
Pigment Production by Zinc Solubilizing Bacteria and their Anti-Microbial Potential

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ABSTRACT

Zinc (Zn) is an essential micronutrient required by plants and animals. But in environment soluble Zn is in limiting amount. To fulfill the requirement of available Zn, biotransformation of insoluble form is required. On the other hand, the synthetic dye is very toxic environment. So, our effort was to isolate some bacteria having potential ability to solubilize Zn and produce pigment. In our study we had taken thirteen bacteria among them E₁₆, E₃, E₉, E₁₀ and E₃ solubilize significant high amount of Zn in presence of ZnO. Among all these isolates E₂₃ E₃, E₁₆ D₁₁, could produce different type of pigment of white, pale yellow, yellow or pink color. The produced pigments have ability to color fabrics. The E₁₆ pigment was highly capable for dyeing fabric than other. Additionally, isolates have antimicrobial ability. E₁₆ is highest efficiency index in case of *E. coli* & *Staphylococcus* sp. So, our isolates might have multipurpose application with proper management strategy.

Keywords: Zinc solubilization; Bacteria; Pigment; Antimicrobial

INTRODUCTION

Microorganisms require various nutrients for their growth and metabolism. Among the nutrients, zinc is an element present in the enzyme system as co-factor and metal activator of many enzymes (Parisi and Vallee, 1969). The role of zinc in the nutrition and physiology of both eukaryotic and prokaryotic organisms is widely studied, especially its importance for activity of many enzymes (Hughes and Poole, 1989). Zinc deficiency in fungi and bacteria is accompanied by impairment of the formation of pigments such as melanin, chrisogenin, prodigiosin, subtilin and others (Chernavina, 1970). Zinc solubilizing potential of few bacterial genera has been studied. Exogenous application of soluble zinc sources, similar to fertilizer application, has been advocated to various crops. This causes transformation of about (96-99)% of applied available zinc to various unavailable forms. The zinc thus made unavailable can be reverted back to available form by inoculating a bacterial strain capable of solubilizing it. Since zinc is a limiting factor in crop production, this study on zinc solubilization by bacteria has an immense importance in zinc nutrition to plants (Saravanan.V.S et al., 2004)

Recent environmental awareness has again revived the interest in natural dyes, mainly among environmentally conscious people. Excessive use of synthetic dyes, estimated at around 10,000,000 tons per annum, the production and application of which release vast amounts of waste and unfixed colorants, causing serious health hazards and disturbing the nature (Ghorpade et al., 2000). Natural dyes are considered eco-friendly as these are renewable and biodegradable and may also provide health benefits to the wearer. Natural dyes can be used for dyeing almost all types of natural fibers. Recent research shows that they can also be used to dye some synthetic fibers (Saxena and Raja, 2014).

Natural dye exhibit better biodegradability and are generally more compatible with the environment. However, the main disadvantage of these natural dyes or pigments lies in the order of magnitude of their extraction yield factors (a few grams of pigments per kg of dried raw material).

Antibiotics are chemical components (Microbial secondary metabolites) produced by some microorganisms in low concentration affecting the growth of other sensitive organisms. The sensitivity of an antibiotic depends on the type of microorganisms of the age of the culture.

Antibiotic sensitivity is quite significant due to development of resistant strain among various microorganisms. Some microbes are very sensitive to particular antibiotic while other are not.

In agar diffusion method, when the test solution is placed in contact with a solid agar, the solute (antibiotic) diffuse into the agar decreases with increase in distances as the solute moves away from the interface. Ultimately, equilibrium is established and the solute concentration becomes uniform throughout the whole system. Such a system is used in microbial assays of antibiotics, where diffusion of antibiotics results in the formation of inhibited growth zone. The diameter of the zone of inhibition reflects the concentration gradient established by diffusion of the antibiotics, into the agar and the sensitivity of the microorganism to the antibiotic. The several factors that affect the test include –concentration of the antibiotic, composition of the medium and the test organisms.

The word pigment has a Latin origin and initially denoted a colored matter but it was later extended to indicate colored objects such as make-up. In the beginning of the middle ages, the word was also used to describe the divers plant and vegetable extracts, especially those used as food colorants. Pigments are classified as either organic/inorganic or natural/synthetic (Turner 1993). Biological pigments can be classified based on two general classifications namely structural affinities and natural occurrence. Some examples for naturally occurring pigments are anthocyanins (blue-red), carotene (yellow-red), chlorophylls (green) and tannins (brown-red) (Babitha ;2004). All biological pigments selectively absorb certain wavelengths of light while reflecting others.

Several intensity colored compounds have been isolated from certain bacteria that resemble pigments in other biological system. One example of pigments from bacteria is violacein,3-[1,2-dihydro-5-(5-hidroxy-1H-indol-3-yl)-2-oxo-3hpyrrol-3-ilydene]-1,3-dihydro-2H-indol-2-one, a violet pigment produced by *Chromobacterium violaceum* is Gram-negative proteobacteria found in the soil and water in tropical and subtropical environments (Buxbaum;1993).The most pleasurable feature of any article is its color. Various colors are associated with variety of feelings such as red color shows warmth, increases pulse rate and respiration, blue or green suggests cool and peaceful environment and encourages relaxation (Joshi, V.K., et al .2003). Naturally colors are obtained from color imparting compounds in living systems known as pigments which play an important role in physiology and molecular processes of microorganisms e.g. help to adapt at in extreme environment, protection from

solar radiation and photosynthesis. Since several decades, taxonomists have been using pigments as a taxonomic tool for the identification and classification of bacteria (Sutthiwong, N et al.2014). Pigments diversity depends on the differences in their chemical structures and the presence of specific chromophores (Hui, K.M. et al. 1969).Since long time, we get pigments from natural sources, and their use has increased due to the side effects caused by toxic nature of the synthetic pigments. In this way the microbial pigments are a good substitute. Synthetic colors are widely used in foodstuff, dyestuff, beauty products and drugs making processes, comprise various lethal effects. Many limitations of synthetic pigments are synthetic in nature, carcinogenic hazardous. They are also non-environment friendly and non- biodegradable (Kumar, A.,et al 2015). Microbial pigments are a promising alternative source for natural food grade pigments and have huge potential for applications in foodstuff due to their natural color and safety to use; these have medicinal properties, nutrients like vitamins. Their production is independent of season and graphical conditions with controllable and predictable yield (Goswami, B., and Bhowal, J. 2014) In nature, a great number of microorganisms e.g. yeast, fungi, algae, protozoa and bacteria produce pigments .Nevertheless, appropriate species should satisfy some criteria: i) ability to use a variety of C and N sources, ii) tolerance to growth conditions (e.g. pH, temperature, nutrient concentration), iii) providing expected color at reasonable yield, iv) non - toxic and non-pathogenic product, and v) simple extraction of pigment from cell biomass if not excreted (Sutthiwong, N et al.2014).

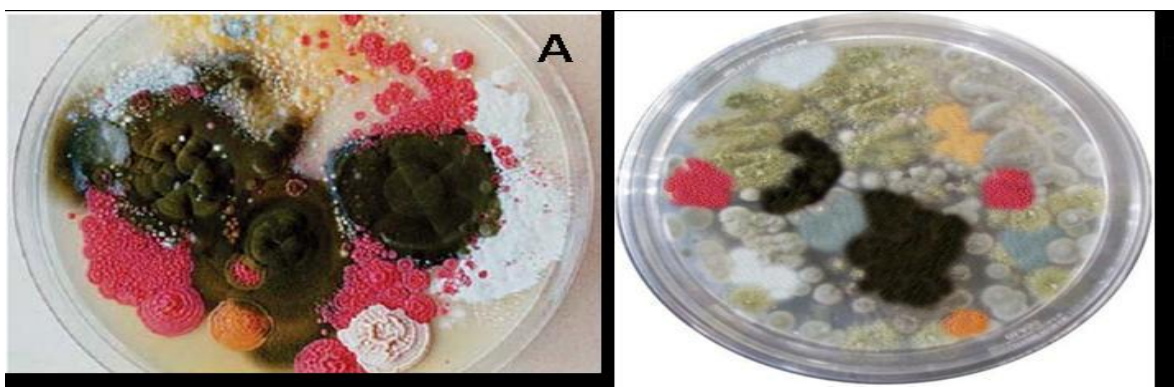


Figure 1. Representation of various colors producing microorganisms on a Petri dish.(Dufosse, L. 2009, Venil, C.K). and(Lakshmanaperumalsamy, P. 2009)

MATERIALS AND METHODS

Bacterial isolates and growth

Thirteen bacterial isolates have been provided for this study. All bacteria were routinely maintained in mineral salt medium (MSM) with the following composition (g/L): $(\text{NH}_4)_2\text{SO}_4$ -1.0, KCl-0.2, K_2HPO_4 -0.1, MgSO_4 -0.2, Dextrose (1%) at 30°C, either in liquid or on agar surface in presence of 1% ZnO. Provided cultures were E₁₁, E₁₈, E₂₃, E₁₆, E₁₄, E₂₁, E₃, E₁₇, E₁, E₂₀, E₉, E₁₀, D₁₁.

Preparation of culture medium

Screening of ZSB isolates

To determine zinc solubilization potential, bacteria were grown in MSM medium supplemented with 0.1% ZnO until observable growth. Well grown cultures were spot inoculated (drop) on the agar surface of the same medium contain 0.5% ZnO in laminar air hood. Inoculated agar plates were incubated at 30°C for 7 days for appearance of halo zone around the colonies. Zone diameter was measured and zinc solubilization efficiency was calculated.

After 72 hrs of incubation, halo-zone was observed around the colonies on mineral salt agar medium supplemented with ZnO were picked up from plates and was streaked onto MS and ZnO containing agar plates for purifying the culture, were then incubated at 30°C for 72 hrs. and then stored under refrigerator condition for further use. 10 different types of colonies were isolated from all the plates E₁₁, E₂₃, E₁₆, E₂₁, E₃, E₁₇, E₁, E₉, E₁₀, D₁₁.

Sterilized mineral salts agar medium supplemented with insoluble source of ZnO [1%, 0.2%, 0.5%, 0.7%, and 0.8%] were poured into sterile Petri plates individually. After the solidification of medium a loop full of selected bacterial isolates were transferred onto the plates, those plates were incubated at 30°C for 7 days. After the incubation, diameter of bacterial colony and halo zone around the colony was measured. These values were calculated using solubilizing index formula $S = (\text{Colony diameter} + \text{Halo zone diameter}) / \text{Colony diameter}$.

Bulk culture prepare (100ml)

Six bacterial isolates (E₃, E₁₀, E₁₁, E₁₆, E₂₁, and D₁₁) were taken for this study. Those bacteria were routinely maintained in mineral salt medium (MSM) with the following composition

(g/L (NH₄)₂SO₄-1.0, KCl-0.2 K₂HPO₄-0.1, MgSO₄-0.2, ZnO-1% Dextrose (1%)) in 100 ml medium at 30°C.

Optimization of pigment production use in Zinc

To optimize the pigment production in presence of ZnO, the bacteria were grown in liquid and solid media (MSM) using different concentration of ZnO (0.2%, 0.5%, 0.7%, and 0.8%).

Bacterial pigment scan

To determine the bacterial pigment potential, bacteria were grown in MSM liquid and solid medium supplemented with 0.2%, 0.5%, 0.7%, and 0.8% of ZnO until observable growth, for liquid medium, 2ml culture was taken from MSM liquid medium culture for centrifugation at 12,000rpm for 10 minutes, then supernatant was taken carefully in another 1.5 ml centrifuge tube scan the supernatant of various wavelength 200nm-1100nm. After scan supernatant was preserve at 4°C.

Then scraped bacterial colony from solid MSM agar plate in 2 ml sterile water, then cell suspended and vortex, this mixture was sonicated and then centrifuge at 12,000 rpm for 10 minutes, then supernatant was taken carefully in another 1.5 ml centrifuge tube scan the supernatant of various wavelength 200nm-1100nm. After scan supernatant was preserve at 4°C.

Antimicrobial assay

Preparation of culture soup

Two milliliter culture was taken from MSM liquid medium culture for centrifugation at 13,000 rpm for 10 minutes, then supernatant was taken carefully in another 1.5 ml centrifuge tube.

Preparation of indicator culture

Indicator cell culture (*E. coli*, & *Pseudomonas* sp.) are grown in 5ml LB in test tube, then incubate at 37°C over night shaker. Then taken 100 µl indicator culture was spread on LB

agar plate and cut the well on the agar surface by cup borer. Then fill the well by 80 µl of culture soup each. These agar plates were incubated at 30°C for 1 day for appearance of halo zone around the well. Zone diameter was measured and zone of inhibition was calculated.

Characterization of Zinc solubilizing bacteria-

After sufficient growth the isolates were observed for colony morphology and Gram's property.

Gram staining

A slide was made grease free using detergents. Then using inoculation loop, two - three loop full of single distilled water was put on the slide. Then using the sterile loop, a single colony of bacteria was picked and mixed with the water taken on the slide. Then small smear was made on the slide. Then air dried or heat fixed, then added crystal violet kept for 1 minute. This stain decanted and Gram's iodine was added and kept for 45 seconds, then addition of 95% Ethyl alcohol in an inclined way until blue color is removed. This color washed with single distilled water. Then Safranin was added and kept for 30 seconds. This color was removed by single distilled water, and then slide was air dried and observed under microscope.

Dyeing of fabric

The produce pigments by the bacteria are (E₁₀(0.8% ZnO), E₂₁(0.2% ZnO), E₁₆(0.5% ZnO), E₁₁(0.2% ZnO), E₃(0.2% ZnO), D₁₁(1% ZnO), and Control) were also tested for textile dyeing. Selected bacteria were allowed to produce pigments in MSM medium in large volume. Cells were removed by centrifugation (at 12,000 rpm for 10 minutes) and supernatant was taken into 1.5 ml centrifuge tube for dyeing. Collected pre-treatment fabric was carried out to remove cotton wax contained in fabrics was UV sterilized for dyeing. These sterilized fabrics dipped into the supernatant fill 1.5 ml Eppendorf. Then boiling of fabric with supernatant at 80°C for 2 hr. The clothes were washed after boiling.

RESULT & DISCUSSION

Bacterial growth medium Observation table

All bacteria were routinely maintained in mineral salt medium (MSM) at 30°C temperature.

<u>TUBE NO.</u>	<u>OBSERVATION DAY-7</u>
E ₁	White
E ₃	White
E ₉	White
E ₂₀	No observation
E ₁₀	Yellow
E ₁₁	Yellow
E ₁₇	White
E ₁₄	No observation
E ₁₈	No observation
E ₁₆	Grey
E ₂₁	White
D ₁₁	Pink
E ₂₃	White

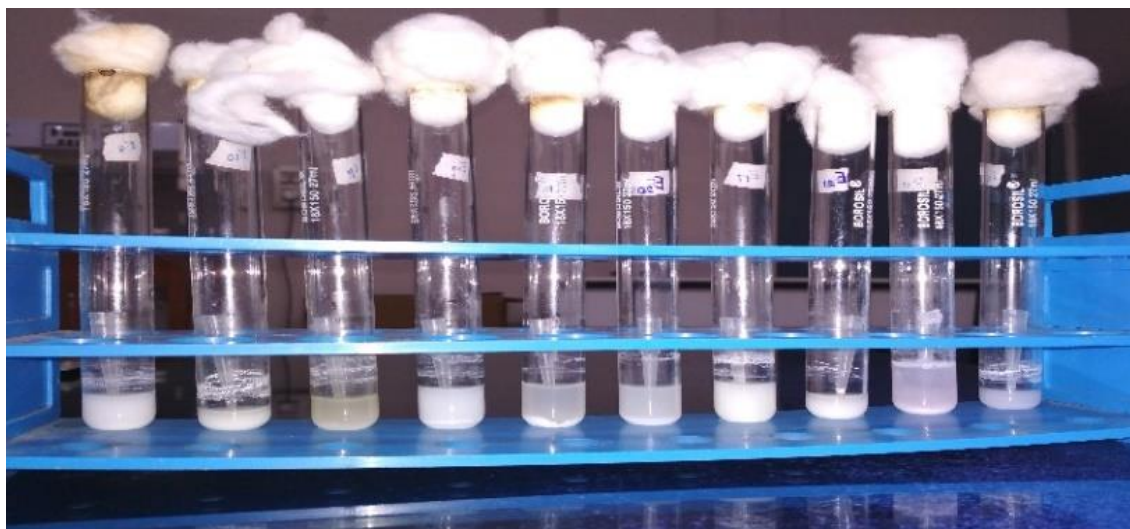
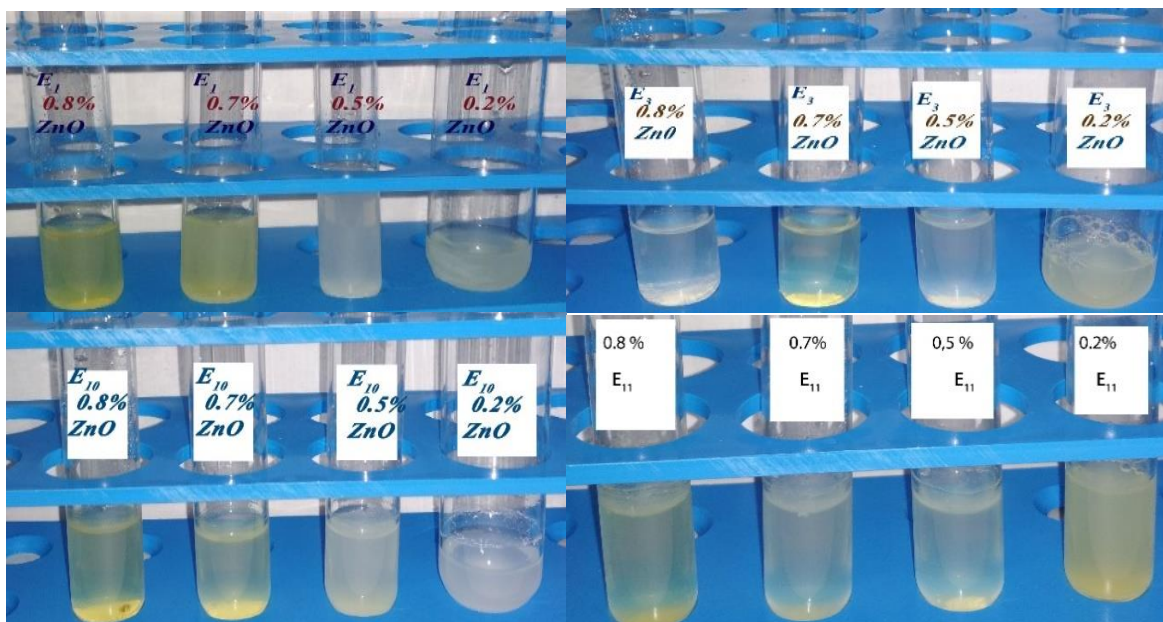


Fig1-Bacterial growth medium Observation

Optimization of pigment production using various concentration of Zinc and screening of ZSB isolates

Day-6 Culture	Percentage of ZnO			
	0.2%	0.5%	0.7%	0.8%
Isolates	0.2%	0.5%	0.7%	0.8%
E ₁	White	White	Pale yellow	yellow
E ₃	White	White	yellow	White
E ₉	yellow	White	yellow	yellow
E ₁₀	White	Pale yellow	White	yellow
E ₁₁	Pale yellow	Pale yellow	Pale yellow	Pale yellow
E ₁₇	No observation	Pale yellow	Pale yellow	Pale yellow
E ₁₆	White	Pale yellow	Pale yellow	Pale yellow
E ₂₁	Dark yellow	Pale yellow	White	White
D ₁₁	No observation	No observation	No observation	No observation
E ₂₃	No observation	White	White	White



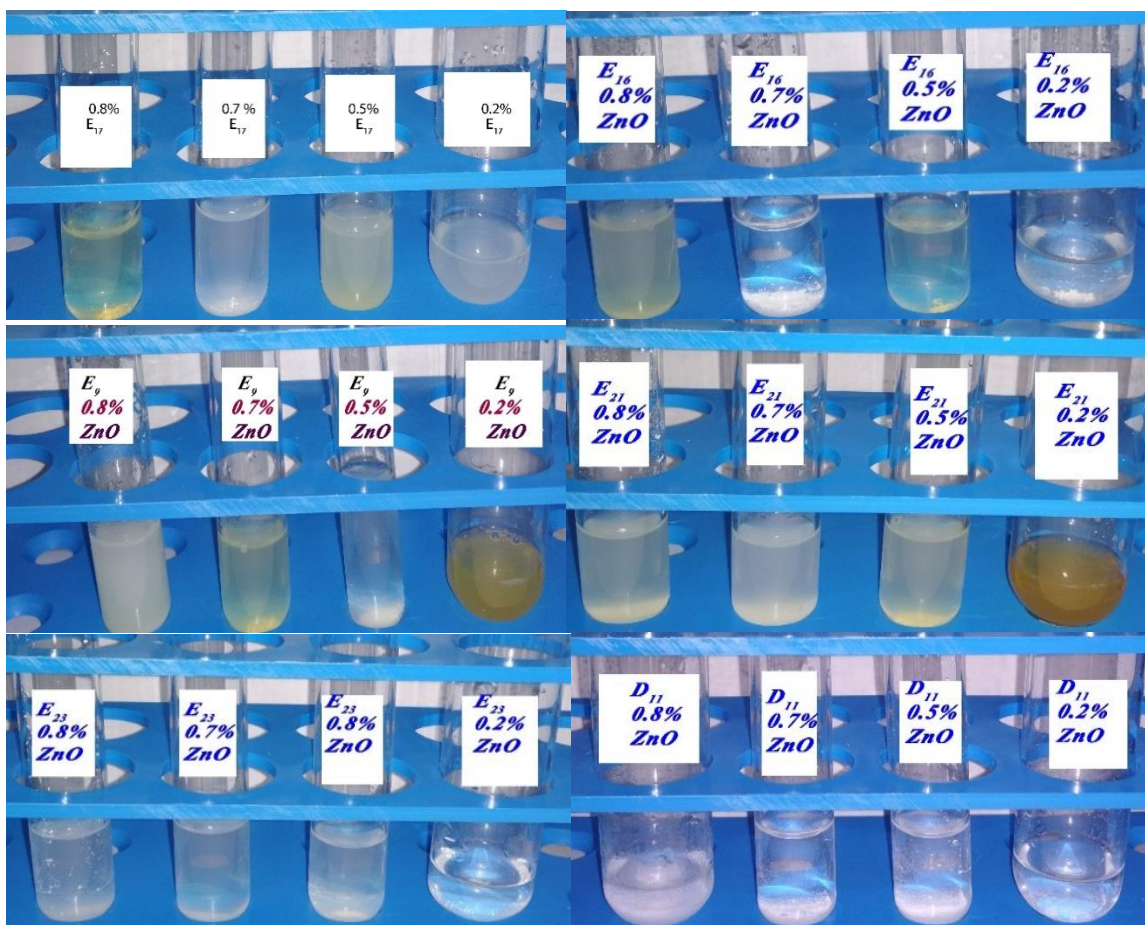


Fig-2 Bacterial culture showing pigment production at various ZnO concentration

Pigment production in MSM Agar plate using different ZnO concentration

Isolates	Percentage of ZnO (MSM Agar plate)			
	0.2%	0.5%	0.7%	0.8%
E ₁	yellow	Pale yellow	Pale yellow	Pale yellow
E ₃	yellow	Pale yellow	Pale yellow	Pale yellow
E ₉	White	yellow	yellow	yellow
E ₁₀	yellow	yellow	yellow	yellow
E ₁₁	yellow	yellow	yellow	yellow
E ₁₇	White	yellow	yellow	yellow
E ₁₆	yellow	yellow	yellow	yellow
E ₂₁	White	White	White	White
D ₁₁	Pink	Pink	Pink	Pink
E ₂₃	White	White	White	White

Bacterial pigment scan

E21: In presence of 0.2% ZnO E21 produce metabolites that showed absorbance around 350 nm. But in higher percentage of ZnO this peak abolished, however new peak were found around 250 to 350 nm.

E16: In presence of 0.5% ZnO E16 produce metabolites that showed absorbance around 350 nm. But in 0.2% and 0.7%percentage of ZnO this peak abolished, however new peak were found around 300 to 350 nm.

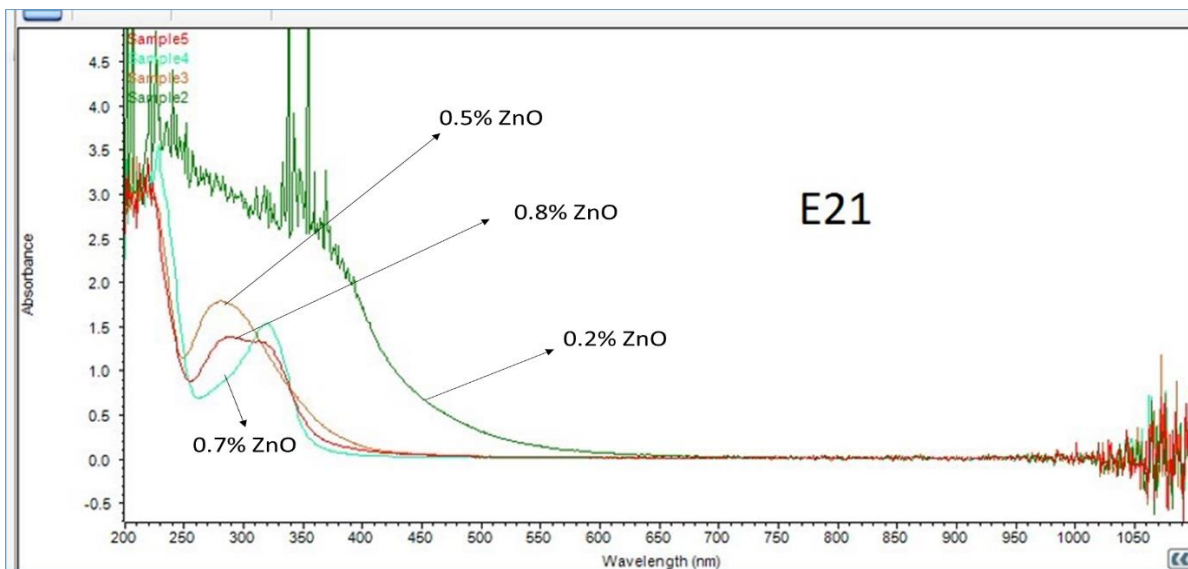
E11: In presence of 0.2% and 0.8% ZnO E11 produce higher metabolites that showed absorbance around 350 nm. But in 0.5% and 0.7% of ZnO this peak were found 250 to 300nm.

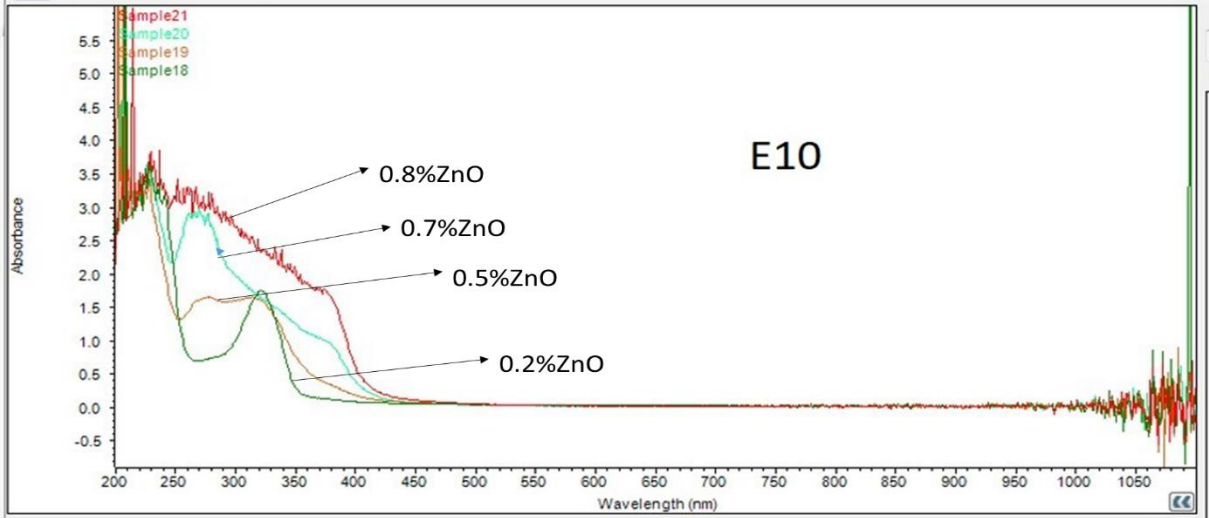
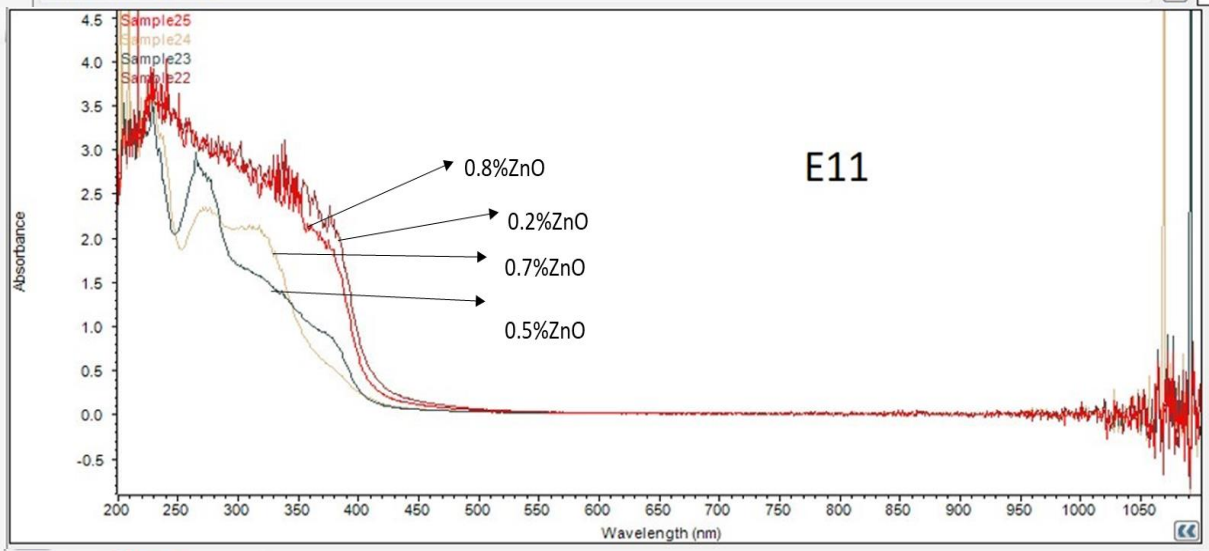
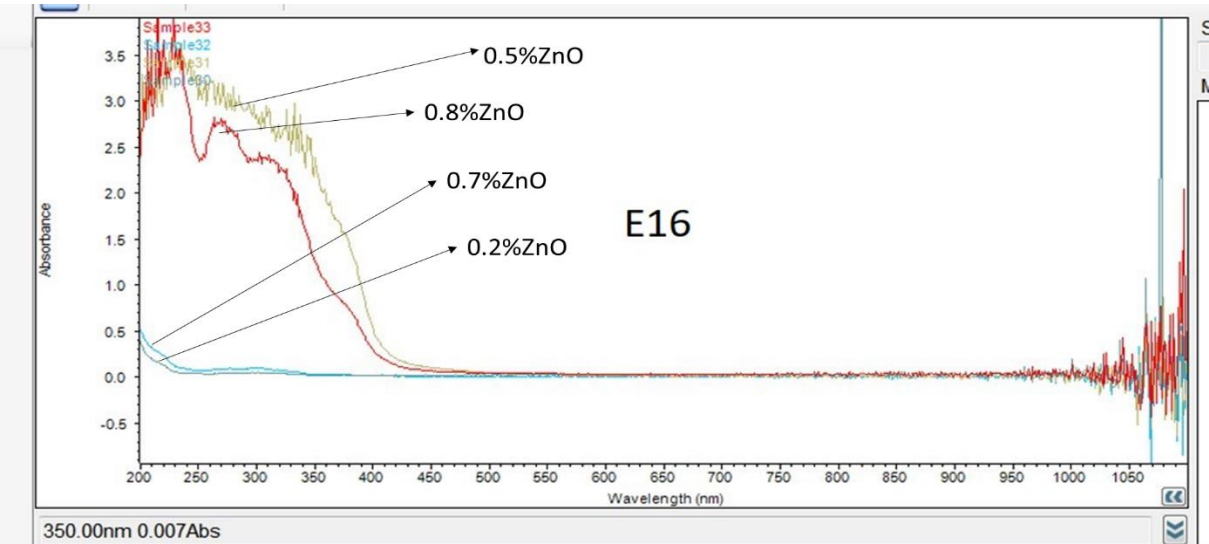
E10: In presence of 0.8% ZnO E10 produce metabolites that showed absorbance around 280 nm. But in lower percentage of ZnO this peak abolished, however new peak were found around 250 to 350 nm.

E3: In presence of 0.2% ZnO E21 produce metabolites that showed absorbance around 280 nm. However new peak were found around 300 to 350 nm.

D11: Not showed absorbance peak compare to control.

All isolates were absorbance around 280nm to350nm, but D11 not show any peak in presence of different percentage of ZnO.





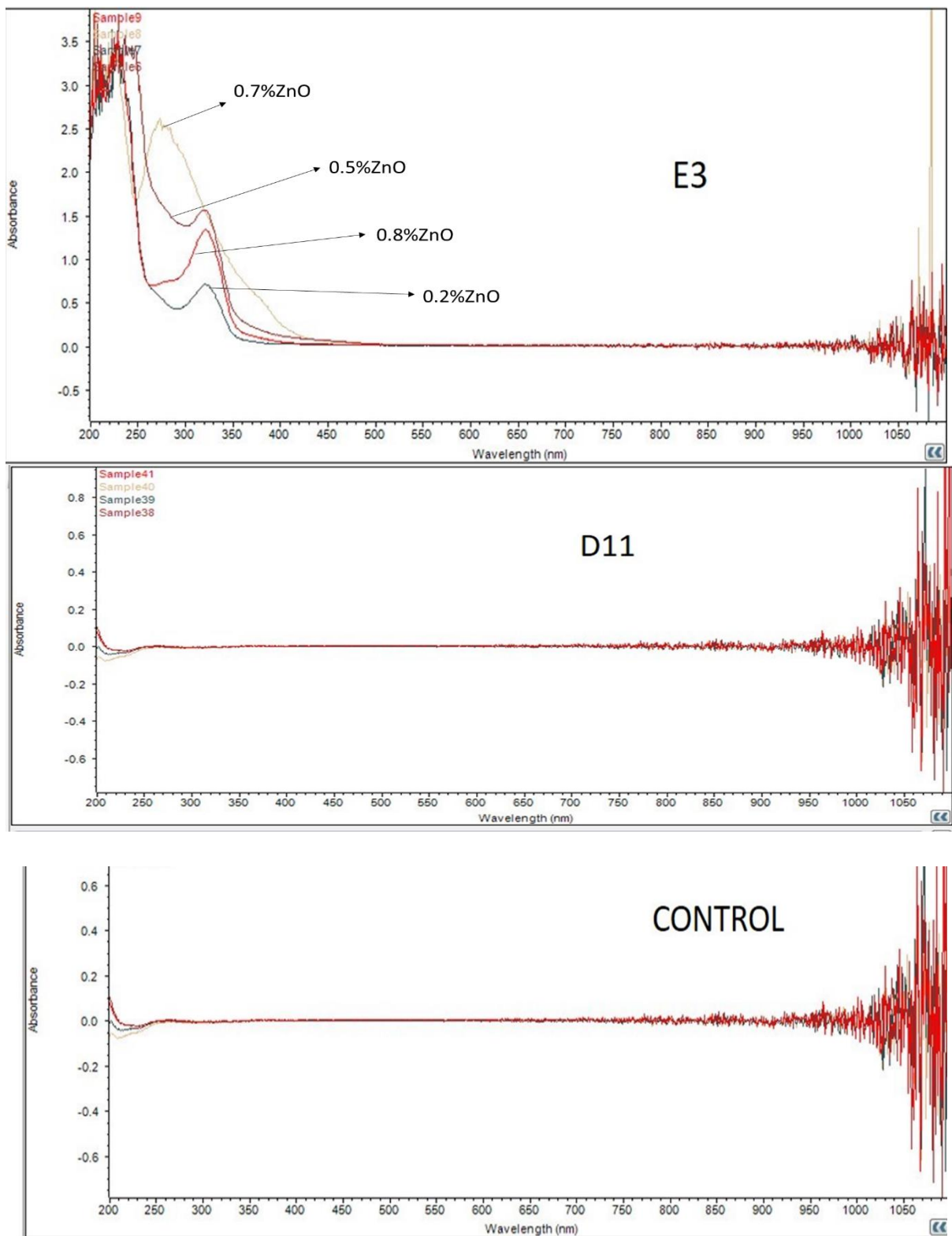


Fig 3: Absorption spectra of pigments produce by different bacteria

Screening of ZSB isolates

A total number of 10 zinc solubilizing bacteria were inoculated in MSM. These ZSB (zinc solubilizing bacteria) were tested for their ability to solubilize Zn using one insoluble Zn compound (ZnO). The isolates showed clear halo zones with a solubilisation diameter [Fig.]. The diameter of halo zone and the zinc solubilizing index for the isolates in medium containing ZnO are shown in table 1. The isolates E₁₆, E₃, E₉, E₁₀ & E₃ were highest solubility index in case of concentration of ZnO are 0.2%, 0.5%, 0.7%, 0.8%, respectively.

Table 1- Zinc solubilizing index of 10 isolates in 0.5% ZnO containing MS media after 7 day incubation

Name of isolates	E ₁₇	E ₁	D ₁₁	E ₂₁	E ₉	E ₁₆	E ₁₁	E ₁₀	E ₃	E ₂₃
Colony Diameter (cm)	0.8	0.8	0.5	0.9	0.6	0.5	0.7	0.5	0.5	0.8
Halo Diameter (cm)	1	0.6	0.6	1.1	0.8	0.7	0.8	0.8	0.9	0.9
Zinc Solubilizing Index*	2.25	1.75	2.2	2.2	2.3	2.4	2.1	2.6	2.8	2.1

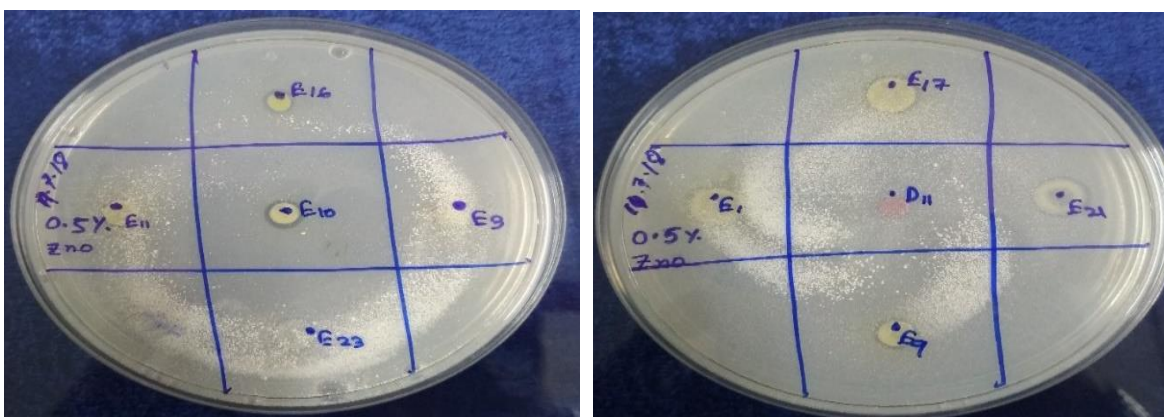


Fig.4: Zinc solubilisation in solid media Solubility of ZnO(0.5%).

Table 2- Zinc solubilizing index of 10 isolates in 0.2%ZnO containing MS media after 7 day incubation

Name of isolates	E ₁₇	E ₁	D ₁₁	E ₂₁	E ₉	E ₁₆	E ₁₁	E ₁₀	E ₃	E ₂₃
Colony Diameter (cm)	0.7	0.7	0.7	0.7	0.6	0.5	0.7	0.7	0.7	0.7
Halo Diameter (cm)	1	0.7	0.7	1.1	0.7	1	1	0.8	1.1	0.8
Zinc Solubilizing Index*	2.4	2	2	2.5	2.1	2.6	2.4	2.1	2.5	2.1

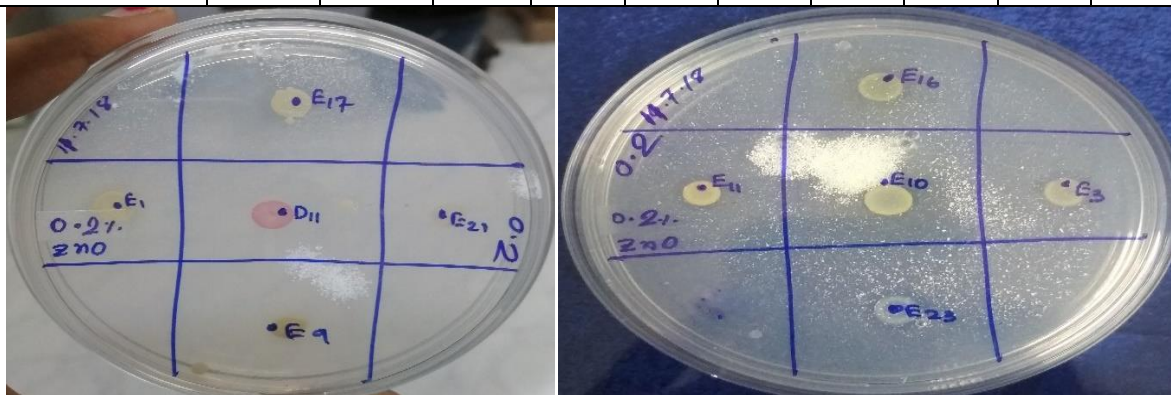


Fig.5: Zinc solubilisation in solid media Solubility of ZnO(0.2%).

*Zinc solubilizing index formula $S = \text{Colony diameter} + \text{Halo zone diameter} / \text{Colony diameter}$.

Table 3- Zinc solubilizing index of 10 isolates in 0.7%ZnO containing MS media after7 days Incubation

Name of isolates	E ₁₇	E ₁	D ₁₁	E ₂₁	E ₉	E ₁₆	E ₁₁	E ₁₀	E ₃	E ₂₃
Colony Diameter (cm)	0.6	0.8	0.5	0.8	0.5	0.6	0.6	0.7	0.7	0.7
Halo Diameter (cm)	1.5	1.7	0.6	2.1	1.8	1.1	2	1.9	1.7	1.2
Zinc Solubilizing Index*	3.5	3.12	2.2	3.6	4.6	2.8	4.3	3.7	3.4	2.7

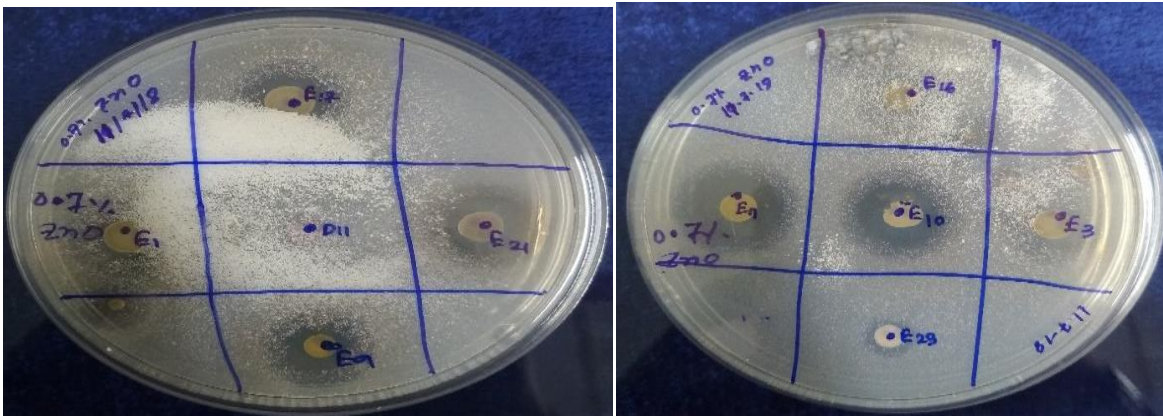


Fig.6: Zinc solubilisation in solid media Solubility of ZnO(0.7%).

Table 4- Zinc solubilizing index of 10 isolates in 0.8%ZnO containing MS media after 7 days incubation.

Name of isolates	E17	E1	D11	E21	E9	E16	E11	E10	E3	E23
Colony Diameter (cm)	0.7	0.9	0.5	0.9	0.6	0.6	0.8	0.7	0.6	0.6
Halo Diameter (cm)	1	2.2	0.6	1.9	1.3	1.3	2	1.9	2	1.4
Zinc Solubilizing Index*	2.7	3.4	3	3.1	3.1	3.1	3.5	3.7	3.7	3.3

*Zinc solubilizing index formula $S = \text{Colony diameter} + \text{Halo zone diameter} / \text{Colony diameter}$

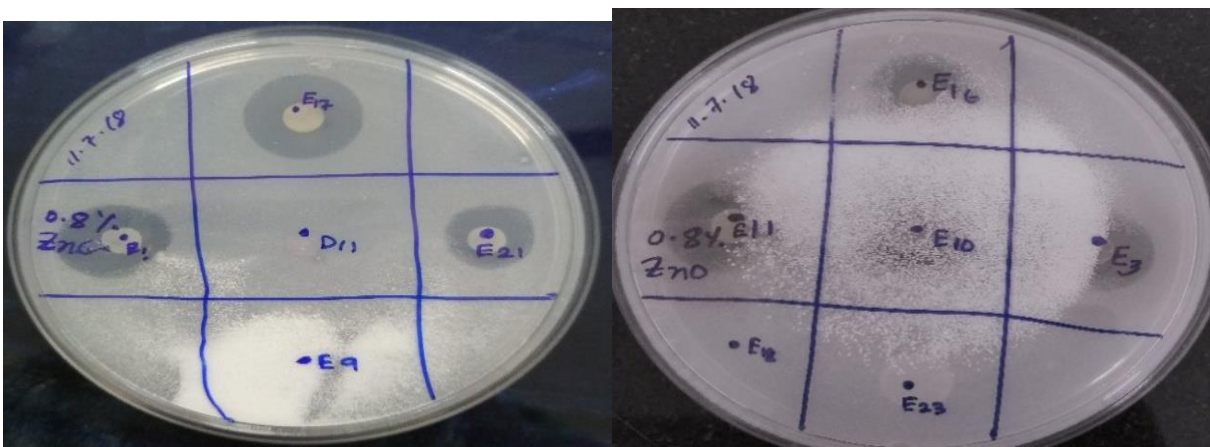


Fig.7: Zinc solubilisation in solid media Solubility of ZnO(0.8%).

Zn solubilization efficiency

Table 5-Zn solubilization efficiency

Name of isolates	Zinc Solubilizing Index*for ZnO (0.5%)	Zinc Solubilizing Index*for ZnO (0.2%)	Zinc Solubilizing Index* ZnO (0.7%)	Zinc Solubilizing Index* ZnO (0.8%)
E ₁₇	2.25	2.4	3.5	2.7
E ₁	1.75	2	3.12	3.4
D ₁₁	2.2	2	2.2	3
E ₂₁	2.2	2.5	3.6	3.1
E ₉	2.3	2.1	4.6	3.1
E ₁₆	2.4	2.6	2.8	3.1
E ₁₁	2.1	2.4	4.3	3.5
E ₁₀	2.6	2.1	3.7	3.7
E ₃	2.8	2.5	3.4	3.7
E ₂₃	2.1	2.1	2.7	3.3

*Efficiency index formula $S = \frac{\text{Cup diameter} + \text{Zone of inhibition diameter}}{\text{Colony diameter}}$.

*ZSB=Zinc solubilizing bacteria

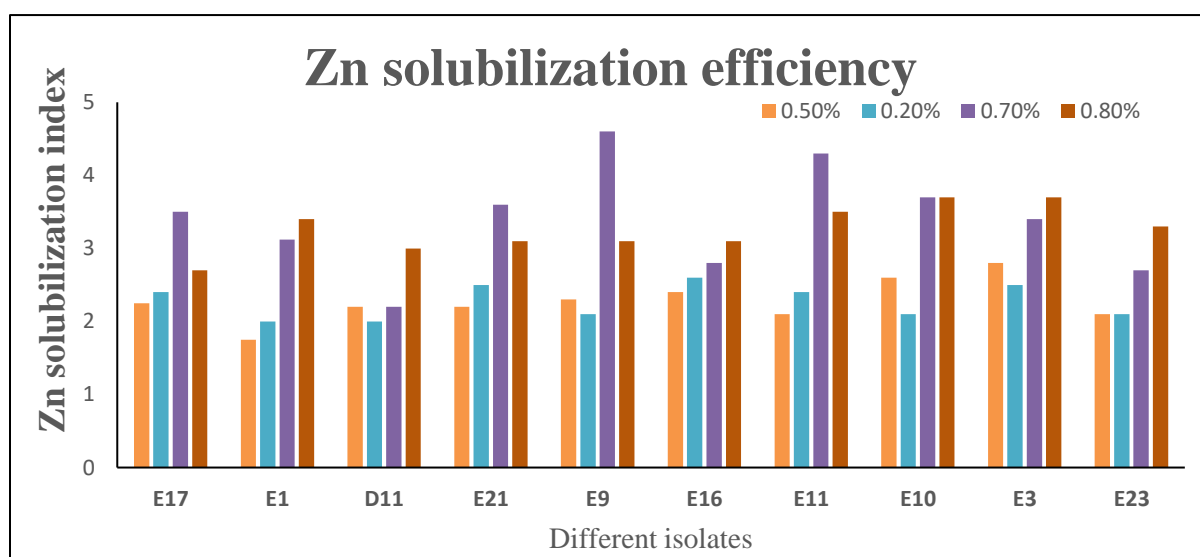


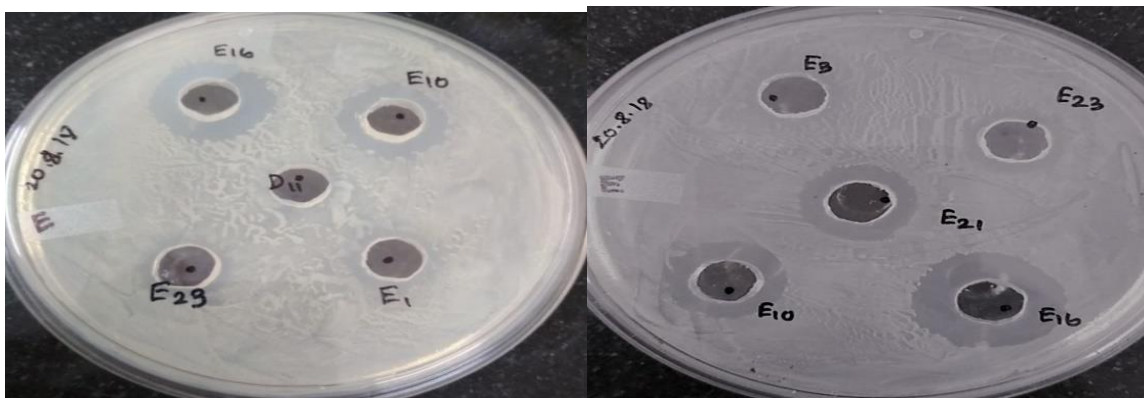
Fig8- Zn solubilization efficiency graph

Antimicrobial assay

Total numbers of 10 zinc solubilizing bacteria were inoculated in MSM medium. These ZSB (zinc solubilizing bacteria) were tested for their antimicrobial effect using their supernatant after centrifugation using Indicator cell culture (*E.coli* & *Staphylococcus* sp.) on LB plate. The isolates showed clear halo zones with a inhibition diameter [Fig-9 and 10]. The diameter of halo zone and the efficiency index for the isolates on LB plate are shown in table 8. The isolates E₁₆ is highest efficiency index in case of *E.coli* & *Staphylococcus* sp.

Table 6-Efficiency Index of ZSB* by using *E.coli*

Name of isolates	E ₁₇	E ₁	D ₁₁	E ₂₁	E ₉	E ₁₆	E ₁₁	E ₁₀	E ₃	E ₂₃
Cup Diameter (cm)	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Zone of inhibition Diameter (cm)	1.8	1.6	No Zone of inhibition	1.6	1.6	2	1.8	1.6	1.3	1.3
Efficiency Index*	3.25	3		3	3	3.5	3.25	3	2.6	2.5



*ZSB=zinc solubilizing bacteria

*Efficiency index formula $S = \frac{\text{Cup diameter} + \text{Zone of inhibition diameter}}{\text{Colony diameter}}$

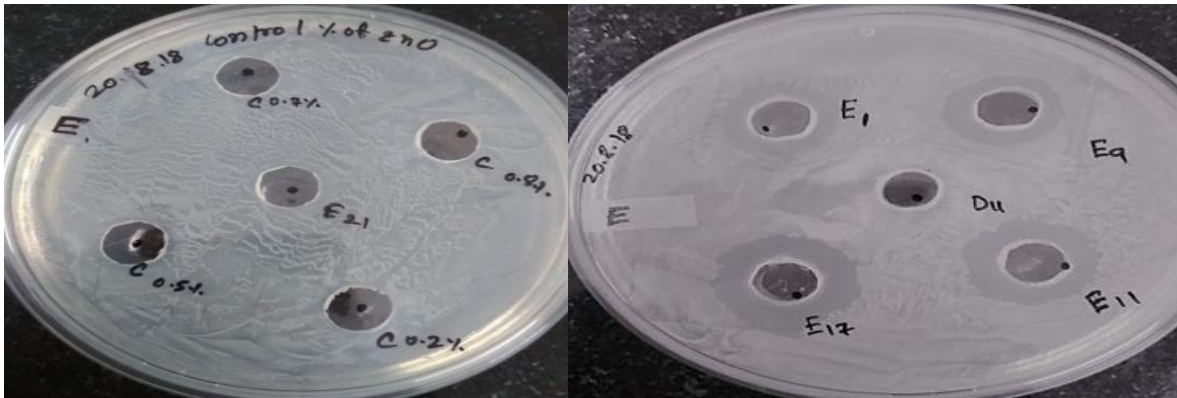


Fig 9-Efficiency Index of ZSB* by using *E.coli*

Table 7-Efficiency Index of ZSB* by using *Staphylococcus* sp.

Name of isolates	E ₁₇	E ₁	D ₁₁	E ₂₁	E ₉	E ₁₆	E ₁₁	E ₁₀	E ₃	E ₂₃
Cup Diameter (cm)	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Zone of inhibition Diameter (cm)	1.5	1.2	1.3	1.4	1.4	1.8	1.5	1.5	1.1	1.1
Efficiency Index*	2.87	2.5	2.62	2.75	2.75	3.25	2.87	2.87	2.37	2.37

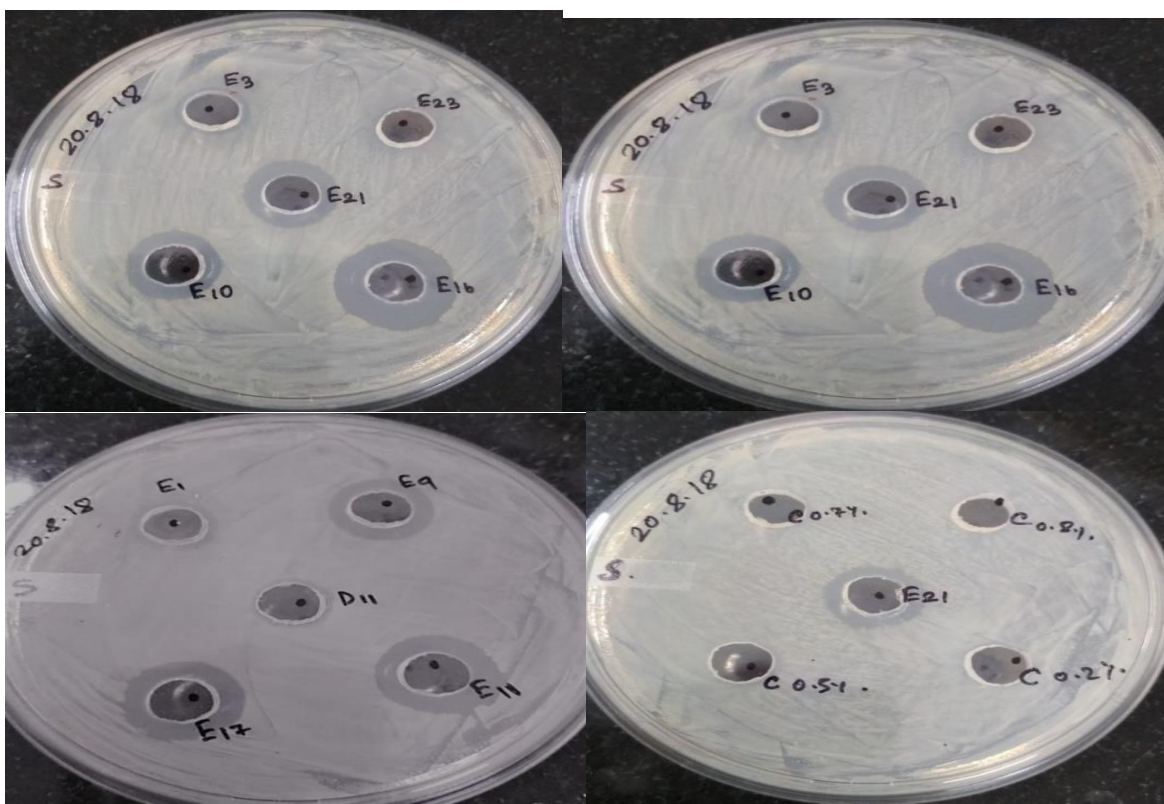


Fig 10-Efficiency Index of ZSB* by using *Staphylococcus* sp.

*Efficiency index formula $S = \frac{\text{Cup diameter} + \text{Zone of inhibition diameter}}{\text{Colony diameter}}$

*ZSB=zinc solubilizing bacteria

Efficiency Index of ZSB* by using *E.coli* & *Staphylococcus* sp.-

Table 8-Efficiency Index of ZSB* by using *E.coli* & *Staphylococcus* sp.

Name of isolates	Efficiency Index of ZSB* by using <i>E.coli</i>	Efficiency Index of ZSB* by using <i>Staphylococcus</i> sp.
E ₁₇	3.25	2.87
E ₁	3	2.5
D ₁₁	No zone of inhibition	2.62
E ₂₁	3	2.75
E ₉	3	2.75
E ₁₆	3.5	3.25
E ₁₁	3.25	2.87
E ₁₀	3	2.87
E ₃	2.6	2.37
E ₂₃	2.5	2.37

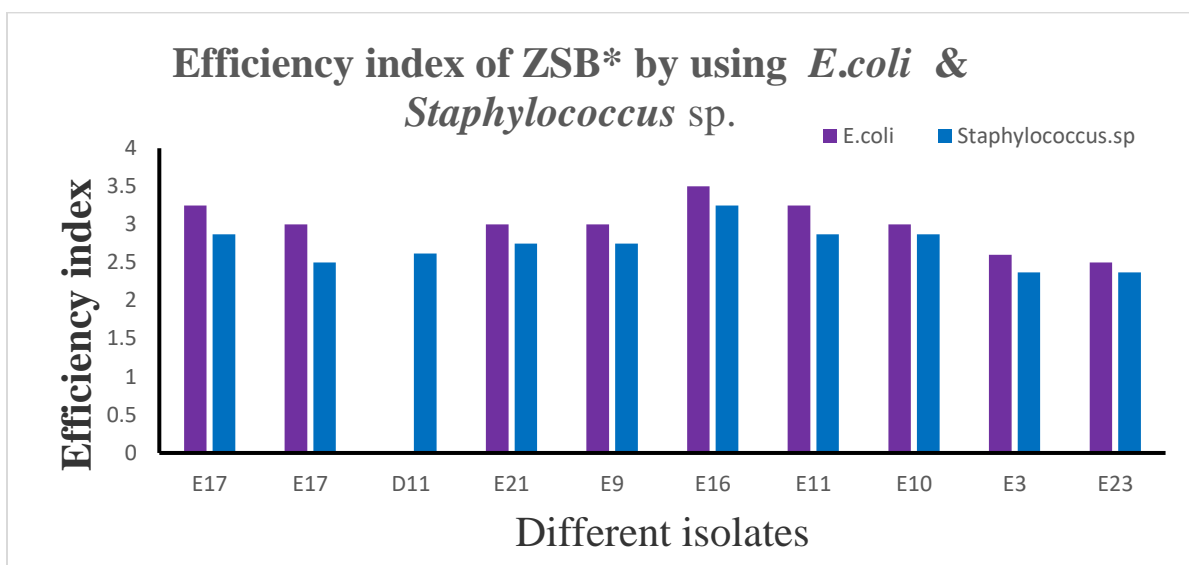


Fig11- Efficiency index of ZSB* by using *E. coli* & *Staphylococcus sp.*

Six bacterial isolates (E₃, E₁₀, E₁₁, E₁₆, E₂₁, and D₁₁) inoculate in 100 ml MSM bulk culture. These ZSB (zinc solubilizing bacteria) were tested for their antimicrobial effect using their supernatant after centrifugation using Indicator cell culture (*E.coli* ,& *Staphylococcus sp.*) on LB plate. The isolates showed clear halo zones with a inhibition diameter [Fig-12 and 13].The diameter of halo zone and the efficiency index for the isolates on LB plate are shown in table 6 .The isolates E₁₀ and E₁₁ are highest efficiency index in case of *Staphylococcus sp.* plate and & the isolate E₁₁ is the highest efficiency index in case of *E.coli* plate.

Table 9-Efficiency Index of ZSB* by using *Staphylococcus sp.*

Name of isolates	E ₁₀	E ₂₁	E ₁₆	D ₁₁	E ₃	E ₁₁
Cup Diameter (cm)	0.8	0.8	0.8	0.8	0.8	0.8
Zone of inhibition Diameter (cm)	1.8	1.4	1.5	No zone Of inhibition	1.3	1.8
Efficiency Index*	3.25	2.75	2.87		2.62	3.25

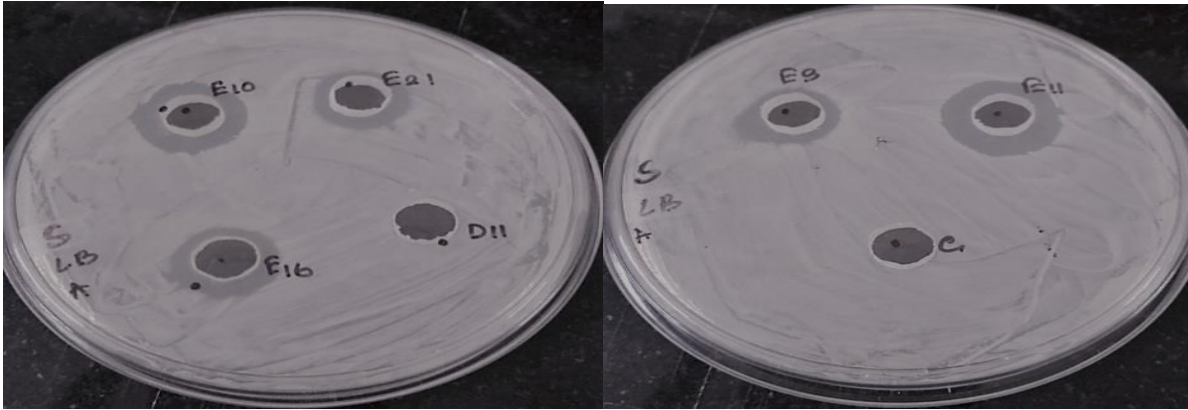


Fig12-Efficiency Index of ZSB* by using *Staphylococcus* sp.

Table 10-Efficiency Index of ZSB* by using *E.coli*.

Name of isolates	E10	E21	E16	D11	E3	E11
Cup Diameter(cm)	0.8	0.8	0.8	0.8	0.8	0.8
Zone of inhibition Diameter(cm)	1.7	1.4	1.5	No zone Of inhibition	1.6	2
Efficiency Index*	3.12	2.75	2.87	0	3	3.5

*ZSB=Zinc solubilizing bacteria

*Efficiency index formula $S = \text{Cup diameter} + \text{Zone of inhibition diameter} / \text{Colony diameter}$.

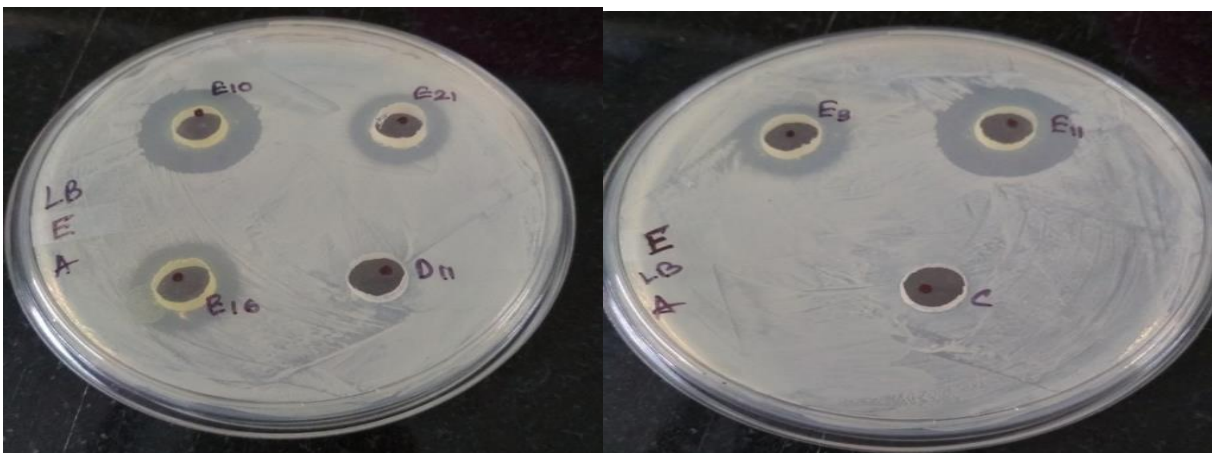


Fig13-Efficiency Index of ZSB* by using *E.coli*.

Efficiency Index of ZSB* by using *E.coli* & *Staphylococcus* sp.-

Table 11-Efficiency Index of ZSB* by using *E.coli* & *Staphylococcus* sp.

Name of isolates	Efficiency Index of ZSB* by using <i>E.coli</i>	Efficiency Index of ZSB* by using <i>Staphylococcus</i> sp.
E ₁₀	3.12	3.25
E ₂₁	2.75	2.75
E ₁₆	2.87	2.87
D ₁₁	No zone of inhibition	No zone of inhibition
E ₃	3	2.62
E ₁₁	3.5	3.25

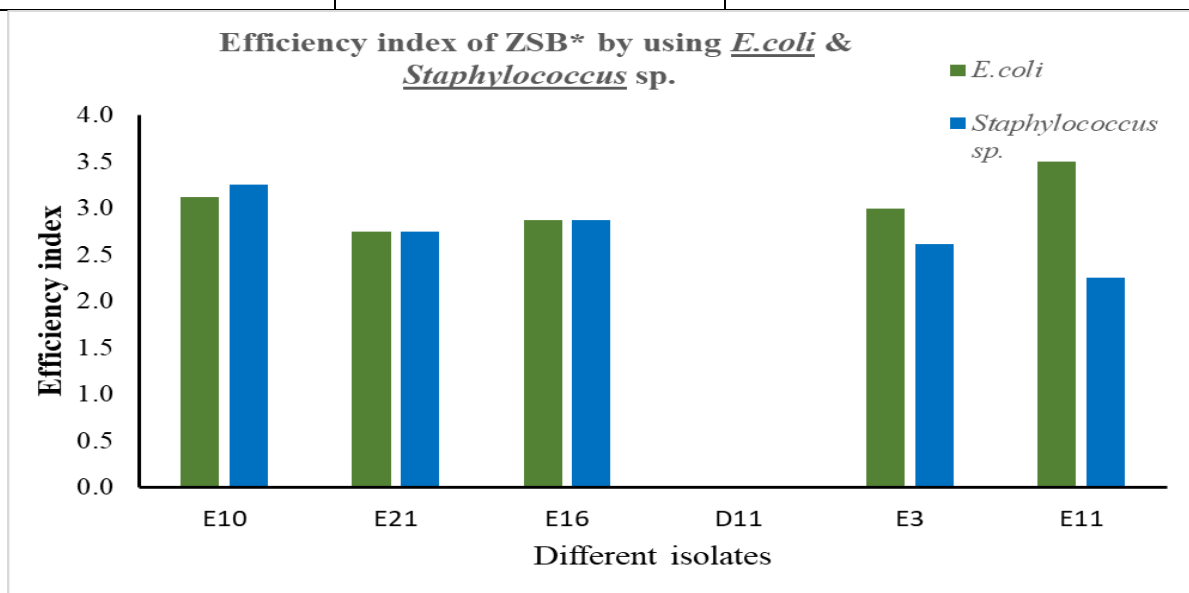


Fig14-Efficiency index of ZSB* by using *E.coli* & *Staphylococcus* sp.

*Efficiency index formula $S = \frac{\text{Cup diameter} + \text{Zone of inhibition diameter}}{\text{Colony diameter}}$.

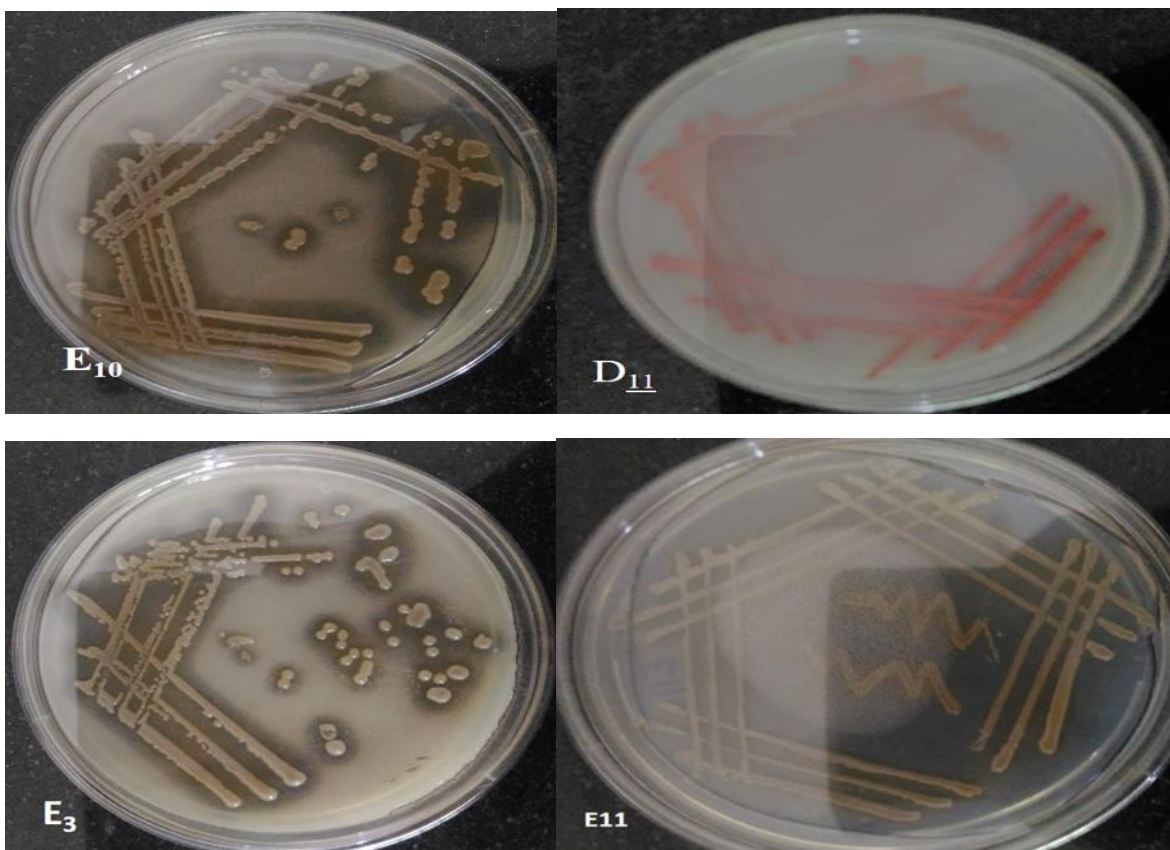
*ZSB=Zinc solubilizing bacteria

Characterization of the given isolates

Morphologically 2 isolates were gram positive, 4 isolates were gram negative and in case of motility test both motile and non-motile varieties were present among all six isolates. The colonies were mostly circular in shape, shiny in appearance and brown in color. All morphological characteristics are under microscope shown below.

Table 12-Morphological study and Gram's reaction

Isolate name	Shape	Gram staining property
D ₁₁	brunch	Gram negative
E ₃	circular	Gram positive
E ₁₀	circular	Gram positive
E ₁₁	rod	Gram negative
E ₁₆	brunch	Gram negative
E ₂₁	rod	Gram negative



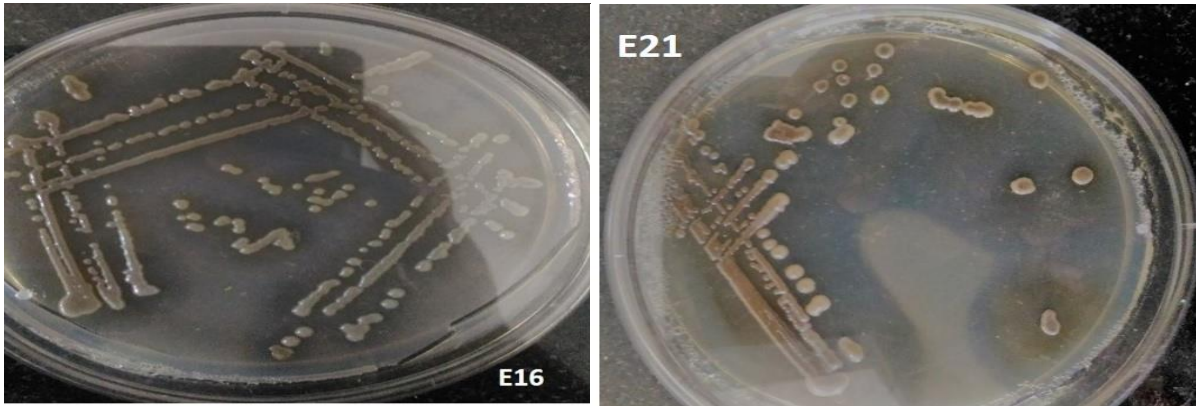


Fig15- Characterization of the given isolates

Dyeing of fabric-

The pigments were used to dye six fabrics. The E₁₆ pigment was highly capable for dyeing fabric than other. It was organic dye.

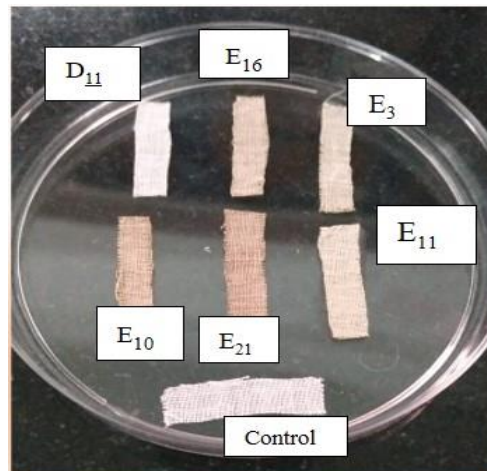


Fig 16-Dyeing of fabric

Conclusion-

All the isolated bacteria have potentiality to solubilize Zinc. They are produced pigment, presence in antimicrobial effect. The bacteria which were isolated through enrichment into medium showed more activity. All the isolates are grown in different concentration of ZnO and their rate of production are different. Zinc solubilizing bacterial production depends on concentration of ZnO.

Bacterial pigment dyeing fabrics, may be these pigment have commercial demand in the textile industry. These pigment are non-toxic eco-friendly dye but not sure to these dye of fabrics is quick washable or not.

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