to survive in the presence of trehalose is independent of the growth phase of the cells because log-phase cells subjected to heat shock rapidly synthesize trehalose and also acquire the ability to survive dehydration (Hottinger *et al*., 1987). These results are also applicable to a variety of other organisms ranging from brine shrimp (Leopold, 1986) to the resurrection plant (Zentella *et al*., 1999).

This use of trehalose to enable cells to survive dehydration (and other stresses) may be an ancient adaptation because even *Archaebacteria* have been found to accumulate trehalose in response to stress (Nicolaus *et al*., 1988). Interestingly, in plants the disaccharide sucrose plays a similar role to that of trehalose in yeast and nematodes (Anandarajah and Mckersie, 1990).

According to Leopold (1986), trehalose is preferred over sucrose by most organisms because it has fewer tendencies to form crystals than dose sucrose.

The two primary stresses that are proposed to destabilize lipid bilayers during dehydration are fusion and lipid phase transitions. Studies by laser light scattering or other techniques demonstrate that trehalose and other sugars inhibit fusion between the vesicles during drying, but the inhibition of fusion alone is not sufficient to preserve the dry vesicles. Thus trehalose is also necessary to prevent phase transitions (Crowe and Crowe, 1990).

The evidence suggests that trehalose depresses the phase transition temperature of the dry lipids, which maintains them in the liquid crystalline phase in the absence of water (Crowe and Crowe, 1988). A large body of evidence indicates that the stabilizing effect of trehalose is due to its structure and stereochemistry. X-ray diffraction studies show that trehalose fits well between the polar head groups with multiple sites of interaction and suggests that the strong stabilizing effects of trehalose are related to its stereochemistry, which provides the most favorable fit with the polar head groups (Rudolph *et al*., 1990).

Trehalose also preserves labile proteins during drying. For example, phosphofructokinase is a tetramer that irreversibly dissociates to inactive dimers during the drying process. Although many compounds that stabilize proteins can prevent this dissociation when excess water is still present, only disaccharides are effective in stabilizing this protein during extreme drying. As in the case of membranes, trehalose probably interacts directly with the dry protein by hydrogen bonding between its hydroxyl groups and polar residues in the protein (Carpenter and Crowe, 1989).

It is not clear why this interaction results in stabilization, but the fact that trehalose is a no reducing sugar means that it cannot undergo the typical browning reaction between the aldehyde group on reducing sugars and amino groups on proteins. The browning reaction usually leads to denaturation of proteins.An increased resistance to withstand drought conditions has been conferred on several different plants and also animal cells by increasing their levels of intracellular trehalose. For example, transgenic tobacco plants were engineered to produce substantial amounts of trehalose by introducing the *E. coli OtsA* and *OtsB* genes for trehalose synthesis. The introduction of these genes had a pronounced effect on plant morphology and growth performance under drought conditions. Thus the transgenic plants showed considerably improved growth (Pilon-Smits *et al*., 1998).

The same results were obtained with human fibroblasts that had the *OtsA* and *OtsB* genes inserted and expressed. These cells could be maintained in the dry state for up to 5 days, as compared with controls cells that were very sensitive to drying (Guo *et al*., 2000).

1.11.2: Protection against heat.

Trehalose has been described to act as the best stabilizer of structure and function of several macromolecules. Although other sugars also stabilize macromolecules, none of them are as effective as trehalose. The extraordinary effect of trehalose has been attributed to several of its properties such as making hydrogen bonds with membranes or the ability to modify the salvation layer of proteins. However, the explanations always result in a question: Why is trehalose more effective than other sugars? Here, we show that trehalose has a larger hydrated volume than other related sugars. According to our results, trehalose occupies at least 2.5 times larger volume than sucrose, maltose, glucose, and fructose. We correlate this property with the ability to protect the structure and function of enzymes against thermal inactivation. When the concentrations of all sugars were corrected by the percentage of the occupied volume, they presented the same effectiveness. results suggest that because of this larger hydrated volume, trehalose can substitute more water molecules in the solution, and this property is very close to its effectiveness. Finally, these data drive us to conclude that the higher size exclusion effect is responsible for the difference in efficiency of protection against thermal inactivation of enzymes .

Yeast cells have had to develop mechanisms in order to protect themselves from chemical and physical agents of the environment to which they are exposed. One of these physical agents is thermal variation. Some yeast cells are known to accumulate high concentrations of trehalose when submitted to heat shock (Elbein, 2003).

In yeast, stimuli that trigger the heat shock response also cause the accumulation of trehalose. In fact at least two subunits of the trehalose-6-P synthesis complex of *S. cerevesiae* are actively synthesized during heat shock (Bell *et al*., 1992), and physiological concentrations of trehalose (up to 0.5 M) were found to protect enzymes of yeast and other organisms from heat inactivation *in vitro*. Trehalose also reduced the heat-induced formation of protein aggregates. Trehalose was as good or better as a protein stabilizer than any of a number of comparable solutes, including polyols, sugars, and amino acids (DeVirgilio *et al*., 1994).

Yeast mutants were prepared that were defective in genes coding for key enzymes in trehalose metabolism (*TPS1, TPS2*), and these mutants showed an inability to accumulate trehalose on mild heat shock and were much less resistant to heating than was the wild-type organism. These various studies strongly implicate trehalose as playing a key role in thermo tolerance and also indicate that the enzymes that synthesize trehalose are induced in response to the stress in order to increase the levels of trehalose. An important *in vivo* and *in vitro* study showed that trehalose protects cells from heat by stabilizing proteins at high temperatures. Using two different temperature-sensitive reporter proteins, these investigators showed that enzymes are better able to retain activity during heat shock in cells that are producing trehalose (Singer and Lindquist, 1998).

These studies showed an additional and important role of trehalose, that is, the ability to suppress aggregation of proteins that have already been denatured. Based on these studies, these researchers also explained why trehalose must be degraded rapidly after the heat shock has ended, that is, if the unfolded luciferase, one of their reporter proteins, is removed from or

diluted out of the trehalose, and it can be refolded by molecular chaperones. On the other hand, if the trehalose concentration remains high, it interferes with the refolding process, and the protein is not renatured by the chaperone (Singer and Lindquist, 1998). Thus, it may be important to have active trehalase present once the heat stress is alleviated.

1.11.3: Protection from damage by oxygen radicals:

Another role for trehalose is in protecting cells against oxygen radicals. Exposure of *S. cerevesiae* to a mild heat shock or to a proteosome inhibitor induced trehalose accumulation and also markedly increased the viability of cells on exposure to a free radical–generating system (H_2O_2/iron). However, when the cells were returned to the normal growth temperature, both the trehalose content and resistance to oxygen stress decreased rapidly and returned to the wild-type level. A mutant cell line defective in trehalose synthesis was much more sensitive to oxygen killing than was the wild type, but adding trehalose to the medium enhanced the resistance of these cells to H_2O_2. The effect of oxygen radicals on these cells was to damage amino acids in cellular proteins and the presence of high concentrations of trehalose in the cells prevented this damage. The trehalose-defective mutant showed a much higher content of damaged proteins even after only a brief exposure to oxygen stress. The suggestion is that trehalose acts as a free radical scavenger. In these studies, mannitol and galactose also protected but less so than trehalose, whereas sucrose was ineffective. This lack of protection by sucrose may have to do with its inability to quench oxygen radicals or be taken up by cells (Banaroudj *et al.*, 2001).

As indicated earlier in this review, trehalose and sucrose are both no reducing disaccharides that may have considerable similarity in synthesis and function. Both are stored in the cytosol of cells, and both may be present in their respective cells in high concentrations depending on various environmental conditions. Another commonality is that when either of these oligosaccharides are present in high concentration, the cells become quite resistant to a variety of stress conditions, including heat, dehydration, oxygen stress, and so on (Hincha *et al*., 2002).

In plants, oligosaccharides of the raffinose series (Gal$_{\infty}$1-6Sucrose and higher) can accumulate in cells to a level of 15% of the dry weight, and these plants may have considerable stress resistance (Hincha *et al*., 2002).

This striking correlation cannot be ignored and needs further investigation to determine whether raffinose and stachyose and other sucrose oligosaccharides are in fact produced as a protection against different stress conditions. The same may be true of the trehalose oligosaccharides that have been isolated from the cytosol of *M. smegmatis*. Although their concentrations in these cells were fairly low, that could be because those cells were not stressed. It will be important to examine the levels of these various trehalose analogs after cells have been exposed to stress. (Ohta *et al*., 2002).

1.11.4: Protection from cold.

Nature has developed numerous strategies for the long-term survival of organisms. Among the most intriguing are the biological mechanisms that preserve living organisms exposed to damaging conditions like extreme cold, dryness, or heat, or the absence of oxygen (Feofilova, 2003).

This phenomenon, named cryptobiosis, involves a reversible suspension of the metabolism and an effective isolation from the environmental changes (Keilin, 1959; Clegg, 2001). Cryptobiosis is widespread in the animal and plant kingdoms, and occurs for example in tardigrades, nematodes, cysts of crustaceans, yeasts, bacteria, fungi, mosses, pollens, seeds, and even in entire higher plants (Clegg, 2001; Crowe *et al*., 1992; Guppy and Withers, 1999; Feofilova, 2003).

A common feature of cryptobiotic processes is the production of large amounts of saccharides triggered by the detection of stressful conditions (Crowe *et al.*, 2002; Crowe, 2002). However, the mechanisms whereby sugars may stabilize living systems during freeze-thaw, heat- cooling, or dehydration-rehydration cycles remain a matter of debate. Studies had shown that a combination of the bio-antioxidant catalase and the membrane stabilizer trehalose in the conventional freezing mixture affords better cryoprotection to hematopoietic cells as judged by clonogenic assays (Sasnoor, 2003).

A mutant strain of *E. coli* that was unable to produce trehalose died much faster that did the wild type at 4°C. However, transformation of this mutant with *OtsA*/*otsB* genes restored the ability to synthesize trehalose and also cell viability in the cold (Kandror *et al*., 2002).

Additional studies showed that downshifting cells from 37°C to 16°C caused an eightfold increase in trehalose levels and a marked increase in mRNA levels for *OtsA* and *OtsB*. The authors speculate that because a number of proteins denature and precipitate in the cold where the hydrophobic effects are relatively weak, it is possible that trehalose also prevents the denaturation and aggregation of specific proteins at cold temperatures. Trehalose may also stabilize cell membranes whose fluidity decreases during temperature downshift. Thus, exogenous trehalose has been shown to protect a variety of organisms against freezing, with maximum protection seen when trehalose is present on both sides of the membrane (Kandror *et al*., 2002).

1.11.5: As a sensing compound and/or growth regulator:

Although tobacco plants transformed with the genes for enzymes of trehalose synthesis (TPS or TPP) do exhibit a slight increase in drought tolerance (still somewhat controversial), the expression of the microbiol genes for trehalose synthesis causes severe growth defects, such as dwarfism and aberrant root development (Vogel *et al*., 2001).

These findings have led these researchers to postulate that trehalose or related metabolites might function as regulators of plant growth and development. This effect could be similar to the effect of trehalose-6-P on hexokinase and glycolysis in yeast, or it could be due to an effect on other metabolic pathways. Interestingly, similar growth defects were observed with transformed rice plants even though these plants did not accumulate large amounts of trehalose (Muller *et al*., 1999).

The authors provide three possible explanations as follows: (1) the pleiotropic phenotype may be due to a disturbance of normal plant metabolism, such as exhaustion of UDP-glucose; (2) even the small amounts of trehalose or trehalose-P might be toxic to plants; or (3) trehalose metabolism may be a signal in sugar sensing and partitioning of assimilates.

1.11.6: As a structural component of the bacterial cell wall:

In mycobacterium and corynebacteria, trehalose is the basic component of a number of cell wall glycolipids (Lederer, 1976).

The best known and most widely studied of these trehalose lipids is cord factor, a cell wall lipid of *M. tuberculosis* that contains the unusual fatty acid mycolic acid esterified to the 6-hydroxyl group of each glucose to give trehalose-dimycolate. This lipid is considered to be one of the major toxic components of the cell wall and is also largely responsible for the low permeability of the mycobacterium cell wall, which confers considerable drug resistance to these organisms (Brennan and Nikaido, 1995).

Trehalose-monomycolate is the proposed precursor to trehalose-dimycolate (cord factor), but it also appears to serve as the donor of mycolic acid residues to the cell wall arabinogalactan to form the mycolyl-arabinogalactan-peptidoglycan complex (Chatterjee, 1997).

A mycolyl transferase was isolated, and this enzyme was shown to catalyze the transfer and exchange of mycolic acid from trehalose-monomycolate to free trehalose to produce both mono- and dimycolyl-trehalose (Belisle *et al*., 1997).

Whether this enzyme or a similar transferase is involved in transferring mycolic acid residues to cell wall polymers remains to be determined. Corynebacteria and nocardia also contain trehalose glycolipids that have fatty acids that are related to but not identical with the mycolic acids, and these fatty acids are referred to as corynomycolic or nocardomycolic acids (Lederer, 1976). The function of these lipids, besides their obvious structural role, is not known. There are other antigenic glycolipids in the mycobacterial cell wall that also have trehalose as the base. For example there are a variety of acylated-trehaloses that have three major types of fatty acids attached to the 2 and 3 hydroxyl groups of the same glucose. These fatty acids are: n-$C_{16–19}$ saturated fatty acids, $C_{21–25}$ α-methyl branched fatty acids, and $C_{24–28}$ α-methyl branched, ß-hydroxy fatty acids (Besra *et al*., 1992).

M. tuberculosis and other mycobacteria also have trehalose lipids that contain sulfate, such as 2,3,6,6'-tetra-acyl-2-sulfate trehalose (sulfatide I) (Alugupalli *et al*., 1995), or other types of fatty acids, such as phthienoic acids (Daffe *et al*., 1988).

This great variation in the types of fatty acids found in these organisms and as cell wall components suggests a probable function, but so far specific functions have not been demonstrated. Finally, mycobacterium, such as *Mycobacterium kansasii*, is characterized by the presence of seven species-specific neutral lipooligosaccharide antigens. These oligosaccharide antigens have a common tetraglucose core which is distinguished by an α,α-trehalose substituent to which are linked such various sugars as xylose, 3-O-methylrhamnose, fucose, and a novel N-acyl aminosugar. The exact structure of these compounds has not been determined. Analogous but specific

lipooligosaccharides typify a host of other typical *Mycobacterium* (Hunter *et al*., 1983).

1.11.7: Protection against freeze-drying (lyophilization)

Freeze-drying is often used for preservation and storage of biological samples; however, it has some undesirable side effects, such as denaturation of sensitive proteins and decreased viability for many cell types. To prevent or reduce these adverse effects, protective substances such as skim milk, sucrose, glycerol, and dimethyl sulfoxide are commonly added to samples before freezing or freeze-drying. While the addition of solutes is known to increase the number of viable cells in a freeze-dried sample, viability remains below that of the initial culture, and the physical mechanism of their protective action remains to be established. Previous work has shown that no reducing disaccharides such as sucrose and trehalose can protect liposome, isolated biological membranes, and some intact cells from the adverse effects of freezing and drying. Liposome dried and rehydrated without the addition of a disaccharide suffer imbibitional damage and leak their contents to the surrounding media, while those dried with a disaccharide retain their contents. Vesicles of isolated lobster sarcoplasmic reticulum dried without a disaccharide suffer fusion and a total loss of Ca_{21} transport activity. Vesicles from the same sarcoplasmic reticulum preparation dried with a disaccharide exhibit no adverse effects. Work with intact pollen from the cattail *Typha latifolia* has shown that sucrose plays a vital role in pollen's ability to tolerate drying and storage (Richard *et al*., 2002).

2.1 Equipments and apparatus:

The following equipment and apparatus were used during this study:

Equipments	Company(origin)
Autoclave	Tomy(Japan)
Balance	Ohans(France)
Compound light microscope	Olympus(Japan)
Distillator	GFL (Germany)
Electrical Oven	Sanyo (Japan)
Electroporator	(Iraq)
Incubator	Termaks(U.K)
Lyophilizor	Labconco(England)
Micropipette	Witey(Germany)
Millipore Filter paper unit	Millipore corp(U.S.A)
pH-meter	Mettler Toledo(U.K)
Refrigerated centrifuge	MSE (UK)
Sensitive balance	Delta Range(Switzerland)
Spectrophotometer	Miltonroy(USA)
UV-transilluminator	Vilber Lourmat(France)
Water bath	GFL(Germany)

2.2 Chemicals

The following chemicals were used in this study:

Chemicals	Company(origin)
Chloroform,NaOH,$BaCl_2$,H_2SO_4, KI, Na_2HPO_4 , KCl, NaCl, Glucose, β-mercaptoethanol,toludine blue, Iodine.	BDH-England
Nutrient Broth, Nutrient Agar	Biolife-Italy
Trehalose	Difco-USA
HEPS, phenol, crystal violate, sufranine, iodine.	Fluka-Switzerland
Ethanol	Iraq
Agar	Sigma-USA

2.3 Antibiotics

The following antibiotics discs were used in this study:

Antibiotics	Abbreviations	Concentration (μg)	Company(origin)
Amikacin	AK	30	Al-Razzi(Iraq)
Carpencillin	PY	100	Al-Razzi(Iraq)
Cestazidime	CZC	30	Al-Razzi(Iraq)
Chloramphenicol	C	30	Bioanalysis(UK)
Erythromycin	E	15	Bioanalysis(UK)
Gentamycin	CA	10	Al-Razzi(Iraq)

Nalidixic acid	NA	30	AL – Nadear(Iraq)
Norfloxacillin	Nor	10	AL – Nadear(Iraq)
Penicillin	P	10(U)	AL – Nadear(Iraq)
Streptomycin	S	10	AL – Nadear(Iraq)
Tetracycline	TE	30	AL – Nadear(Iraq)
Vancomycin	VA	30	AL – Nadear(Iraq)

-Antibiotics powders:

Antibiotic	Abbreviations	Company (origin)
Tetracycline	TE	Oxoid(England)

2.4 Bacterial Isolates:

The following bacterial isolates were used in this study:

Bacterial isolates	phenotype	Source
Escherichia coli	Wild type	Central of childhood Hospital
Staphylococcus aureus	Wild type	Central of childhood Hospital
Staphylococcus epidermidis	Wild type	Central of childhood Hospital

2.5. Media

2.5.1 Ready-made culture media:

The following media were ready-made media prepared according to the manufactures instructions and autoclaved at 121°C for 15 minutes.

Medium	Company (Origin)
Brain heart infusion agar	Difco (U.S.A)
Brain heart infusion broth	Difco (U.S.A)
EMB media	Oxoid (England)
Nutrient agar	Oxoid (England)
Nutrient broth	Oxoid (England)
Mannitol salt agar	Difco(U.S.A)

2.5.2 Prepared culture media:

- **Blood Agar Medium** (Atlas *et al*., 1995)

It was prepared by autoclaving blood agar base (Difco U.S.A) after adjusting pH to 7.0, cooled to 45°C, then 5% blood was added and mixed well.

- **DNase Medium** (Atlas *et al*., 1995)

It was prepared by dissolving 42g DNase agar(Sigma U.S.A) with 0.1g toludine blue in 1 litter of distilled water , pH was adjusted to 7.2 and sterilized by autoclaving at 121°C for 15 min.

2.6 Reagent and Stains:

- **Hydrogen Peroxide Reagent** (Atlas *et al*., 1995)

This reagent used for catalase test at 3% concentration.

- **Oxidase Reagent** (Atlas *et al*.,1995)

This reagent was composed of 1% of tetra methyl-p-phenylenediamine dihydrochloride (Freshly prepared solution).

- **Crystal violate stain** (Atlas *et al*., 1995)

This stain was prepared by dissolving 2g of crystal violate in 20ml of 95% ethanol and the final volume was completed to 100ml with distilled water and filtered before use.

- **Safranine Counter Stain** (Atlas *et al*., 1995)

This stain was prepared by dissolving 0.25g of safranine *O* in 10ml of 95% ethanol and the final volume was completed to 110ml with distilled water allow to stand several days and filtered before used.

- **Lugol's Iodine Reagent** (Atlas *et al*., 1995)

It was prepared by mixing 2g of potassium iodide with 1g of iodine in 300 ml of distilled water.

2.7 Buffers and solution:

- **Electroporation buffer (Internet # 1):**

HEPES (pH 7.0) 20 mM

NaCl 137 mM

KCl 5 mM

Na_2HPO_4 0.7 mM

Glucose 6 mM

ß-mercaptoethanol 0.1 mM

Dissolved in distilled water, Sterilized by Filtration

- **Phosphate Buffer ph 7.0** (Atlas *et al*., 1995)

Na_2HPO_4 9.52 g/L

NaH_2PO_4 6.00 g/L

Sterilized by autoclaving at 121 °C for 15 min.

- **Antibiotic solution** (Maniatis *et al*., 1982).

Tetracycline

It was prepared as stock solution of 12.5 mg/ml of tetracycline hydrochloride in solution of ethanol/ water (50% V/V). Sterilized by filtration and stored in aliquots at -20°C in the dark.

Methods

2.8 Collection of Samples

Skin sample (pus) were collected from wound infection of patient in central childhood hospital, during the period from 10/04 to 11/04, one sample was collected and diagnosed, and after that the isolate was used for this study.

2.9 Maintenance of bacterial isolate:-

Maintenance of bacterial isolate was performed according to (Atlas *et al*., 1995), as the following:

2-9-1 short-term Storage:

Colonies of bacteria were maintained for periods of few weeks on the surface of agar medium (Nutrient agar). The plates were tightly warped with parafilm and stored at 4°C.

2-9-2 Medium-term Storage:

Bacterial isolates were maintained in stab cultures for long periods (few months). Such medium were prepared in screw-capped vials containing 5-8ml of nutrient agar medium and stored at 4°C.

2-9-3 long-Term Storage:

Ten ml of 15-20% glycerol were added to screw tubes containing nutrient broth. After autoclaving, inoculate with bacteria and incubate at 37°C for 24 hr. aerobically then kept in freezer, bacteria can be stored for many years in this medium without significant loss of viability (Contreras *et al*., 1991).

2.10 Inoculum's preparation:

Cells of bacterial isolates grown in nutrient broth until mid exponential phase (O.D600nm = 1), were pelleted from 10ml by centrifugation at 6000 rpm for 10 min. washed and resuspended with normal saline, 1% (v/v) This prepared inoculums were then used in our experiment.

2.11 Measurement of Bacterial Growth:

Growth of bacterial was monitored by MacFarland tube No. 5 turbidity standard (which prepared by adding 0.6 ml of 0.048 M $BaCl_2$ [1.175% w/v $BaCl_2$ $2H_2O$] to 99.5 ml of 0.36 N H_2SO_4), which is equivalent to bacterial concentration for inoculums 1.5 x 10^8 organisms/ml.

2.12 Identification of bacterial isolates (Holt *et al*., 1994; Atlas *et al*., 1995)

2.12.1 Morphological and Cultural Characteristics.

Morphology of colonies were studied on brain heart infusion agar (Collee *et al*., 1996).

Color, shape, size and edge of colonies were recorded after 24 hrs. Of incubation at 37°C.

- **Gram's Stain** (Atlas *et al*., 1995)

A single colony was transferred by a loop to a clean slide. The smear was stained with crystal violet, treated with iodine, decolorized with 95% alcohol, and counterstained with safranine, then examined by light microscope.

2.12.2 Biochemical and Physiological Characteristics.

2.12.2.1 Catalase Production test (Maza *et al*., 1997)

This test was performed by adding (2-3) drops of hydrogen peroxide (H_2O_2) (3%) on a single colony grown on BHI agar plate. Appearance of bubbles was regarded as positive result.

2.12.2.2 Oxidase Production test (Harely and Prescott, 1996)

Filter paper was saturated with the substrate (tetramethyle-p-phneylene-diamine-dihydrochloride), colony of bacterial isolate to be test was rubbed on the filter paper with a sterile wooden applicator stick. An immediate color change to deep blue indicates a positive result.

2.12.2.3 Growth at 45°C.

Test tubes containing BHI broth were inoculated with overnight culture of bacterial isolate, incubated at 45 °C for 24 hrs. Bacterial growth (turbidity) was regarded as positive result.

2.12.2.4 Coagulase Production Test.

Plasma diluted 1:10 in normal saline (0.85%NaCl) was prepared and placed in small tube. The bacterial isolate under test was inoculated into the tube, by adding 0.l ml of overnight culture. The tube was incubate at 37°C and examined for coagulation after 1, 3 and 6 hrs.

The tubes that showed negative results were left at room temperature overnight and reexamined. The conversion of the plasma into a soft gel was observed by tilting the tube to a horizontal position.

2.12.2.5 DNase Production Test (Collee *et al*., 1996)

DNase agar plate containing toluidine blue (100mg/ml) indicator, was inoculated with the bacterial culture, and then incubated at 37°C for 24-48 hrs.

Development of a rose pink halo surrounding the areas of bacterial growth indicated the hydrolysis of DNA.

2.12.2.6 Hemolysis Patterns on Blood Agar (Atlas *et al*., 1995)

A single colony of overnight growth culture was streaked on blood agar. The type of hemolysis produced by the growing colonies was observed and recorded.

2.12.2.7 Aerobic Mannitol Fermentation Test (Atlas *et al*., 1995)

The isolated bacterium was streaked on the plate of mannitol salt agar and the plate was incubated at 37°C overnight. The change in color from deep pink to yellow was a positive indication of mannitol fermentation.

2.12.2.8 Tolerance to NaCI (7-9%) (Atlas *et al*., 1995)

A loop full of an overnight culture of bacterial isolate was inoculated into brain heart infusion broth supplemented with 7-9% NaCI, incubated at 37°C for 24hrs. Bacterial growth was regarded as positive result.

2.13 Effect of Trehalose on bacterial isolate preserved in the dry state at room temperature:

A novel procedure was used to evaluate the effect of Trehalose on the *S. aureus* and *E.coli* isolates. These were used viable dried bacteria produced by natural drying in the presence of Trehalose.

A novel method was provided for preserving live bacteria by subjected an aqueous system containing the growing bacteria to drying without special equipment, in the presence of trehalose, a dried compound for preservation of aerobic bacteria in a viable state is provided. The dried composition consists essentially of dried via aerobic bacteria and an appropriate growth medium. The bacteria and growth medium were initially placed in an aqueous solution of 100mM, and dried in room temperature.

Bacterial isolate with the following phenotypic characteristics were each tested for their ability to be preserved and reconstituted using trehalose as preservation agent: *E.coli, S.aureus*. The two bacterial isolates listed above were each individually inoculated into standard culture media in wells of a 96-well microtiter dish. They were grown overnight at 37^{o}C. In the following day an equal volume of preservation media, with 10 mM of divalent cation ($CaCl_2$) containing a trehalose was added to the growing cultures giving a final concentration of trehalose 100mM. The cultures were gently rocked over a 96 hour period and the contents allowed drying. The dish was then covered and placed at room temperature. After one week the individual cultures were rehydrated with sterile water, pre-warmed to 37^{o}C.added to nutrient broth media to test for viability. After 1 month,

individual cultures were rehydrated with sterile water, pre-warmed to 37^{o}C.added to nutrient broth media to test for viability.

2.14 Trehalose protection against UV light

Culture of *S.aureus* grown in Brain Heart medium with 100mM trehalose and without trehalose(control) until the mid exponential phase, were pelleted from 5ml by centrifugation at 4000 rpm for 10 min., washed twice and resuspended in the same volume of phosphate buffer (pH=7.0).

The UV source was UV-Transiluminator-Cross Linker [FLX-20-M, Vibler Lourmat, France].

The tray size for irradiation was approximately 15x25 cm. Then the samples were exposed on Petri dishes, multi-well plates, membrane, etc, to direct irradiation from(for 15 watts) 254nm bulbs. A UV photoelectric cell detects actual intensity. The dose rate of UV irradiation was 2-5 J/m^2/s.The experiments in this study were preformed under red light. The distance between UV source and irradiated suspension was 11cm.

Three ml samples of the bacterial suspension in phosphate buffer contain Trehalose and the control was irradiating in sterile Petri dish for the following doses: (0, 10, 20, 30, 40, and 50) J/m^2.

Two samples of each treatment were taken and as follows : 0.1 ml sample diluted properly and spread on BHA, then incubated at 37ºC for 24 hr. to determine the total viable count(survival fraction).

2.15 Antibiotic sensitivity Test (Baron and Finegold, 1994):

Disk Diffusion Test:

Two test tubes contain 10 ml of nutrient broth medium one with 100mM of trehalose and the other without(control) inoculated with the bacterial isolate, *S.aureus*, *E.coli* the cultures were incubated at 37°C to mid log phase (O.D600nm a bout 1) giving $1x10^8$ bacterium/ml. 0.1 ml of inoculated broth transferred to BHA. A sterile cotton swab was used to streak the inoculums on the plate surface in 3 different planes (by rotating the plate approximately 60 each time to obtain an even distribution of the inoculums). The inoculated plates were then placed at room temperature for 10 minutes to allow absorption of excess moisture. With sterile forceps the selected antibiotic disks were placed on the Inoculated plates and incubated the plates at 37°C for 18 hrs. in an inverted position.

After incubation, the diameter of inhibition zone were noted and measured by a ruler in mm, results were determined according to the National Committee for laboratory standards (NCCLS, 1986).

Minimum Inhibitory Concentration (MIC)

Twenty test tubes containing nutrient broth were prepared. Ten test tubes have been added 100mM trehalose and the other ten test tubes treatments left without trehalose (control), to each have been added quantities of antibiotics, serially diluted from 0.4µg/ml to 100µg/ml. The last tubes were free of antibiotics and serves as a growth control. Twenty tubes were inoculated with a calibrated suspension of the *S.aureus*, following by incubation at 37°C for 18 hrs. At the end of the incubation period, the tubes

were visually examined for turbidity. Cloudiness indicates that bacterial growth has not been inhibited by the concentration of antibiotic contained in medium.

2.16 Effect of Trehalose on Antibacterial activity:

Antibacterial Agents Test: Inhibitory effect was determined by the agar diffusion method described by (Nathan *et al*., 1978).

Using the bacterial inoculation technique. Commercially available 60 mm. petri dishes containing BHA were inoculated with *S.aureus* isolate with 100mM trehalose and without trehalose as control by using sterile swabs. Two evenly spaced holes 3 mm. in diameter were made in the agar of each plate with sterile cork borer. To identify the intrinsic antibacterial activity of the diluents, wells were filled with *S.epidermidis.* An equal volume (200ml) of each agent was expressed into each well (Two replicate plates were prepared for each agent). Test plates were then incubated at 37°C for 24 hours and zones of inhibition were measured with millimeters. A clear inhibition zone indicates that the agent had retained its antibacterial activity.

2.17 Effect of Trehalose against freezing

The two tests isolate *S.aureus* and *E.coli* were cultured in nutrient broth medium for 18 hrs. After the incubation period a 100 mM Trehalose was then added to the culture, while the control left without any addition.

All the tubes were then sealed and stored in -20ºC for 40 days; a sample were then lifted each 10 days, recultured on nutrient agar medium to determine its viability.

2.18 Effect of Trehalose after freeze drying (Lyophilized Bacteria)

Two flasks containing 50 ml of nutrient broth culture medium were prepared, and inoculated with *S.aureus* 18 hrs. at 37°C.

A 100 mM trehalose was added to the culture while the control left without any addition.

The two flask then transfer to sterilize lyophilizes flask and then lyophilized. After the lyophilizing the flasks sealed and stored for 4 months, each month sample were then diluted and cultured in Brain Heart Agar to determine the viable cells after incubating for 18 hrs. at 37°C.

2.19 Effect of Trehalose on Electroporated bacteria

Two tubes containing 10 ml of nutrient broth culture medium were prepared, and inoculated with *S.aureus* 18 hrs. at 37°C

100 mM trehalose was added to the culture while the control treatment left without any addition.

The cluttered were centrifuged (3000rpm for 10 min.) and wash twice with electroporation buffer, then transferred to a cuvette. Gently to avoided any bubbles formation in the gap between the plate electrodes. The cuvette inserted into cuvatte holder of the local handmade electroporator fig.(2-1) , which the plastic nose of the cuvatte fits into slit of the cuvatte holder. The cuvatte holder was pushed into the electroporator until it clicks into position. The voltage were selected and switched on. After electroporation the cuvatte

was removed from the cuvatte holder. The bacterial solution was then transferred into BHI broth media to determine the viable cells.

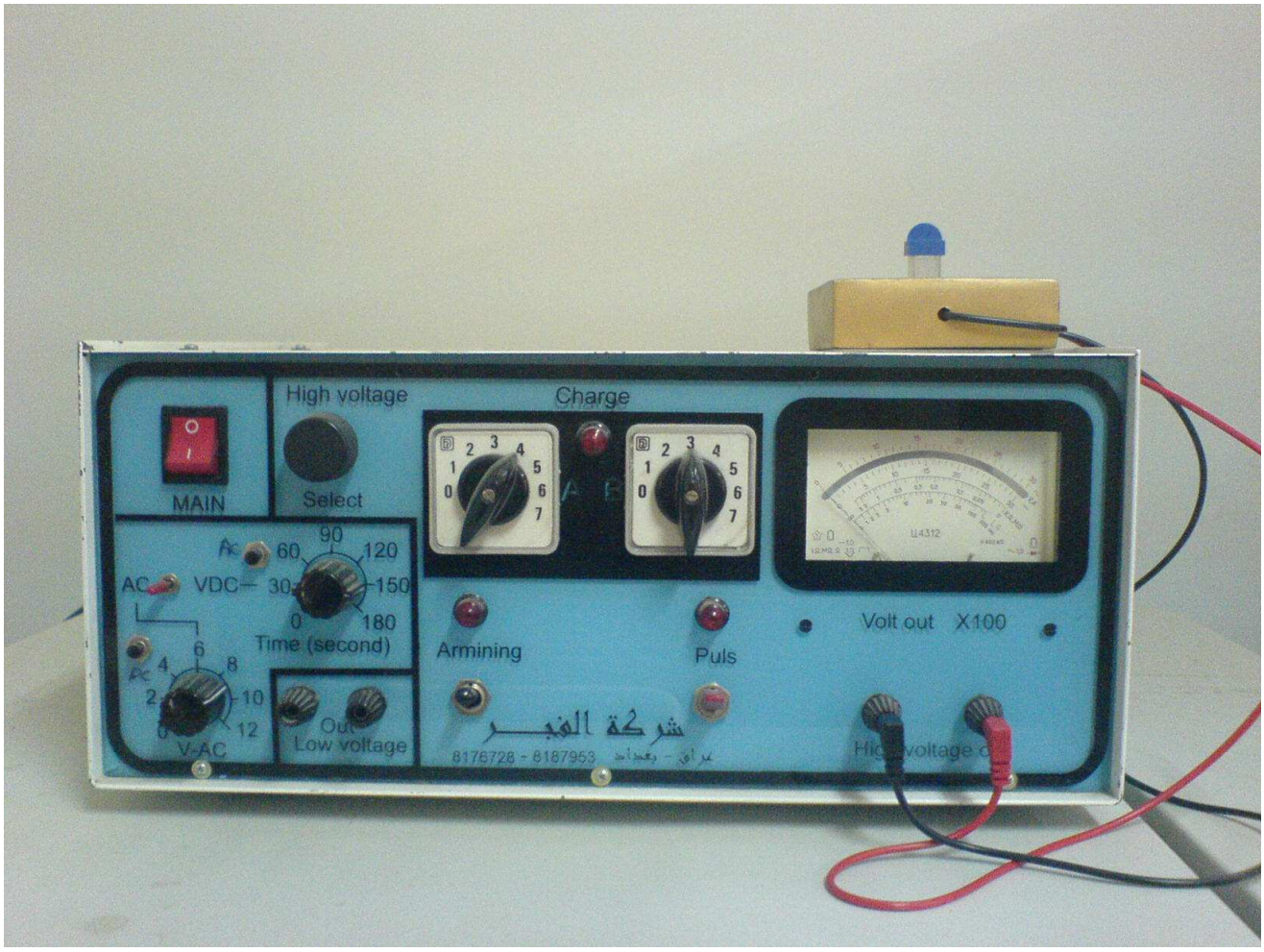
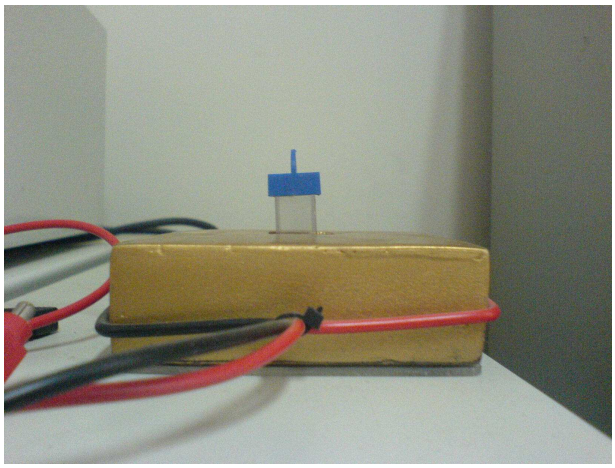
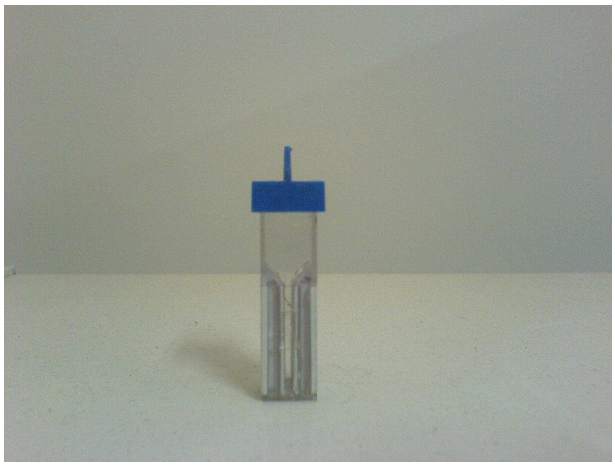


Figure (2-1): (A) local handmade electroporator.



(B) Cuvatte holder



(C) Cuvatte

3.1 Identification of Bacterial Isolates:

The bacterium *Staphylococcus* sp. was isolated from skin smear (pus) and identified by biochemical and physiological characteristic, while the bacterium E.coli was isolated from previous study.

3.1.1 Morphological and Cultural Characteristics:

The isolate had shown golden pigmented colonies, which were 2-3 mm in diameter, on (B.H.I.) agar at 37°C for 24 hrs. Moreover, microscopically examination was demonstrated that grape like clusters of cells with Gram-positive reaction (Figure 3-1).

3.1.2 Biochemical and Physiological Characteristics:

Results (Table 3-1) of biochemical and physiological tests performed on *Staphylococcus* sp. showed positive to coagulase and catalase, but negative to oxidase. Furthermore, it was able to ferment mannitol aerobically, produce DNase, able to produce β-hemolysis on blood agar, grow on media containing 7-9% NaCl and grow at 45°C.

Morphological and biochemical characterization agreed with these stated by Holt *et al.,* (1994) and Atlas *et al.,* (1996).

The results in table (3-1) showed that the isolate belong to the species of *S.aureus.*

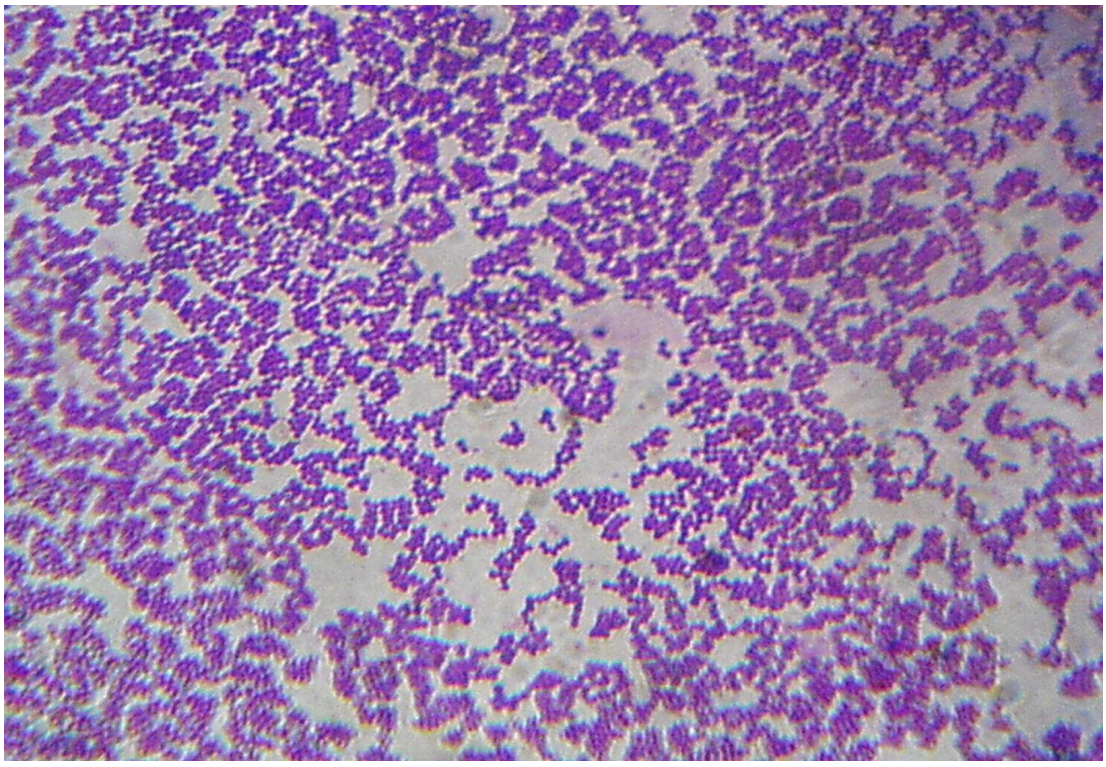


Figure (3-1) A smear of Gram-Positive bacteria *Staphylococcus* sp.

Table (3-1) Biochemical and physiological characteristics of *S. aureus*:

Test	Result
Coagulase	**+ve**
Catalase	**+ve**
Oxidase	**-ve**
Aerobic mannitol fermentation	**Ferment**
DNase production	**+ve**
Haemolysis on blood agar	**ß-hemolysis**
Growth in 7-9%NaCl	**+ve**
Growth at 45°C	**+ve**

3.2 Effect of Trehalose on drying (dehydrations):

The results were shown in fig (3-2) .Isolate of *S.aureus* had a higher level of viability after one month when preserved in the presence of Trehalose than control. The two primary stresses that were proposed to destabilize lipid bilayers during dehydration are fusion and lipid phase transitions. Studies by laser light scattering or other techniques demonstrate that Trehalose and other sugars inhibit fusion between the vesicles during drying, but the inhibition of fusion alone is not sufficient to preserve the dry vesicles. Thus Trehalose is also necessary to prevent phase transitions (Crowe and Crowe, 1990).

Trehalose depresses the phase transition temperature of the dry lipids, which maintains them in the liquid crystalline phase in the absence of water (Crowe and Crowe, 1988).

While the result of *E.coli* isolate shown no effect of Trehalose preservation in dry state. The disaccharide Trehalose acts as a carbon source in *E.coli.* At high osmolarity of the growth medium, however, they can also degrade Trehalose as the sole source of carbon under both high- and low-osmolarity growth conditions. The modes of Trehalose utilization are different under conditions and have to be well regulated (Boos *et al.,* 1990)

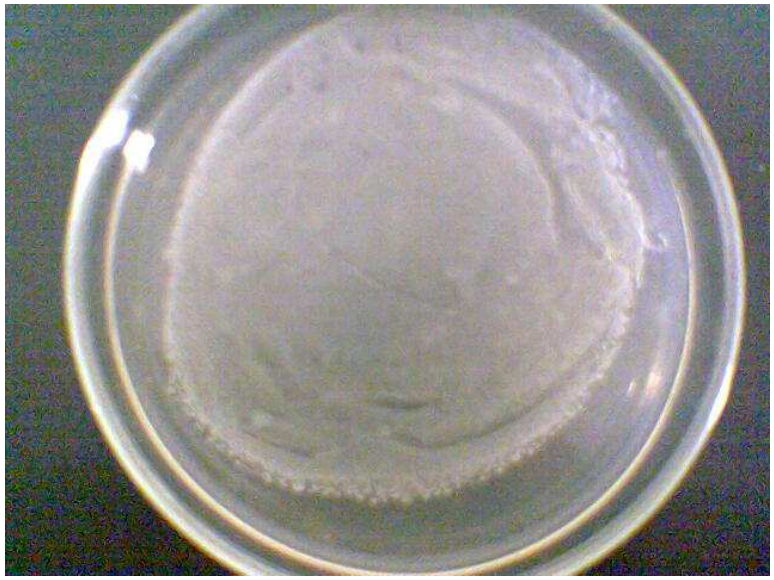


Figure (3-2): (A) Effect of trehalose in preservation of *S.aureus* after one month of drying.

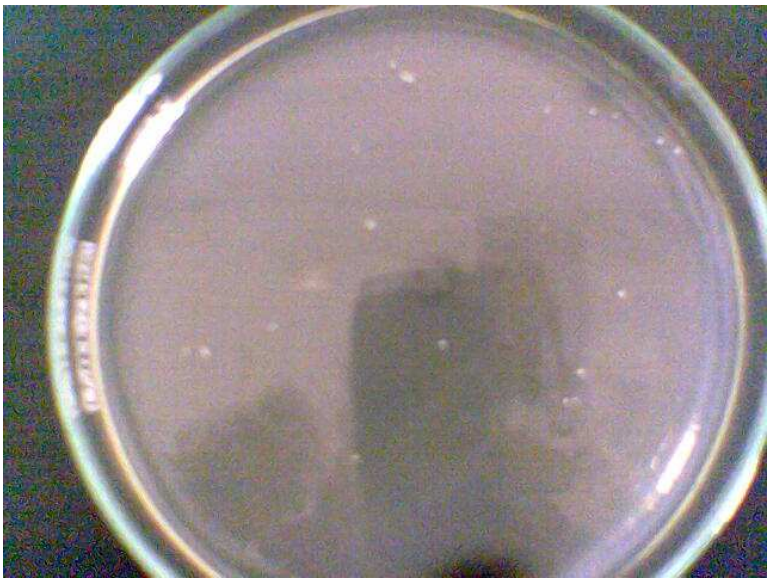


Figure: (B) drying of *S.aureus* after one month.

3.3 UV Survival Curve of *Staphylococcus aureus* with and without addition of Trehalose:

The survival curve of *S.aureus* after exposed to different doses of UV irradiation is shown in figure (3-3). The result indicated that *S.aureus* lost it's viability exponentially while addition of Trehalose make the bacterial isolate more resistance to UV. The survival curve of *S.aureus* without addition of Trehalose (control) showed increases of lethal percentage exponentially with the increase of UV doses and the highest lethal percentage or the less survival fraction is $l.lxl0^{14}$ when the bacteria exposed to 40 J/m^2 of UV irradiation. But the survival curve of *S.aureus*. With the addition of Trehalose showed more resistance to the UV (The most serious damage to cells exposed to radiation is attributed mostly to effects on the structure of cellular DNA. We found that trehalose protects DNA from irradiation, In the presence of Trehalose, DNA can be protected from about 4 times higher doses of irradiation, The protective effect increases with the amount of the sugar , Yoshinaga, 1997).

Other study of lethal effect of UV light on *S.aureus* showed that this bacterium was sensitive (Al-Bakri and Umrann, 1994; Al-Zubaei, 2001).

Results indicated that *S.aureus* without addition of Trehalose was sensitive to mutation induced by UV, while the addition of Trehalose, the bacteria become more resist to UV irradiation(Yoshinaga, 1997).

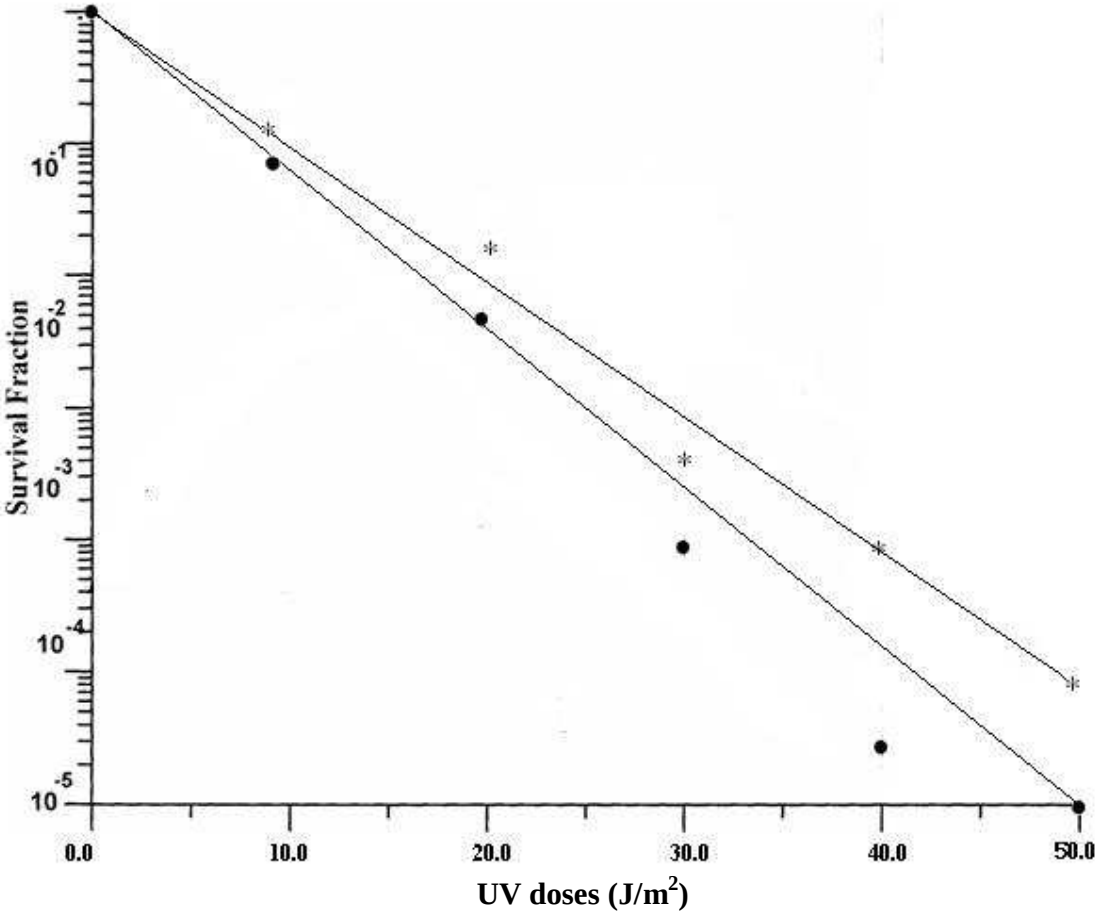


Figure (3-3): UV survival of *S.aureus* with and without Trehalose.

* ***S.aureus*** **with trehalose**

• ***S.aureus*** **without trehalose**

3.4 Antibiotic Sensitivity:

Isolate of *S.aureus* and *E.coli* were tested using eight different antibiotic disks. Decision for considering an isolate resistant or sensitive with and without addition of trehalose (control) were taken in comparison of the diameter of inhibition zone with that of standard value of (NCCLs, 2001).

Results shown in table (3-2) indicate that resistance to antibiotics was widely increased among isolate of *S.aureus* treatment with trehalose than the control.

The results showed that isolate of *S.aureus* treated with trehalose was resist to Norfloxacin, Erythromycin, Streptomycin and Gentamycin while the control was sensitive to these antibiotics.

Table (3-2): Result of antibiotics disc test by using 8 antibiotics against isolate of *S.aureus.*

Bacterial isolates	Antibiotics							
	Nor	**E**	**S**	**TE**	**GM**	**VA**	**CZC**	**ZOX**
S.aureus treated with trehalose	R	R	S	R	R	S	R	S
S.aureus without trehalose(control)	S	S	S	S	S	S	R	S

While Results shown in table (3-3) indicate that Trehalose had no effect on antibiotics resistance to *E.coli.*

The result shown in table (3-3) Trehalose had no effect on antibiotics resistance to *E.coli* because of Trehalase enzyme. The disaccharide Trehalose acts as a carbon source in *E.coli.* At high osmolarity of the growth medium, however, they can also degrade Trehalose as the sole source of carbon under both high- and low-osmolarity growth conditions. The modes of Trehalose utilization are different under conditions and have to be well regulated (Boos *et al.*, 1990).

Table (3-3): Result of antibiotics disc test by using 8 antibiotics against isolate of *E.coli.*

Bacterial isolates	**Antibiotics**							
	Nor	**E**	**S**	**TE**	**GM**	**VA**	**C**	**CF**
E.coli treated with Trehalose	S	S	R	R	S	R	S	S
E.coli without Trehalose(control)	S	S	R	R	S	R	S	S

Testing of resistance in microorganism is important for categorizing their behavior in accordance to kinds of antibiotics as well as their

medical applications concerning their effectiveness in treatment of diseases and distribution of resistance among isolates for designing pattern of resistance, serve as a picture for following transfer of genetic elements among species and hence spread of resistance (Baron *et al.*, 1994).

For screening purposes, test is required for separation of huge numbers of isolates. Accordingly, disc method is useful to do so. The test is fast and simple, however it suffer from lowerness of accuracy as long as zone of inhibition widely effected by medium composition and interference of some ions with spread of antibiotics through the medium (Baron *et al.*, 1994).

For research studies, Minimum Inhibitory Concentrations (MICs) is useful to specify minimum concentration of antibiotic showing inhibition of growth. Such concentration could also be useful for medical purposes. Accordingly, for testing antibiotic sensitivity, the two methods were used wherever it is needed.

3.5 Minimal Inhibitory Concentration (MIC) Test:

Isolates were selected due to multiple antibiotic resistances, thereby; these isolates were selected in order to evaluate bacterial susceptibility to antibiotic as in the table (3-4). The breakpoints were applied following (NCCLs, 1991), recommendations, when resistance level were calculated, **"MIC"** in both the intermediate and resistant ranges (as defined by the (NCCLs, 1991) were considered as non susceptible in this study. MIC was defined as the lowest drug concentration in microgram per milliter that inhibited the visible growth of bacteria (Kinoshita *et al.*, 1997).

MICs were determined using the agar dilution method. From results of table (3-4) one could concluded that Trehalose increasing antibiotics resistance of *S.aureus* while had no effect on *E.coli* resistance.

Table (3.4) The MICs values for tetracycline antibiotics:

Isolate	Antibiotics	MIC Values µg/ml
S.aureus + Trehalose	Tetracycline	12.5
S.aureus	Tetracycline	16.25

3.6 Effect of Trehalose on antibacterial activity (antagonism)

The isolated of pathogenic bacteria *S.aureus* with trehalose and without Trehalose (control) were inhibited by the crude antimicrobial substance produced by *S.epidermidis.*(antagonism activity) From Gram-positive microorganisms, it doesn't inhibit any Gram-negative bacteria, but does inhibit several other genera (Galiono and Hinsdill, 1970).Trehalose showed no effect on the protection of bacteria *S.aureus* from antimicrobial substance produced by *S.epidermidis.*

3.7 Store of bacterial isolates from freezing temperature in the presence and absence of Trehalose:

Two isolates of bacteria *S.aureus, E.coli* were stored by using the freezing technique and the results appears by the diagrams bellow for storing of the bacterial isolates in -20ºC in addition of 100mM Trehalose and without(control).There was increasing capability of viability of bacterial isolate *S.aureus*, when added 100mM of Trehalose before freezing compared with the control. Figure (3-4) showed that the bacterial isolate *S.aureus* keeps its viability after 4 weeks of freeze preservation while the control loss more of it's viability after preservation for the same time. In preservation of *E.coli* with Trehalose, showed the same effect in preservation against freeze than the control as shown in figure (3-5). The plasma membrane is a key component of the cell and must be maintained during freezing conditions if the cell is to be kept alive. It has been demonstrated with artificial membranes, such as unilamellar vesicles, that damage measured by intermixing and fusion can be reduced by a series of cryoprotectants, with trehalose and sucrose being more protective than glycerol. Thus, these sugars probably play a key role in preventing deleterious alteration to the membrane during reduced-water states (Anchordoguy *et al*., 1987).

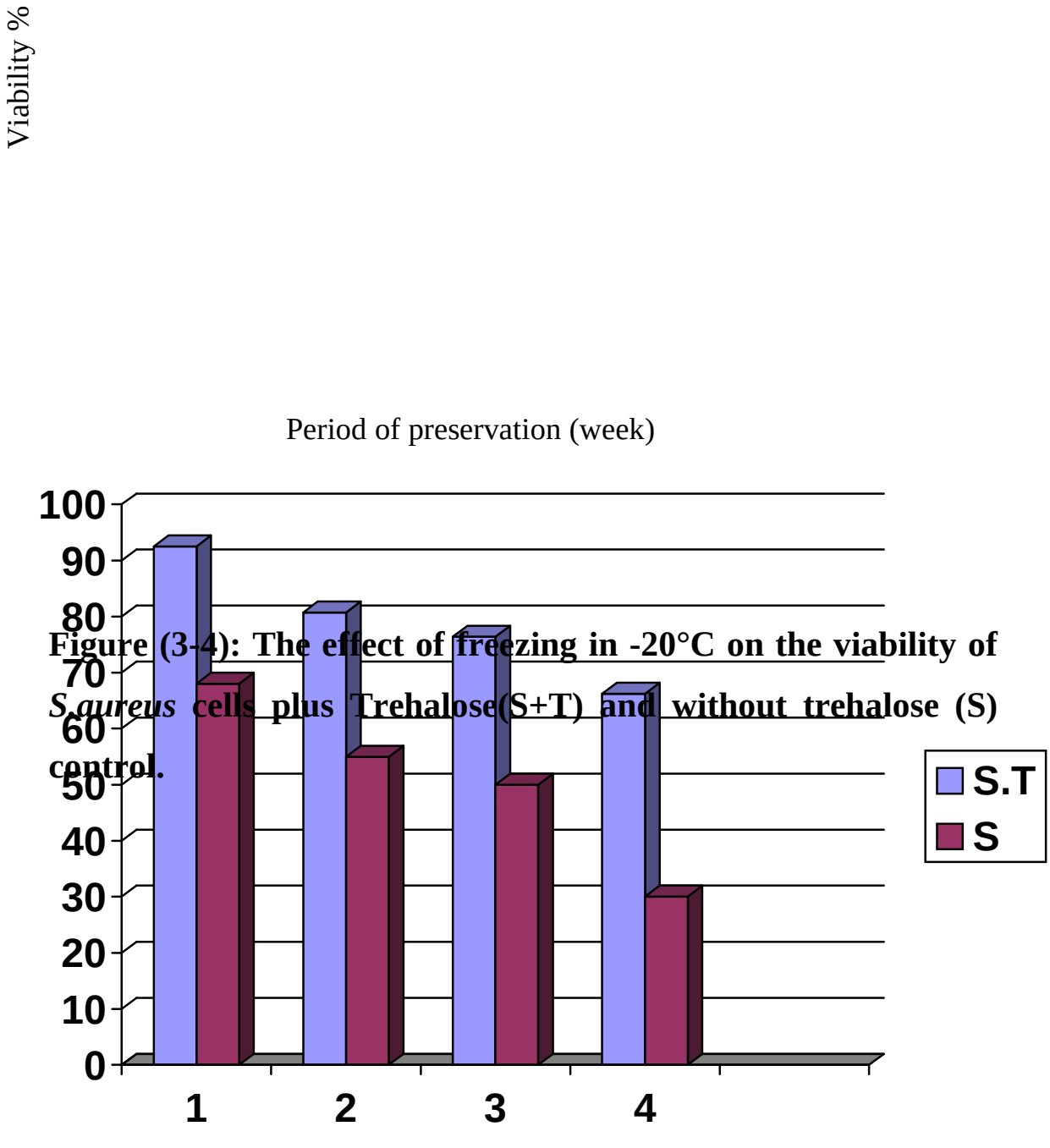


Figure (3-4): The effect of freezing in -20°C on the viability of *S.aureus* cells plus Trehalose(S+T) and without trehalose (S) control.

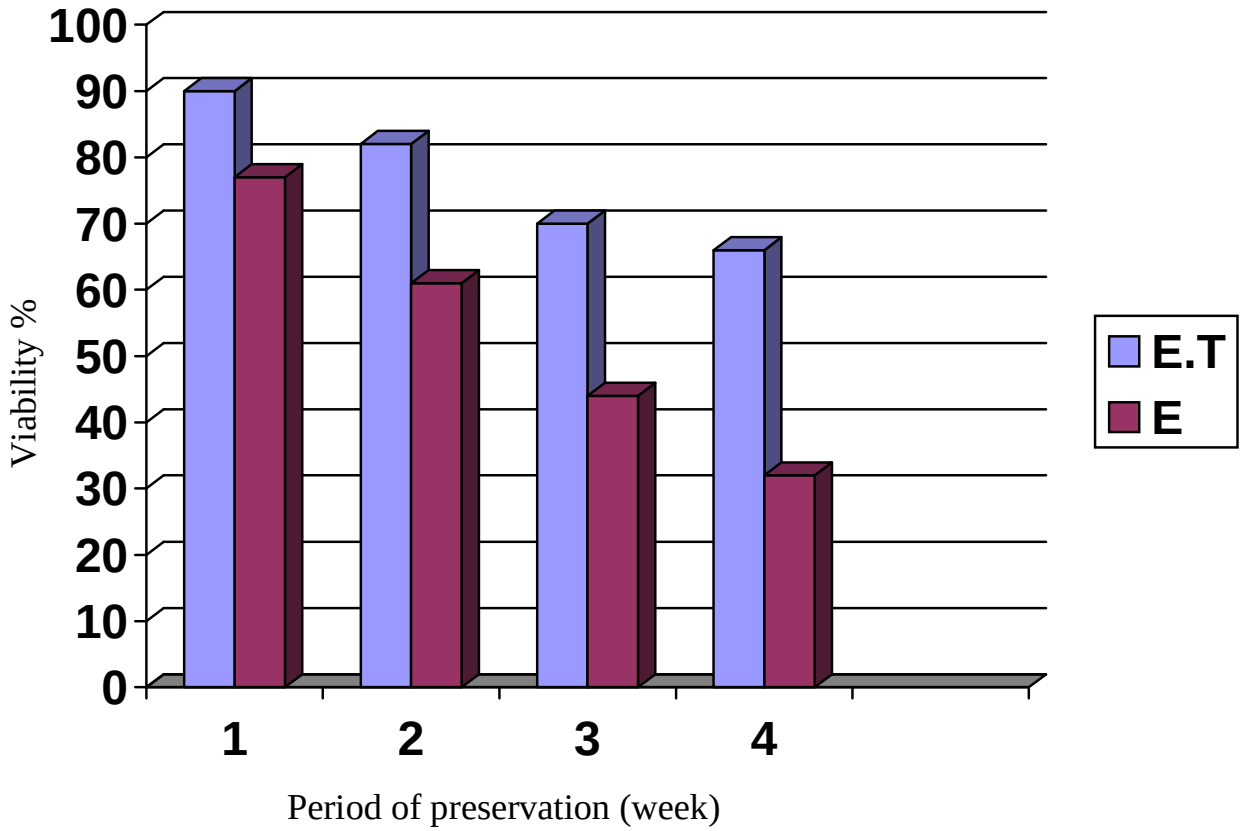


Figure (3-5): The effect of freezing in -20°C on the viability of *E.coli* cells plus Trehalose (E+T) and without trehalose (E) control.

3.8 Protection of bacterial isolate from freeze-drying (lyophilization):

S.aureus was lyophilized with Trehalose and without Trehalose (control). The experiment was proved that Trehalose keeps the bacteria more viable after lyophilization and stored in room temperature after 4 months of storing higher decrease was observed on viability of bacteria preserved without Trehalose, while the bacteria that preserved with Trehalose keep more of it's viability after the same time fig. (3-6).

The diagram shows the effect of Trehalose for preservation of bacteria after lyophilization and stored in room temperature.

Molecular simulations of model, hydrated phospholipids bilayers (e.g., dipalmitoylphosphatidylcholine) are performed over wide ranges of temperatures with and without protectants. The area per head group increases with temperature from 58 to 77 $Å^2$. Other properties such as hydration number, alkyl tail order-parameter, diffusion coefficients and radial distribution functions exhibit a clear dependence on temperature. The simulated area per head group of the bilayer is not affected by the presence of disaccharides, suggesting that the overall structure of the bilayer remains undisturbed. The results of simulations reveal that the interaction of disaccharide molecules with the bilayer occurs primarily at the surface of the bilayer, and it is governed by the formation of multiple hydrogen bonds to specific groups of the lipid. Disaccharide molecules are observed to adopt specific conformations to fit onto the surface topology of the bilayer, often interacting with up to three different lipids simultaneously. At high disaccharide concentrations, the results of simulations indicate that disaccharides can serve as an effective replacement for water under

anhydrous conditions, which helps explain their effectiveness as lyophilization agents for liposome and cells (MTSM).

The protection of biological molecules during freezing and freeze-drying (lyophilization) is a subject of considerable practical importance, particularly in the pharmaceutical industry. Much work has been conducted on the use of a wide variety of compounds as cryoprotectants for these types of processes. They have been found to protect proteins during freezing and drying stresses. They have also been shown to prevent damage to cells during freezing and drying. Trehalose has been found to be a highly effective. Several structural and thermodynamic arguments have been put forward to explain the mechanisms of action of molecules such as Trehalose (MTSM).

Damage to biological systems resulting from freeze-drying can be attributed to two primary causes: changes in the physical state of membrane lipids and changes in the structure of sensitive proteins. Removal of hydrogen-bonded water from the head group region of phospholipid bilayers increases the head group packing (Crowe *et al*., 1985), and forces the acyl chains together, increasing the probability of Van Der Waals interactions. As a result, the lipid may undergo a transition from liquid crystalline to gel phase (Crowe *et al*., 1988).

Upon rehydration, dry membranes, which are in gel phase at room temperature, undergo a transition from gel to liquid crystal phase. As the membranes pass through this phase transition there are regions with packing defects, making the membranes leaky (Byler and Susi 1985).

Adding a disaccharide such as trehalose before drying lowers the transition temperature (*Tm*) of the dry membranes by replacing the water between the lipid head groups, preventing the phase transition and its accompanying leakage upon rehydration (Crowe *et al*., 1985, Crowe *et al*., 1989).

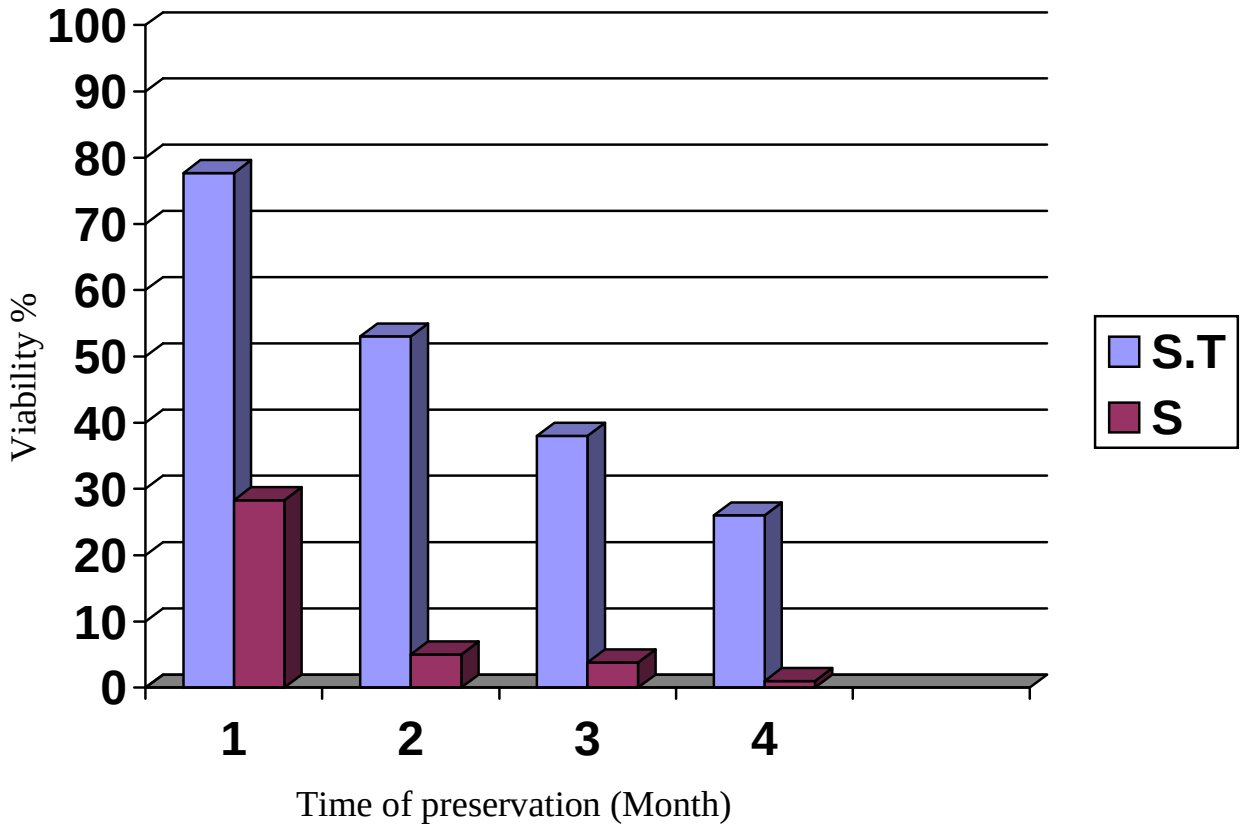


Figure (3-6): The effect of freeze-drying on the viability of *S.aureus* cells plus Trehalose(S+T) and without Trehalose(S) control.

3.9 Effect of Trehalose on Electroporated bacteria

Trehalose showed an effect on protection of bacteria after electroporation. Result showed in fig. (3-7) indicate Trehalose increase the resistance of the bacteria against electroporation.

Electroporation is widely used for introduction of DNA and other foreign molecules into eukaryotic cells. However, conditions yielding the greatest molecule uptake and gene expression can result in low cell survival. In this study, we assessed the efficiency of Trehalose for enhancing cell viability after excessive Electroporation. This disaccharide was chosen because of its capability of stabilizing cell membranes under various stressful conditions, such as dehydration and freezing However, electroporation is very damaging to the cell membranes. (Mussauer *et al*., 1999).

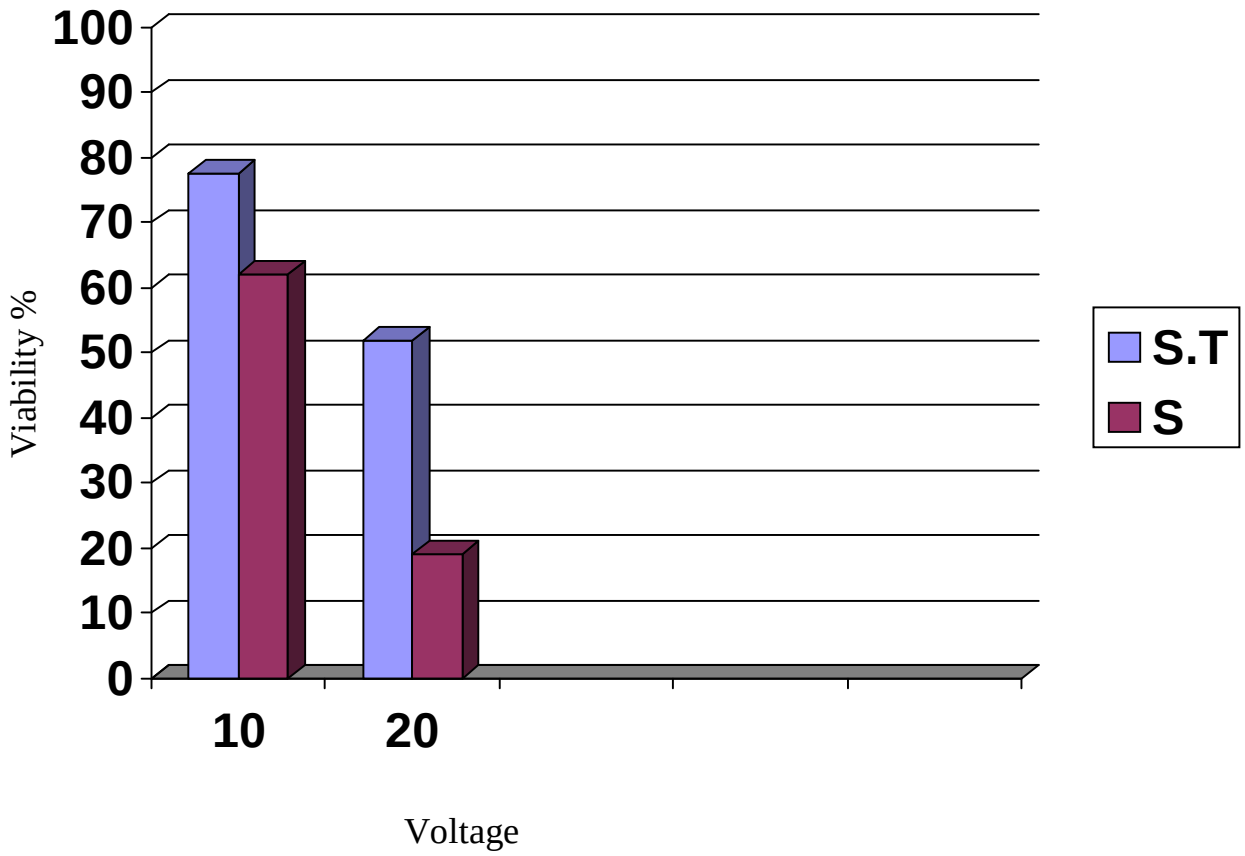


Figure (3-7): The effect of electroporation on the viability of *S.aureus* cells plus Trehalose (S+T) and without Trehalose (S) control.

اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ (1) خَلَقَ الْإِنسَانَ مِنْ عَلَقٍ (2) اقْرَأْ وَرَبُّكَ الْأَكْرَمُ (٣) الَّذِي عَلَّمَ بِالْقَلَمِ (٤) عَلَّمَ الْإِنسَانَ مَا لَمْ يَعْلَمْ (٥)

صَدَقَ اللهُ العلي العَظيمِ

سورة العلق من أية(١-٥)

Recommendations

1. The study of the effect of in vivo injected Trehalose and its role in the protection of living cells.
2. Increase the production of Trehalose from microorganisms produced it.
3. Study the effect of Trehalose in protection of bacteria from curing agents.

Abbott P.T. (1999) Low trehalase activity is associated with abdominal symptoms caused by edible mushrooms. *Scand. J. Gastroenterol*., 34, 898–903.

Al-Bakri G.H.; and Umran M.A. (1994). Mutagenesis of noval halotolerant bacteria (*micrococcus* sp.) using ultraviolet light and N-methyl-N-nitrosoguanidine. Iraq. *J. Microbial* 6:55-65.

Anandarajah K. and Mckersie B.D. (1990) Manipulating the desiccation tolerance and vigor of dry somatic embryos of *Medicago sativa L* with sucrose, heat shock and abscissic acid. Plant Cell Rep., 9, 451–455.

Anchordoguy T. J.; Rudolph A.S.; Carpenter J.F.; Crowe J.H.(1995). Modes of interaction of cryoprotectants with membrane phospholipids during freezing. *Cryobiology* 24:324-331.

Andersson U.(2002). Beta-glucose 1-phosphate interconverting enzymes in *Lactococcus lactis*: physiological role and regulation in maltose, trehalose and glucose metabolism, Center for Chemistry and Chemical Engineering, Hall K:C.

Argüelles J.C. (2000). Physiological roles of trehalose in bacteria and yeasts: a comparative analysis 174: 217 – 224.

Atlas R.M. (1995). Principles of microbiology. *Mosby-year Book, Inc*., USA.P:547 and 668.

Ballone P.; Marchi M.; Branca C.; and Magazu S. (2000) . Structural and vibrational properties of trehalose: a density functional study. *J. Phys. Chem. B.* 104:6313–6317.

Banaroudj, N., Lee, D.H., and Goldberg, A.L. (2001) Trehalose accumulation during cellular stress protects cells and cellular proteins from damage by oxygen radicals. J. Biol. Chem., 276, 24261–24267.

Behm C. A. (1997). The role of trehalose in the physiology of nematodes. *Int. J. Parasitol.* 27:215–229.

Becker A.; Schloeder P.; Steele J.E.; and Wegener G. (1996) The regulation of trehalose metabolism in insects. *Experientia*, 52, 433–439.

Belisle, J.T., Vissa, V.D., Sievert, T., Takayama, K., Brennan, P.J., and Bersa, G.S. (1997) Role of the major antigen of *Mycobacterium tuberculosis* in cell wall biogenesis. Science, 276, 1420–1422.

Belton P. S.; and Gil A. H. (1994). IR and Raman spectroscopic studies of the interaction of trehalose with hen egg white lysozyme. *Biopolymers.* 34:957–961.

Besra, G.S., Bolton, R.C., McNeil, M.R., Ridell, M., Simpson, K.E., Glushka, J., van Halbeek, H., Brennan, P.J., and Minnikin, D.E. (1992) Structural elucidation of a novel family of acyltrehaloses from *Mycobacterium tuberculosis*. Biochemistry, 31, 9832–9837.

Birch, G. G. (1972). Mushroom sugar. *In* Health and Food. G. G. Birch, L. F. Green, and I. G. Plaskett, editors. Elsevier Science, New York. 49–53.

Boos W.; Ehmann U.; Forki W.; Klein M.; Rimmele M.; and Postma P. (1990) Trehalose transport and metabolism in *Escherichia coli*. *J. Bacteriol.*, 172, 3450–3461.

Branca C.; Magazu S.; Maisano G.; and Migliardo P.(1999). Anomalous cryoprotective effectiveness of trehalose: Raman scattering evidences. *J. Chem. Phys.* 111:281–287.

Branca C.; Magazu S.; Maisano, G.; Bennington, S. M.; and Fak B. (2003). Vibrational studies on disaccharide/H2O systems by inelastic neutron scattering, Raman, and IR spectroscopy. *J. Phys. Chem. B.* 107:1444–1451.

Bredereck, H.; Hoschele, G.; and Ruck, K. (1953) Synthesis of non-reducing disaccharides. *Chem. Ber.*, 86:1277–128.

Brennan, P.J. and Nikaido, H. (1995) The envelope of *mycobacteria*. Annu. Rev. Biochem., 64, 29–63.

Bryant, G.; and Wolfe, J.(1992). Interfacial forces in cryobiology and anhydrobiology. *Cryo-Letters.* 13:23–36.

Byler, D. M.; and Susi H. (1986). Examination of the secondary structure of proteins by deconvolved FTIR spectra. *Biopolymers* 25:469–487.

Carpenter J.F. and Crowe J.F. (1989) An infrared spectroscopic study of the interactions of carbohydrates with dried proteins. Biochemistry, 28, 3916–3922

Carpenter, J. F.; Prestrelski, S. J.; Anchordoguy, T. J.; and Arakawa, T. (1994). Interactions of stabilizers with proteins during

freezing and drying. *In* Formulation and Delivery of Proteins and Peptides. J. L. Cleland and R. Langer, editors. *American Chemical Society, Washington, DC*. 134–147.

Cerda, J.J.; Preiser, H.; and Crane, R.K. (1972) Brush border enzymes and malabsorption: Elevated disaccharides in chronic pancreatic insufficiency with diabetes mellitus. *Gastroenterology*, 62: 841.

Chandrasekhar, I.; and Gaber, B. P. (1988). Stabilization of the bio-membrane by small molecules: interaction of trehalose with the phospholipids bilayer. *J. Biomol. Struct. Dyn.* 5:1163–1171.

Chatterjee, D. (1997) The mycobacterial cell wall: structure, biosynthesis and sites of drug action. Curr. Opin. Chem. Biol., 1, 578–588.

Clegg, J. S. (1965). The origin of trehalose and its significance during formation of encysted dormant embryos of *Artemia salina. Comp. Biochem. Physiol.* 14:135–143.

Clegg, J. S. (1997). Embryos of *Artemia franciscana* survive four years of continuous anoxia: the case for complete metabolic rate depression. *J. Exp. Biol.* 200:467–475.

Clegg, J. S. (2001). Cryptobiosis—a peculiar state of biological organization. *Comp. Biochem. Physiol.* 128B:613–624.

Clegg, J. S.; and Jackson, S. A. (1992). Aerobic heat-shock activates trehalose synthesis in embryos of *Artemia franciscana. FEBS Lett.* 303:45–47.

Collee, J.C.; Fraser, A.G.; Marminon, B.P. and Simmons, A. (1996). Staphylococcus: cluster-forming Gram-positive cocci. In practical medical microbiology 14th edition. Churchill livigstone. UK. P: 245.

Conrad, P. B.; and de Pablo, J. J. (1999). Computer simulation of the cryoprotectant disaccharide alpha, alpha-trehalose in aqueous solution. *J. Phys. Chem. A.* 103:4049–4055.

Cottone, G.; Ciccotti, G.; and Cordone, L. (2002). Protein-trehalose-water structures in trehalose coated carboxy-myoglobin. *J. Chem. Phys.* 117:9862–9866.

Cottone, G.; Cordone, L.; and Ciccotti, G. (2001). Molecular dynamics simulation of carboxy-myoglobin embedded in a trehalose-water matrix. *Biophys. J.* 80:931–938.

Crowe J. H.; Carpenter J. F.;and Crowe L. M. (1989a). The role of verification in anhydrobiosis. *Annual Review of Physiology* 60: 73-103.

Crowe, J. H.; Carpenter, J. F.; and Crowe, L. M. (1998b). The role of vitrification in anhydrobiosis. *Annu. Rev. Physiol.* 60:73–103.

Crowe, J. H.; Clegg, J. S.; and Crowe, L. M. (1998). Anhydrobiosis: the water replacement hypothesis. *In* The Properties of Water in Foods (ISO-POW 6). D. S. Reid, editor. Chapman and Hall, New York. 440–455.

Crowe, J. H.; Crowe, L. M.; and Chapman, D. (1984). Preservation of membranes in anhydrobiotic organisms: the role of trehalose. *Science.* 223:701–703.

Crowe, J. H.; Crowe, L. M.; Carpenter, J. F.; and Wistrom, C. A. (1987). Stabilization of dry phospholipid bilayers and proteins by sugars. *Biochem. J.* 242:1–10.

Crowe, J. H.; Crowe, L. M.; Carpenter, J. F.; Prestrelski, S.; Hoekstra, F. A.; Araújo, P. de; and Panek, A. D. (1997). Anhydrobiosis: cellular adaptation to extreme dehydration. *In* Handbook of Physiology, Vol. 2. W. H. Dantzler, editor. *Oxford University Press, Oxford, UK.* 1445–1477.

Crowe, J. H.; Crowe, L. M.; Oliver, A. E.; Tsvetkova, N.; Wolkers, W.; and Tablin, F. (2001). The trehalose myth revisited: introduction to a symposium on stabilization of cells in the dry state. *Cryobiology.* 43:89–105.

Crowe, J. H.; F. Hoekstra, A.; and Crowe, L. M. (1992). Anhydrobiosis. *Annu. Rev. Physiol.* 54:579–599.

Crowe, J. H.; Oliver, A. E.; and Tablin, F. (2002). Is there a single biochemical adaptation to anhydrobiosis? *Integr. Comp. Biol.* 42:497–503.

Crowe, J. H.; Tablin, F.; Wolkers, W. F.; Gousset, K.; Tsvetkova, N. M.; and Ricker, J. (2003). Stabilization of membranes in human platelets freeze-dried with trehalose. *Chem. Phys. Lipids.* 122:41–52.

Crowe, J.; Crowe, L.; and Chapman, D. (1984) Preservation of membranes in anhydrobiotic organisms. The role of trehalose. *Science*, 223:209–217.

Crowe, J.H.; and Crowe, L.M. (1990) Lyotropic effects of water on phospholipids. In: F. Franks (ed.), *Water science reviews*. Cambridge University Press, Cambridge, England, 1–23.

Crowe, L. M. (2002). Lessons from nature: the role of sugars in anhydrobiosis. *Comp. Biochem. Physiol.* 131A:505–513.

Crowe, L. M.; J. H. Crowe, A.; Rudolph, C. Womersley; and L. Appel. (1985). Preservation of freeze-dried liposomes by trehalose. *Arch. Biochem. Biophys.* 242:240-247.

Crowe, L.M.; and Crowe, J.H. (1988) Trehalose and dry dipalmitoyl phosphatidylcholine revisited. *Biochim. Biophys. Acta*, 946:193–201.

Daffe, M., Lacave, C., Laneelle, M.-A., Gillois, M., and Laneele, G. (1988) Polyphthienoyl trehalose, glycolipids specific for virulent strains of the tubercle bacillus. Eur. J. Biochem., 172, 579–584.

Dahlqvist, A. (1974) Enzyme deficiency and malabsorption of carbohydrates. In: Sipple, H.L & McNutt, K.W., eds, *Sugars in Nutrition*, New York: Academic Press, 187–214.

Damore, T. G.; R. Crumplen, G.; and Stewart, G. G. (1991). The involvement of trehalose in yeast stress tolerance. *J. Ind. Microbiol.* 7:191–195.

Demelier, J.-F.; Labat, J. and Courtois, J.-E. (1975) Influence of parenteral injection of trehalose on mammals with distinct trehalose equipment. *C.R. Acad. Sc. (Paris) Serie D*, 280: 669–672.

De Virgilio, C., Hottinger, T., Dominiguez, J., Boller, T., and Wiemken, A. (1994) The role of trehalose synthesis for the acquisition of thermotolerance in yeast. Eur. J. Biochem., 219, 179–186.

Eastmond,P.J.; Yi Li , Graham,I.A.(2003) Is trehalose-6-phosphate a regulator of sugar metabolism in plants? Journal of Experimental Botany, 54:533-537.

Elbein, A.D. (1974) The metabolism of α,α-trehalose. *Adv. Carbohyd. Chem. Biochem.*, 30:227–256.

Elbein, A.D.; and Mitchell, M. (1973) Levels of glycogen and trehalose in *Mycobacterium smegmatis*, and the purification and properties of the glycogen synthase. *J. Bacteriol.*, 113:863–873.

Elbein,A.D.; Pan ,Y.T.; Pastuszak,I ; Carroll,D.(2003). New insights on trehalose: a multifunctional molecule, 13:17-27.

Engelsen, S. B.; and Perez, S. (2000). Unique similarity of the asymmetric trehalose solid-state hydration and the diluted aqueous-solution hydration. *J. Phys. Chem. B.* 104:9301–9311.

Eroglu, A.; Toner, M.; and Toth, T. L. (2002). Beneficial effect of microinjected trehalose on the cryosurvival of human oocytes. *Fertil. Steril.* 77:152–158.

Eze, L.C. (1989) Plasma trehalase activity and diabetes mellitus. *Biochem. Gen*., 27:487–495.

Felix,C.F.; Moreira, C. C.; Oliveira, M. S.; Sola-Penna, M. ; Meyer-Fernandes, J. R. ; Scofano, H. M.; Ferreira-Pereira, A. (1999). Protection against thermal denaturation by trehalose on the plasma

membrane H^+-ATPase from yeast Synergetic effect between trehalose and phospholipid environment*Eur. J. Biochem.* 266:660-664

Feofilova, E. P. (2003). Deceleration of vital activity as a universal biochemical mechanism ensuring adaptation of microorganisms to stress factors: a review. *Appl. Biochem. Microbiol.* 39:1–18.

Fernandez J.; Sato T.; Franco A.; Vicentre-Soler J.; Cansado J.; Gacto M. (1998). Enhancement of neutral trehalose activity by oxidative stress in the fission yeast *Schizosaccharmyces pombe.* Fungal Genet Biol 25:79-86.

Fukuse, T.; Hirata, T.; Nakamura, T.; Ueda, M.; Kawashima, M.; Hitomi, S.; and Wada, H. (1999). Role of saccharides on lung preservation. *Transplantation.* 68:110–117.

Gadd G.M.; Chalmers K. and Reed R.H. (1987) The role of trehalose in dehydration resistance of *Saccharomyces cerevesiae*. FEMS Microbiol. Lett., 48, 249–254.

Garland, G. (1989) Brush border membrane sucrase–isomaltase, maltase–glucoamylase and trehalase in mammals. *Comp. Biochem. Physiol*., 94B:1–11.

Gudmand-Høyer, E. and Skovbjerg, H. (1996) Disaccharide digestion and maldigestion. *Scand. J. Gastroenterol*., 31 : 111–121.

Gudmand-Høyer, E.; Fenger, H.J.; Skovbjerg, H.; Kernhansen, P. and Rørbaek Madsen, P. (1988) Trehalase deficiency in Greenland. *Scand. J. Gastroenterol*., 23:775–778.

Guo, N., Puhlev, I., Brown, D.R., Mansbridge, J., and Levine, F. (2000) Trehalose expression confers desiccation tolerance on human cells. Nature Biotechnol., 18, 168–171.

Guppy, M.; and Withers, P. (1999). Metabolic depression in animals: physiological perspectives and biochemical generalizations. *Biol. Rev.* 74:1–40.

Harely, P.J. and Prescott, M.L. (1996). Laboratory exercises in microbiology. Mc grow-Hill, USA.

Helferich, B.; and Weis, K. (1956) The synthesis of glucosides and of nonreducing disaccharides. *Chem. Ber*., 89:314–321.

Hietanen, E. (1973) Interspecific variation in the levels of intestinal alkaline phosphatase, adenosine triphosphatase and disaccharidases. *Comp. Biochem. Physiol*., 46A:359–369.

Higashiyama,T.(2002). Novel functions and applications of trehalos. *Pure Appl. Chem.*, 74: 1263–1269.

Hincha, D.K., Zuther, E., Hellwege, E.M., and Heyer, A.G. (2002) Specific effects of fructo- and gluco-oligosaccharides in the preservation of liposomes during drying. Glycobiology, 12, 103–110.

Hoekstra, F. A.; Wolkers, W. F.; Buitink, J.; Golovina, E. A.; Crowe, J. H.; and Crowe, L. M. (1997). Membrane stabilization in the dry state. *Comp. Biochem. Physiol.* 117A:335–341.

Holt, J.G. (1994). Bergey´s manual of determinative bacteriology. 9.ed. Philadelphia: Williams & Wilkins,. 787.

Hore, P. and Messer, M. (1968) Studies on disaccharidase activities of the small intestine of the domestic cat and other carnivorous mammals. *Comp. Biochem. Physiol*., 24:717–725.

Hottinger T.; Boller T. and Wiemken, A. (1987) Rapid changes of heat and desiccation tolerance correlated with changes of trehalose content in *Saccharomyces cerevesiae*. FEBS Lett., 220, 113–115.

Hunenberger,P.H.(2004). Interaction of the Disaccharide Trehalose With a Phospholipid Bilayer: A Molecular Dynamics Study Laboratory of Physical Chemistry, ETH-Honggerberg, HCI G233, CH 8093 Zurich, Switzerland.

Hunter, S.W., Murphy, R.C., Clay, K., Goren, M.B., and Brennan, P.J. (1983) Trehalose-containing lipooligosaccharides. J. Biol. Chem., 258, 10481–10487.

Kaasen, I.; McDougall, J.; and Strom, A.R. (1994) Analysis of the otsBA operon for osmoregulatory trehalose synthesis in *Escherichia coli*. *Gene*, 145:9–15.

Kandror, O., DeLeon, A., and Goldberg, A.L. (2002) Trehalose synthesis is induced upon exposure of *Escherichia coli* to cold and is essential for viability at low temperatures. Proc. Natl. Acad. Sci. USA, 99, 9727–9732.

Keilin, D. (1959). The problem of anabiosis or latent life: history and current concept. *Proc. R. Soc. Lond. B Biol. Sci.* 150:149–191.

Kinoshita J.; Yoshida, K.; and Egawa, A. (1997) Valuing Environmental Benefits of Farmland at the Urban Fringe by the Double-

Bounded Dichotomous Choice CVM, Journal of Rural Economics, 69:43-51.

Kizawa H.; Miyazaki J.; Yokoda A.; Kanegae Y.; Miyagawa K.; Sugiyama Y. (1995). Trehalose production by a strain of *Micrococcus varians.* Biosci Biochem 59:1522-1527.

Koch, E. M.; and Koch, F. C.(1998). The presence of trehalose in yeast. *Science.* 61:570–572.(Midline).

Koster, K. L.; Lei, Y. P.; Anderson, M.; Martin, S.; and Bryant, G. (2000). Effects of vitrified and nonvitrified sugars on phosphatidylcholine fluid-to-gel phase transitions. *Biophys. J.* 78:1932–1946.

Koster, K. L.; Webb, M. S.; and Bryant, G. (1994). Interactions between soluble sugars and POPC (1-palmitoyl-2-oleoylphosphatidylcholine) during dehydration: vitrification of sugars alters the phase-behavior of the phospholipid. *Biochim. Biophys. Acta.* 1193:143–150.

Labat-Robert, J. (1982) Trehalases. In: Lee, C.K. & Lindley, M.G., eds, *Developments in Food Carbohydrate*, Vol. 3, *Disaccharidases*, London: Applied Science Publishers. 84–106.

Lambruschini, C.; Relini, N.; Ridi, A.; Cordone, L.; and Gliozzi, A. (2000). Trehalose interacts with phospholipid polar heads in Langmuir monolayers. *Langmuir.* 16:5467–5470.

Lederer, E. (1976) Cord factor and related trehalose esters. Chem. Phys. Lipids, 16, 91–106.

Lee, C. W. B.; Waugh, J. S.; and Griffin, R. G. (1986). Solid-state NMR-study of trehalose/1,2-dipalmitoyl-sn-phosphatidylcholine interactions. *Biochemistry.* 25:3737–3742.

Lemieux, R.U.; and Bauer, H.F. (1954) A chemical synthesis of D-trehalose. *Can. J. Chem.*, 32:340–344.

Leopold A.C. (1986) Membranes, metabolism, and dry organisms. Cornell University Press, Ithaca, NY. , 1–377.

Leslie, S. B.; Crowe, J. H.; Rowe, L. M. (1995).Trehalose and Sucrose Protect Both Membranes and Proteins in Intact Bacteria during Drying Applied and environmental microbiology. 61:3592–3597.

Lins, R. D.; Pereira, C. S.; and Hünenberger, P. H. (2004). Trehalose-protein interaction in aqueous solution. *Proteins.* 55:177–186.

Liu, Q.; Schmidt, R. K.; Teo, B.; Karplus, P. A.; and Brady, J. W. (1997). Molecular dynamics studies of the hydration of alpha, alpha-trehalose. *J. Am. Chem. Soc.* 119:7851–7862.

Luzardo, M. D.; Amalfa, F.; Nunez, A. M.; Diaz, S.; de Lopez, A. C. B.; and Disalvo, E. A. (2000). Effect of trehalose and sucrose on the hydration and dipole potential of lipid bilayers. *Biophys. J.* 78:2452–2458.

Madin, K. A. C.; and Crowe, J. (1975). Anhydrobiosis in nematodes: carbohydrate and lipid metabolism during drying. *J. Exp. Zool.* 193:335–342.

Maestracci, D. (1976) Enzymic solubilization of the human intestinal brush border membrane enzymes. *Biochim. Biophy. Acta*, 433, 469–481.

Maniatis, T.; Fritch, E.F. and Sambrook, J. (1982). Molecular cloning. A laboratory manual. Cold Spring Harbour. NewYourk.

Martin, M.C.; Diaz, L.A.; Manzanal, M.B.; and Hardisson, C. (1986) Role of trehalose in the spores of *Streptomyces*. *FEMS Micro. Lett.*, 35: 49–54.

Maruta, K.; Hattori, K.; Nakada, T.; Kubota, M.; Sugimoto, T.; and Kurimoto, M. (1996a) Cloning and sequencing of trehalose biosynthesis genes from *Rhizobium* sp. M-11. *Biosci. Biotechnol. Biochem.*, 60:717–720.

Maruta, K.; Hattori, K.; Nakada, T.; Kubota, M.; Sugimoto, T.; and Kurimoto, M. (1996b) Cloning and sequencing of trehalose biosynthesis genes from *Arthrobacter* sp. Q36. *Biochim. Biophys. Acta*, 1289:10–13.

Maruta, K.; Mitsuzumi, H.; Nakada, T.; Kubota, M.; Chaen, H.; Fukuda, S.; Sugimoto, T.; and Kurimoto, M. (1996c) Cloning and sequencing of a cluster of genes encoding novel enzymes of trehalose biosynthesis from thermophilic archaebacterium *Sulfolobus acidocaldarius*. *Biochim. Biophys. Acta*, 1291:177–181.

Matsuda, K. (1956) Studies on the unfermentable disaccharides in koji extract. IV. Isolation of α,α- and α,ß-trehalose. *Tohoku J. Agric. Res.*, 6: 271–277.

Matsuo, T. (2001). Trehalose protects corneal epithelial cells from death by drying. *Br. J. Ophthalmol*. 85:610–612.

Matsuo, T.; Tsuchida, Y.; and Morimoto, N. (2002). Trehalose eye drops in the treatment of dry eye syndrome. *Ophthalmology.* 109:2024–2029.

Maza, L.M; Pezzlo, M.T. and Baron, E.J. (1997). Color atlas of diagnostic microbiology Mosby-Year Book, Inc.USA.

Muller, J., Wiemken, A., and Aeschbacher, R. (1999) Trehalose metabolism in sugar sensing and plant development. Plant Sci., 147, 37–47.

Mussauer, H.; Sukhorukov V. L.; Hasse A.; and Zimmermann U.. (1999). Resistivity of red blood cells against high-intensity, short-duration electric field pulses induced by chelating agents. *J. Membr. Biol.* 170:121–133

Nagase, H.; Ueda, H.; and Nakagaki, M. (1999). Relationship between hydrophobic index of saccharide and gel-liquid crystal transition temperature of the L-alpha-dipalmitoyl phosphatidylcholine (DPPC)/saccharide/water system. *Chem. Pharm. Bull.* 47:607–610.

Nagle, J. F.; and Tristram-Nagle, S. (2000). Structure of lipid bilayers. *Biochim. Biophys. Acta.* 1469:159–195.

Nakagaki, M.; Nagase, H.; and Ueda, H. (1992). Stabilization of the lamellar structure of phosphatidylcholine by complex-formation with trehalose. *J. Membr. Sci.* 73:173–180.

Nathan, P.; Law, E.J.; Murphy, D.F. (1978). A laboratory method for the selection of topical antimicrobial agents to treat infected burn wounds. *Burns* 4:177–178.

Nicolaus B.; Gambacorta A.; Basso A.L.; Riccio R.; DeRosa M. and Grant W.D. (1988) Trehalose in Archaebacteria systems. Appl. Microbiol., 10, 215–217.

Niederst, C. and Dauça, M. (1985) [Activities of renal hydrolases during pre- and postnatal development of mice.] *Can. J. Physiol. Pharmacol*., 63: 731–734.

Nishimoto, T.; Nakano, M.; Bnakada, T.; Chaen, H.; Fukuda, S.; Sugimoto, T.; Kurimoto, M.; and Tsujisaka, Y. (1995) Purification and properties of a novel enzyme, trehalose synthase, from *Pimelobacter* sp. R48. *Biosci. Biotechnol. Biochem.*, 60:640–644.

Norcia, M. A. (2000). Compositions and methods for wound management. *Off. Gaz. Pat. Office.* 1232:424–448.

Ohta, M., Pan, Y.T., Laine, R.A., and Elbein, A.D. (2002) Trehalose-based oligosaccharides isolated from the cytoplasm of *Mycobacterium smegmatis*. Eur. J. Biochem., 269, 1–8.

Oliver, A. E.; Leprince, O.; Wolkers, W. F.; Hincha, D. K.; Heyer, A. G.; and Crowe, J. H. (2001). Non-disaccharide-based mechanisms of protection during drying. *Cryobiology.* 43:151–167.

Pereira , C. S.; Lins , R. D.; Chandrasekhar , I.; Freitas, L. C. G. ; Hünenberger,P.H.(2004).Interaction of the Disaccharide Trehalose with

a Phospholipid Bilayer: A Molecular Dynamics Study *Biophysical Journal* 86:2273-2285.

Pilon-Smits, E.A.H., Terry, N., Sears, T., Kim, H., Zayed, A., Hwang, S., van Dun, K., Voogd, E., Verwoerd, T.C., Krutwagen, R.W.H.H., and Goddijn, O.J.M. (1998) Trehalose-producing transgenic tobacco plants show improved growth performance under drought stress. J. Plant Physiol., 152, 525–532.

Qiaofang Chen; and Haddad,G.G.(2004). Role of trehalose phosphate synthase and trehalose during hypoxia: from flies to mammals *Journal of Experimental Biology* 207:3125-3129.

Riby, J.; Sunshine, S. and; Kretchmer, N. (1990) Renal trehalase: Function and development. *Comp. Biochem. Physiol*., 95A:95–99.

Richards A.B.; Krakowka S.; Dexter L. B.; Schmid H.; Wolterbeek A.P.; Waalkens-Berendsen D.H.; Shigoyuki A.; and Kurimoto M. (2002a). Trehalose: areview of properties, history of use and human tolerance, and results of multiple safety studies. Food and Chemical Toxicology 40:871-898.

Richards, A. B.; Krakowka, S.; Dexter, L. B.; Schmid, H.; Wolterbeek, A. P. M.; Waalkens-Berendsen, D. H.; Shigoyuki, A.; and Kurimoto, M. (2002b). Trehalose: a review of properties, history of use and human tolerance, and results of multiple safety studies. *Food Chem. Toxicol.* 40:871–898.

Ricker, J. V.; Tsvetkova, N. M.; Wolkers, W. F.; Leidy, C., Tablin, F.; Longo, M.; and Crowe, J. H. (2003). Trehalose maintains phase separation in an air-dried binary lipid mixture. *Biophys. J.* 84:3045–3051.

Ring R. A.; Danks H.V. (1998). The role of trehalose in cold hardiness and desiccation Crg. Letter 19:257-282.

Rosseau P.; Halvorson H.O.; Bulla L.A. and Jullan, G. (1972) Germination and outgrowth of single spores of *Saccharomyces cerevesiae* viewed by scanning electron and phase contrast microscopy. *J. Bacteriol.*, 109, 1232–1238.

Rudolph, B. R.; Chandrasekhar, I., Gaber, B. P., and Nagumo, M. (1990). Molecular modeling of saccharide-lipid Interactions. *Chem. Phys. Lipids.* 53:243–261.

Ruppin, H.; Domschke, W.; Domschke, S. and Classen, M. (1974) [Intestinal disaccharidase activities in juvenile diabetes mellitus.] *Klin. Wschr*., 52:568–570.

Sasnoor, L.M; Kale, V.P.; Limaye, L.S. (2003). Supplementation of Conventional Freezing Medium with a Combination of Catalase and Trehalose Results in Better Protection of Surface Molecules and Functionality of Hematopoietic Cells. 12: 553-564.

Sato, A.; and Aso, K. (1957) Kojibiose, 2-O-α-D-glucopyranosyl-D-glucose. *Nature*, 180: 984–985.

Schick I.; Fleckenstein J.; Weber H.; Kulbp K.D. (1991). Coenzyme-independent enzymetic synthesis of alpha,alpha trehalose. *Biochem Enz*. Stuttgart 128-129.

Scott, P. (2000). Resurrection plants and the secrets of the leaf. *Ann. Bot.* 85:159–166.

Shimakata, T.; and Minatagawa, Y. (2000) Essential role of trehalose in the synthesis and subsequent metabolism of corynomycolic acid in *Corynebacterium matruchotii*. *Arch. Biochem. Biophys.*, 380:331–338.

Singer, M. A.; and Lindquist, S. (1998). Thermotolerance in *Saccharomyces cerevisiae*: the yin and yang of trehalose. *Trends Biotechnol.* 16:460–468.

Sola-Penna M.; Meyer-Fernandes J. R.(1998).Stabilization against thermal inactivation promoted by sugars on enzyme structure and function: why is trehalose more effective than other sugars? 1;360(1):10-4

Somme, L. (1996). Anhydrobiosis and cold tolerance in tardigrades. *Eur. J. Entomol.* 93:349–357.

Sun, W. Q.; and Leopold, A. C. (1994). Glassy state and seed storage stability: a viability equation analysis. *Ann. Bot.* 74:601–604.

Sun, W. Q.; and Leopold, A. C. (1997). Cytoplasmic vitrification acid survival of anhydrobiotic organisms. *Comp. Biochem. Physiol.* 117A:327–333.

Sun, W. Q.; Irving, T. C.; and Leopold, A. C. (1994). The role of sugar, vitrification and membrane phase-transition in seed desiccation tolerance. *Physiol. Plantarum.* 90:621–628.

Sun, W. Q.; Leopold, A. C.; Crowe, L. M.; and Crowe, J. H. (1996). Stability of dry liposomes in sugar glasses. *Biophys. J.* 70:1769–1776.

Sussich, F.; Skopec, C.; Brady, J.; and Cesaro, A. (2001). Reversible dehydration of trehalose and anhydrobiosis: from solution state to an exotic crystal? *Carbohydr. Res.* 334:165–176.

Takahashi, H.; Ohmae, H.; and Hatta, I. (1997). Trehalose-induced destabilization of interdigitated gel phase in dihexadecylphosphatidylcholine. *Biophys. J.* 73:3030–3038.

Takayama, K.; and Armstrong, E.L. (1976) Isolation, characterization and function of 6-mycolyl-6'acetyltrehalose in the H37Rv strain of *Mycobacterium tuberculosis*. *Biochemistry*, 15:441–446.

Thevelein J.M. (1984) Regulation of trehalose metabolism in fungi. Microbiol. Rev., 48, 42–59.

Thompson, A.; Anno, K.; Wolfrom, M.L.; and Inatome, M. (1954) Acid reversion products from D-glucose. *J. Am. Chem. Soc.*, 76:1309–1311.

Timasheff, S. N. (1982). The control of protein stability and association by weak-interactions with water. *In* Biophysics of Water. F. Franks and S. Mathias, editors. Wiley, New York. 70–72.

Tsvetkova, N. M.; Phillips, B. L.; Crowe, L. M., Crowe, J. H.; and Risbud, S. H. (1998). Effect of sugars on headgroup mobility in freeze-dried dipalmitoylphosphatidylcholine bilayers: solid-state P-31 NMR and FTIR studies. *Biophys. J.* 75:2947–2955.

Van Elburg, R.M.; Uil, J.J.; Kokke, F.T.M.; Mulder, A.M.; Van de Broek, W. G. M.; Mulder, C.J.J. and Heymans, H. S. A. (1995) Repeatability of the sugar-absorption test, using lactulose and mannitol,

for measuring intestinal permeability for sugars. *J. Pediatr. Gastroenterol. Nutr*., 20:184–188.

Vogel, G.; Fiehn, O.; Louis Jean - Richard- dit- Bressel, Boller, T. ; Wiemken, A.; Aeschbacher ,R. A. ;Wingler, A. (2001).metabolism in *Arabidopsis*: occurrence of trehalose and molecular cloning and characterization of trehalose-6-phosphate synthase homologues Journal of Experimental Botany, 52: 1817-1826

Westh, P.; and Ramlov, H. (1991). Trehalose accumulation in the tardigrade adorybiotus-coronifer during anhydrobiosis. *J. Exp. Zool.* 258:303–311.

Williams, R. J.; and Leopold, A. C. (1989). The glassy state in corn embryos. *Plant Physiol.* 89:977–981.

Wingler, A. (2002). The function of trehalose biosynthesis in plants. *Phytochemistry.* 60:437–440.

Wolfe, J.; and Bryant, G. (1999). Freezing, drying and or vitrification of membrane-solute-water systems. *Cryobiology.* 39:103–129.

Wyatt, G. R.; and Kalf, G. F. (1957). The chemistry of isect hemolymph. 2. Trehalose and other carbohydrates. *J. Gen. Physiol.* 40:833–847.

Xie, G. F.; and Timasheff,S. N. (1997). The thermodynamic mechanism of protein stabilization by trehalose. *Biophys. Chem.* 64:25–43.

Yashida M.; Nakamura N.; Herikeshi K. (1995). Production and application of maltose phosphorylase and trehalose phospherase by strain of *Plesiomonus* , Oye toshito kagaka 42:19-25.

Yoshida, K. (1993) Serum trehalase activity in patients with rheumatoid arthritis. *Clin. Chim. Acta*, 215:123–124.

Yoshinaga K.; Yoshioka H.; Kurosaki H.; Hirasawa M.; Uritani M.; Hasegawa K..(1997). Protection by trehalose of DNA from radiation damage. Biosci Biotechnol Biochem.;61(1):160-1.

Zahringer H.; Holzer H.; Nwaka S. (1998). Stability of neutral trehalose during heat stress in *Saccharomyceses cerevisiae* in dependent on the activiyy of the catalytic subunits of cAMP- dependant protein kinase TPK1 and TPK2 eur. J. Biochem. 255:544-551.

Zentella R.; Mascorro-Gallardo J.O.; Van Dijck P.; Folch-Mallol J.; Bonini B.; VanVaeck C.; Gaxiola R.; Covarrubias A.A.; Nieto-Sotelo J.; Thevelein J.M. and Iturriaga, G. (1999) A *Selaginella lepidophylla* trehalose-6-phosphate synthase complements growth and stress-tolerance defects in a yeast tps1 mutant. Plant Physiol., 119, 1473–1482.

Zhang, J. and; Steponkus, P. L. (1996). Proposed mechanism for depression of the liquid-crystalline-to-gel phase transition temperature of phospholipids in dehydrated sugar-phospholipid mixtures. *Cryobiology*. 33:625–626.

الخلاصه

استخدمت عزلتان في هذه الدراسة احدهما تعود الى جنس المكورات الذهبية والاخرى لبكتريا القولون والتي شخصت اعتمادا على صفاتها المظهرية والفسلجية وخصائصها الكيموحيوية . عوملت البكتريا من كلا النوعين ب١٠٠ ملي مولار من التريهالوز لدراسه تاثيرات هذا النوع من السكر في حفظ البكتريا من العوامل المتعدده.

أظهرت النتائج بان التريهالوز له القدره على حفظ بكتريا المكورات الذهبية من الجفاف ولمدة شهر في درجه حراره الغرفة الاعتيادية وكانت البكتريا اكثر بقائيه مقارنة مع معاملة السيطرة .في حين لم يلاخظ اي تأثير في بكتريا القولون المعامله بنفس النسبة من التريهالوز.

وأظهرت النتائج التأثير الحافض السكر التريهالوز في حاله خزن البكتريا في ظروف الانجماد ولمده حوالي ٤ اسابيع كانت نسبة بقائية بكتريا المكورات الذهبية ٦٧% مقارنه مع السيطره بنسبه ٣٠%، بينما في بكتريا القولون كانت النتائج ٦٦% والسيطرة٣٢%.

واشارت النتائج ايضا الى قدره الحمايه التي يوفرها السكر للبكتريا المجفده حتى في ضروف البيئه الغير ملائمه (درجه حراره الغرفه) ولمده ٤ اشهر حيث نسبه بقائيه البكتريا المعامله بالتريهالوز ٢٧% و السيطرة ٢%.

اظهر التريهالوز تأثيرات الحماية للبكتريا المشعه فوق البنفسجيه وكذلك البكتريا المثقبه كهربائيا .

في حين لم يظهر اي نوع لتأثيرات الحمايه من الفعاليه التضاديه في حين على النقيض من ذلك اظهر التريهاوز تاثيرات واضحه في الحمايه من بعض المضادات الحيويه وذلك من خلال زياده نسبه المقاومه اتجاه هذه المضادات،حيث اظهرت النتائج ان بكتريا المكورات الذهبية المعاملة بالتريهالوز اصبحت مقاومة للمضادات: Tetracycline, Gentamycin, Norfloxacin, Erythromycin في حين السيطرة بقيت حساسة لها ولم يظهر اي تأئير للتريهالوز في مقاومة بكتريا القولون للمضادات الحيوية.