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ISOLATION AND SCREENING OF HEAVY METAL ABSORBING BACTERIA FROM THE INDUSTRY EFFLUENT SITES OF THE RIVER NAGAVALI

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ABSTRACT

The present study was designed to evaluate the heavy metal absorbing potential of bacteria isolated from industry effluent sites of river Nagavali, Srikakulam Andhra Pradesh. From the effluent waste water three bacteria were isolated and confirmed as *Staphylococcus aureus* and *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* after performing various biochemical tests. The heavy metal tolerant efficiency was determined by analyzing the growth of the bacteria in presence of heavy metal (Cu, Hg, Co and Zn) solution and their optimum tolerance was determined by measuring the optical density at 600nm after 24hr and 48hr of incubation. *Staphylococcus aureus, Staphylococcus epidermidis* and *Staphylococcus saprophyticus* were found to grow in heavy metal solutions.

INTRODUCTION: An effluent is a out flow of water discarded after industrial operations and manufacturing processes. Industrialization is vital to a Nation's economy because it serves as vehicle for development but the associated problems needs to be addressed using relevant technologies to minimize toxicity and other possible environmental hazards. Industrial waste consists of both organic and inorganic compounds. Organic wastes include pesticide residues, solvents, cleaning fluids, dissolved fluids, lignin from pulp and paper. Effluents can also contain some in organic wastes such as brine salts and metals.

Heavy metal pollution of fresh water bodies arises from various industries and causes various environmental concerns for aquatic life. These effluents even at the low concentrations adversely affect the composition, distribution and diversity of benthic organisms [1-3]. Removal of toxic heavy metals from industrial waste water is essential from the environmental point of view to combat water pollution [4].

Conventional methods for removing metals from industrial effluents include chemical precipitation, chemical oxidation or reduction, ion exchange, filtration, electrochemical treatment, reverse osmosis, membrane technologies and evaporation recovery [5]. These processes may be ineffective or extremely expensive especially when the metals in solution are in the range of 1-100 mg/l [6]. Therefore, it is important to develop an innovative, low cost and ecofriendly method for removal of toxic heavy metal ions from the wastewater. Wide varieties of microorganisms are capable of growing in the presence of heavy metal ions and tolerate high concentrations [7-9]. Since heavy metals are ubiquitously present in the environment microorganisms have developed resistance to these heavy metals [10].

MATERIAL AND METHODS: Waste water samples were collected in screw capped sterilized bottles. 100µl of the effluent water sample was spread on nutrient agar plates. Nutrient agar plates were prepared by dissolving 0.5g peptone, 0.3g beef extract and 0.5g

NaCl in 100ml distilled water, pH adjusted to 7 and 2.0g agar was added in the 250ml flasks. The medium was autoclaved at 121^{o}C for 15min. The growth of the bacterial colonies was observed after 24hrs of incubation at 37^{o}C.

The microorganisms that grow on nutrient agar were randomly isolated based on their morphology and color of the colonies. Pure cultures obtained were preserved at 4^{o}C for further studies. The isolated strains were studied for morphological, cultural and biochemical characteristics using KB002 Hi Assorted Biochemical test kit of Hi-media Laboratories. The morphology was identified by high resolution microscope. The bacterial isolates were identified in accordance with Bergy's manual of systematic bacteriology [11]. Various biochemical tests were performed viz; catalase activity, starch hydrolysis, mannitol fermentation, glucose fermentation, mannitol salt agar test for biochemical characterization.

Determination of optimum Metal Resistance and Antibiotic Sensitivity: To determine the metal resistance 2ml of nutrient broth inoculated with 1ml of suspension culture of isolated strain and mixed with 1ml of metal solution of various concentrations (10mM, 20mM, 100mM, 200mM) for each heavy metal (Cu, Hg, Zn, Co). These cultures were incubated at 37^{o}C followed by analysis of growth by measuring optical density after 24hrs and 48hrs at 600nm.

RESULTS & DISCUSSION: The present study revealed that the isolated strain *Staphylococcus aureus* was a gram positive cocci and found positive for catalase test, starch hydrolysis, mannitol fermentation and mannitol salt agar test, whereas *Staphylococus epidermidis* was a gram positive cocci and found positive for catalase, starch hydrolysis, mannitol fermentation and negative for mannitol salt agar test (**Table 1**).

TABLE 1: MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF THE BACTERIAL ISOLATES

Characteristics	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Staphylococcus saprophyticus*
Gram staining	+ve	+ve	+ve
Shape	Cocci	Cocci	Cocci
Catalase test	+ve	+ve	+ve
Mannitol fermentation	+ve	+ve	-ve
Glucose fermentation	-ve	-ve	-ve
Starch hydrolysis	+ve	+ve	+ve
Mannitol salt agar test	+ve	-ve	-ve

Staphylococcus saprophyticus was found gram positive cocci and gave positive tests for catalase and starch hydrolysis and negative for mannitol fermentation and glucose fermentation respectively. Metal resistance studies showed that *Staphylococcus saprophyticus* is more resistant to Cu metal than *Staphylococus aureus* and *Staphylococus epidermidis.*

Staphylococus aureus has shown maximum growth at 200mM conc. after 48hrs of incubation whereas *Staphylococus epidemidis* showed maximum growth at 100mM after 48hrs of incubation (**Fig. 1**). *Staphylococus saprophyticus* has shown maximum growth at 100mM after 24hrs of incubation (**Fig. 2**).

In the mercury solution higher growth rate was observed at 10mM conc. after 24hrs of incubation for *Staphylococus aureus* and *Staphylococus epidermidis* (**Fig. 3**). *Staphylococus saprophyticus* has shown higher growth at 200mM after 24hrs of incubation (**Fig. 4**). After 24hrs of incubation in zinc metal solution *Staphylococus aureus* and *Staphylococus epidermidis* have shown maximum growth at 100mM conc. (**Fig. 5**) but *Staphylococus saprophyticus* showed maximum growth at 10mM after 24hrs of incubation and on further incubation the growth was gradually decreased (**Fig. 6**).

It was observed that both *Staphylococus aures* and *Staphylococus epidermidis* have maximum resistance to cobalt metal at 100mM conc. after 24hrs of incubation (**Fig. 7**). Similarly *Staphylococus saprophyticus* has shown maximum growth at 200mM after 24hrs of incubation (**Fig. 8**).

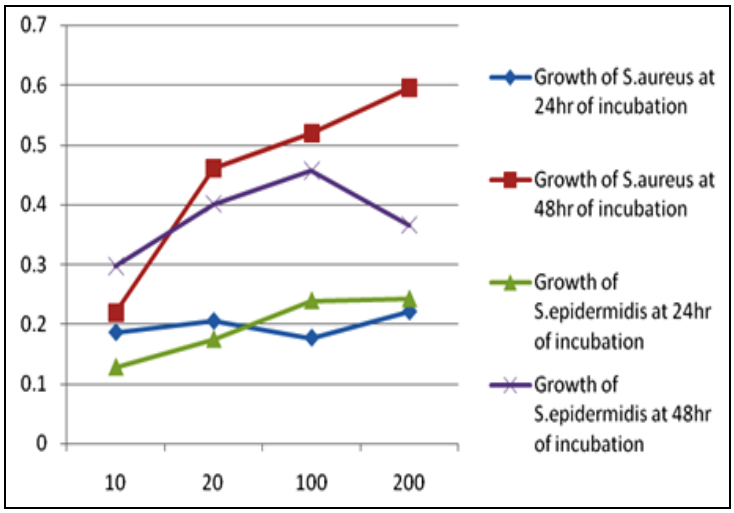

FIG. 1: EFFECT OF CU, CONC. ON THE GROWTH OF *STAPHYLOCOCCUS AUREUS* AND *STAPHYLOCOCCUS EPIDERMIDI*

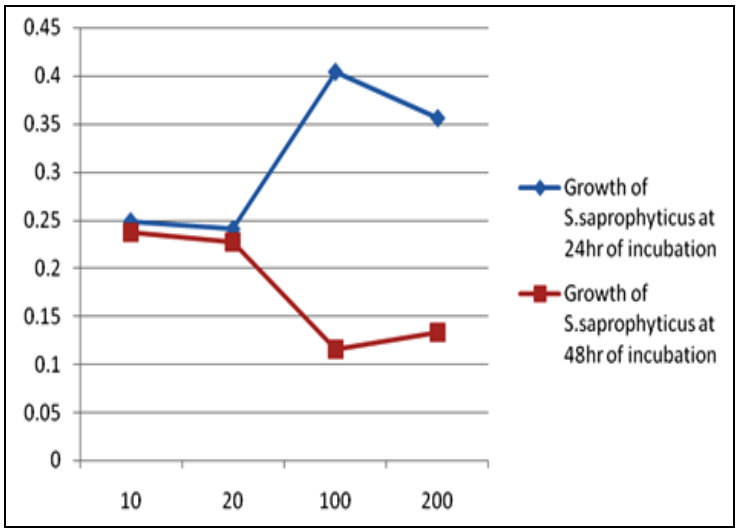

FIG. 2: EFFECT OF Cu, CONC. ON THE GROWTH OF *STAPHYLOCOCCUS SAPROPHYTICUS*

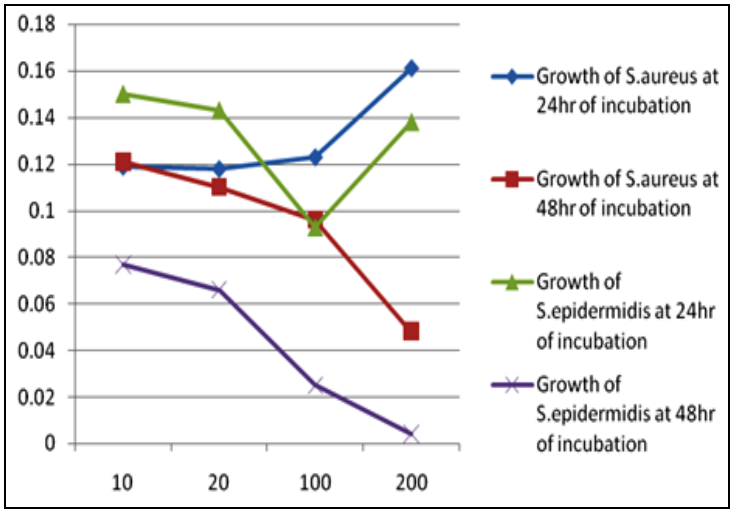

FIG. 3: EFFECT OF Hg, CONC. ON THE GROWTH OF *STAPHYLOCOCCUS AUREUS* AND *STAPHYLOCOCCUS EPIDERMIDIS*

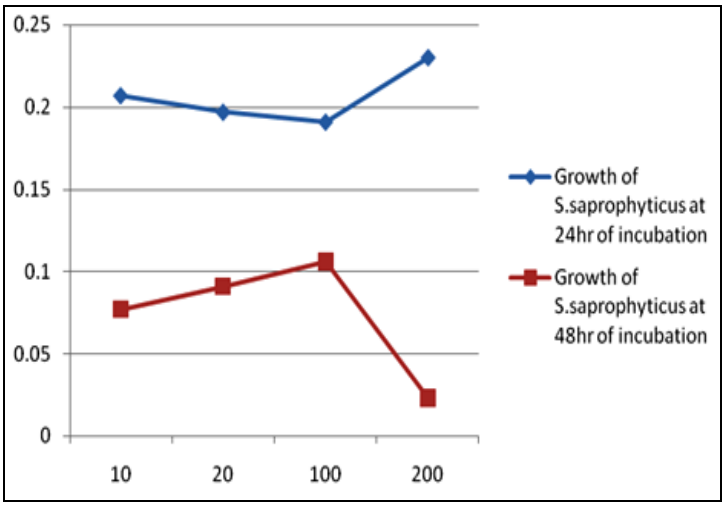

FIG. 4: EFFECT OF Hg, CONC. ON THE GROWTH OF *STAPHYLOCOCCUS SAPROPHYTICUS*

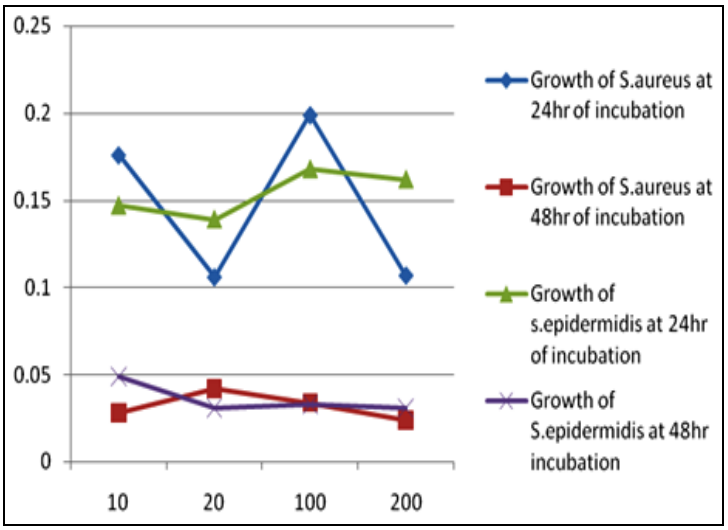

FIG. 5: EFFECT OF Zn, CONC. ON THE GROWTH OF *STAPHYLOCOCCUS AUREUS* AND *STAPHYLOCOCCUS EPIDERMIDIS*

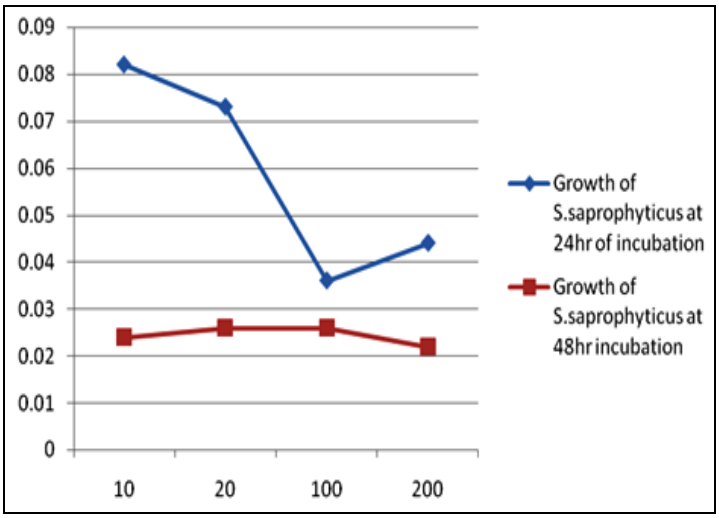

FIG. 6: EFFECT OF Zn, CONC. ON THE GROWTH OF *STAPHYLOCOCCUS SAPROPHYTICU*

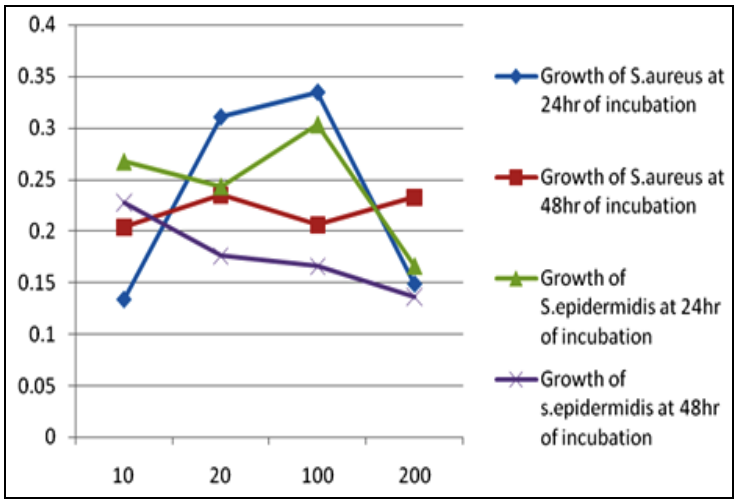

FIG. 7: EFFECT OF Co, CONC. ON THE GROWTH OF *STAPHYLOCOCCUS AUREUS* AND *STAPHYLOCOCCUS EPIDERMIDIS*

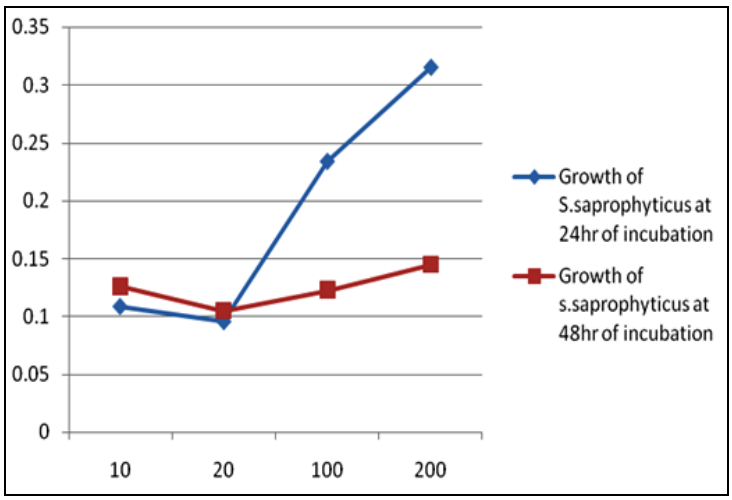

FIG. 8: EFFECT OF Co CONC. ON THE GROWTH OF *STAPHYLOCOCCUS SAPROPHYTICUS*

CONCLUSION: The isolated bacterial strains have shown high degree of heavy metal resistance and could be explored as candidate bacteria for wastewater bioremediation processes special reference to heavy metal contaminated water bodies. The isolates have got the potential to remove and recover the toxic metals economically and effectively from the industrial effluents.

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