



# Exploring Secondary Metabolites to Prevent Growth of Microorganisms Present in Ceramic Slurry Used in Investment Casting

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## Abstract

Investment casting relies on a high-quality ceramic shell for successful production. However, shell-making suffers from a high rejection rate (around 35%) and consumes a significant portion of the total energy used in the process (nearly one-third). This inefficiency is often linked to issues with the ceramic slurry, particularly the growth of microorganisms within the mixture. This study investigates the impact of microbial presence on the quality and performance of ceramic slurry used in investment casting. The typical slurry composition involves a mixture of finely-ground (300-400 mesh) zircon flour (typically 75%) as the primary refractory material and hydrolysed ethyl silicate or colloidal silica (typically 25%) acting as a binder. Maintaining precise slurry properties, including viscosity, pH, and specific gravity, is very crucial for appropriate shell formation. However, microbial growth within the slurry can disrupt these properties, adversely affect factors including life of slurry and cold strength of the resulting mould. Present work provides better insight on presence as well as identification of various microorganisms in ceramic slurry used in investment casting process as well as secondary metabolites to control growth of those microorganisms observed in ceramic slurry. Various secondary metabolites have been tested to control the growth. It was observed that *Enterococcus hirae* has shown promising results to control overall growth of microorganism, and can further be explored for development of antibiotics for industrial purpose.

**Keywords:** Investment casting, Shell making, Ceramic slurry, Microorganisms

## 1. Background

Invented over 5500 years ago, Investment casting derives its name from the fundamental process of investing refractory material around patterns to create molds. Also referred to as the lost-wax process, and it has a rich history. However, when it comes to manufacturing industrial components with superior surface finish, dimensional accuracy and intricate geometry, it stands out as the one of the most suitable manufacturing processes. [1] This process effectively reduces material waste and the need for subsequent machining. Its applications extend to the production of mechanical

components such as gears, cams, ratchets, turbine blades and machinery parts. [2] Additionally, investment casting finds utility in dentistry and jewelry making. Different stages of investment casting process allow it to produce almost any complex geometry. [3]

The process commences by generating a duplicate of the final part, known as a pattern. Wax is a preferred material for patterns due to its melting and molding characteristics, although special plastics or even metals may be utilized. Wax allows for the creation of intricate patterns that can be replicated in metal part. The precision and accuracy of the pattern are critical, as they directly influence the final part. Injection molding is typically employed for



crafting wax and plastic patterns. Multiple patterns are assembled into pattern trees, featuring a central wax sprue through which molten metal flows into the cavity. [4]

This pattern tree is immersed in a ceramic slurry to create a wet layer of fine materials like silica or zircon. Stucco is applied to this layer and solidified. The particle size of the stucco increases with each added coat, aiming to maintain optimal mold permeability. Multiple layers are built up to form a thick and durable ceramic shell around the pattern tree. The assembly is allowed to dry before dewaxing, a crucial step where the wax is completely removed to form the exact hollow shape of the original part. [5]

The resulting hollow ceramic shell is then filled with molten metal after preheating it close to the liquid metal temperature. This preheating allows sufficient time for the molten metal to flow

through the cavity before cooling. The ceramic mold is left to cool for several hours, dependent on the size of the shell and the material of the casting part. Once complete solidification occurs, the shell is removed or broken, typically through methods like vibration. Finally, the casting undergoes a finishing process, including parting off, machining, polishing, heat treatment and other steps based on specific requirements. [6]

The method enables the creation of intricate shapes that could be challenging or unattainable using other casting techniques, while achieving the highest precision and surface finish. Consequently, it is applied in aerospace engineering, automotive components, medical instruments, industrial parts, jewelry, as well as in the crafting of art and sculptures (figure 1). [7]



Fig. 1. Different applications of investment castings  
(Courtesy: various foundries from cluster) [1,2]

Real-world observations in investment casting foundries suggest a one of potential problems that is usually out of untouched in prior research, and is related to developing unwanted foul odour and effects on hygiene environment of ceramic shell making section. Interestingly, despite proper temperature, humidity control, and tank cleaning protocols (though specifics remain unknown), this phenomenon persists. Given the use of binders in slurry, major concern is related to uncontrolled microbial growth. [8] This raises a critical research question: how do microbes present in the slurry impact both the material itself and the foundry of workers' health Addressing this gap in knowledge is crucial. By employing standard microbiological techniques, researchers can identify and understand the specific roles of these microscopic inhabitants. This knowledge can be harnessed to control their influence, potentially leveraging beneficial microbes while mitigating detrimental ones. [9] Notably, existing research on investment casting slurries has primarily focused on mechanical properties, neglecting the potentially significant microbiological aspects. This study delves into this unexplored territory, exploring

the types of microbes present in the slurry and their potential impact. [10]

Microbial control is a critical concern in recirculating systems using low-metal, low-sodium colloidal silica. While conventional slurries may initially appear free of microorganisms, growth increases during recirculation and dilution, especially when organic rate accelerators are involved. Recognizing this issue, the inventors embarked on a mission to develop a new class of colloidal silica slurries. These novel slurries not only eliminate bacterial and fungal growth but also maintain, and potentially even enhance, the polishing rate of the system. Additionally, they offer an extremely low metal content, particularly of aluminium (Al), iron (Fe), potassium (K), sodium (Na), and other transition metals. Through extensive research, the inventors have successfully formulated a unique group of low-sodium, low-metal colloidal silica slurries that effectively inhibit bacterial growth and improve the polishing rate of silicon wafers. These slurries are comprised of a low-metal, ammonium-stabilized silica sol with discrete, spherical particles ranging from approximately 4 to 130 nanometres in size. This

solution finds applications in high-quality investment casting, high-tech refractories, and catalyst use. While existing method mentioned above usually minimizes the growth of microbial in slurries but it leads to unacceptable changes in the desired properties of slurry [11].

It is observed that microbiological aspects in ceramic slurry used in investment casting has not been widely explored in prior research, and appropriate methodology to control overall growth of microorganisms present in ceramic slurry is not traceable. However, specific microorganisms that can be present in ceramic slurry is also not explored in prior research. Present work is mainly focused on identification of various microorganisms present in ceramic slurry used in investment casting process especially for manufacturing of automobile and aerospace industries, and specific metabolise were also tested to control overall growth of microorganism present in ceramic slurry used for manufacturing these castings.

Microbiological methodology adopted for identification of microorganisms followed by testing of specific metabolise to control overall growth of microorganisms are discussed next.

Table 1.  
Sample collection of Slurry

No.	Primary Slurry	Secondary Slurry	
Industry - 1	Type-1	Type-1	Type-2
	Sample-1	Sample-2	Sample-3
Industry- 2	Type-1	Type-1	Type-2
	Sample-4	Sample-5	Sample-6

## 2.2. Isolation and Identification

Streak plate technique was used to isolate individual colonies on nutrient (NA) TM Media-TM341 and potato dextrose agar (PDA (TM-Media-TM344)) plates. Colonies with distinct morphologies were chosen for further characterization [16]. Gram staining was performed on isolated colonies to differentiate them as Gram-positive or Gram-negative based on cell wall structure [17]. This initial identification step provided valuable information for further analysis of the isolated bacteria [18, 19].

Table 2.  
Results of Gram staining of 12 isolates

Nutrient agar Isolates				Potato Dextrose agar Isolates			
1	Gram negative	4	Gram negative	1	Gram negative	4	Gram negative
2	Gram negative	5	Gram negative	2	Gram negative	5	Gram negative
3	Gram negative	6	Gram negative	3	Gram negative	6	Gram positive

## 3. Inhibitory Potential

An inhibition test was conducted to assess the inhibitory potential of the isolated bacterial strains. Fresh N-broth (HiMedia-M002) tubes were prepared by autoclaving the media, and each isolated colony was inoculated into a separate tube using sterile wire loop following aseptic technique. These inoculated tubes were then incubated at 37°C for 24 hours with shaking in an orbital shaker to promote bacterial growth (Kumar, et al., 2016). In parallel, fresh NA (TM Media-TM341) and PDA (TM-

## 2. Methodology

### 2.1. Sample Collection and Enrichment

Samples for exploring secondary metabolites to prevent growth of microorganisms present in ceramic slurry have been collected from regional investment casting foundries as per details mentioned in table 1. To ensure sterility, N-broth (HiMedia-M002) was sterilized and transferred aseptically into centrifuge tubes before transport to the site. Sterile droppers collected representative slurry samples into the broth. After sealing, samples were incubated at 37°C for 24 hours to promote bacterial growth [12]. Following this, a two-step centrifugation process at 10000 revolutions per minutes (rpm) and 12000 rpm concentrated the target bacteria in the supernatant. The enriched fraction was then transferred to fresh sterile N-broth (HiMedia-M002) for another 24-hour incubation at 37°C, further enriching the desired bacterial population [13, 14, 15].

Diversified microorganisms present in slurry are isolated and identified (table 2). Gram staining (e.g. scientific procedure for identifying the group of microorganisms) has revealed the presence of 15 bacterial colonies [20], from which bacterial colonies with same morphology are removed from the list and with that there are 12 diversified bacterial colonies [21]. These findings suggest a complex microbial community within the slurry Further research is necessary to understand the specific roles and potential impact of these microbes on both the slurry itself and the foundry environment [22].

Media-TM344) plates were autoclaved. Four known bacterial cultures (*Escherichia coli* (MTCC NO 452), *Staphylococcus aureus* (MTCC NO 741), *Bacillus subtilis* (MTCC NO 411), and *Klebsiella pneumoniae* (MTCC NO 2403)) were used as stock cultures which is identified and confirmed by biochemical test. Each stock culture was inoculated onto a separate agar plate (NA (TM Media-TM341) or PDA (TM-Media-TM344) depending on the culture's requirements) using a micropipette with 100 µl of culture. The plates were then spread with a sterile glass spreader to ensure even distribution (table 3 and 4) [23].

Table 3.  
Potato Dextrose Agar Composition

	Ingredients	gm/L
1	Infusion from potatoes	5.000
2	Dextrose(glucose)	5.000
3	Agar	15.000
4	pH (at 25°C)	5.6±0.2

Table 4.  
Nutrient Agar Composition

	Ingredients	gm/ L
1	Peptone	5.000
2	Sodium chloride	5.000
3	HM peptone B#	1.500
4	Yeast extract	1.500
5	Agar	15.000
6	pH (at 25°C)	7.4±0.2

The cup borer method was employed to create wells in the agar. A sterile cup borer was used to remove agar plugs from the inoculated plates, creating wells for the isolated bacterial cultures. Finally, under aseptic conditions within a laminar airflow cabinet, 25 µl of the grown bacterial cultures (from the incubated N-broth tubes) were inoculated into the wells created on the agar plates containing the stock cultures. Following inoculation, following a 24-hour incubation at 25°C. This setup allowed for observation of whether the isolated bacteria could inhibit the growth of the known bacterial cultures [24].

#### 4. Interaction of antibacterial strains with slurry samples

To assess the inhibitory potential of isolated strains against the original slurry microbes, a zone of inhibition test was conducted. Fresh NA (TM Media-TM341) and PDA (TM-Media-TM344) plates were sterilized through autoclaving. The initial slurry sample, containing a diverse bacterial community, was inoculated onto a separate agar plate using a micropipette with 100 µl of culture. A sterile glass spreader ensured even distribution of the slurry across the plate. Subsequently, the cup-plate method was

employed to create wells in the agar [20]. Using a sterile cup borer, agar plugs were removed from the inoculated plate, forming wells for the isolated bacterial cultures. Under aseptic conditions within a laminar airflow cabinet, 25 µl of each of the six potent bacterial cultures were inoculated into separate wells on the agar plate containing the slurry sample [24]. Following incubation for 24 hours at 25°C, the plates were examined for zones of inhibition surrounding the wells containing the isolated cultures. The presence of clear zones around the wells would indicate the ability of the isolated strains to inhibit the growth of bacteria present in the original slurry [23].

#### 5. Results and Discussion

Following isolation and identification procedures, Gram staining revealed a predominance of Gram-positive bacteria. Microscopic analysis indicated the presence of eleven Gram-positive and one Gram-negative strain among the twelve isolated bacterial species. Assuming the accuracy of the Gram stain results, the eleven Gram-positive isolates could potentially belong to the following species, listed species here in table 5. However, definitive identification of all isolates requires further testing beyond Gram staining.

Table 5.  
Predicted microorganism species and their pathological characteristics

	Predicted microorganism species	Pathological characteristics
1	<i>Staphylococcus sp.</i>	Skin Infections and Respiratory Infections
2	<i>Enterococcus sp.</i>	Opportunistic Infections and Urinary tract infections (UTIs)
3	<i>Streptococcus sp.</i>	Skin infections and Pneumonia
4	<i>Micrococcus sp.</i>	Skin Infections and Respiratory Infections Bloodstream infections
5	<i>Bacillus sp.</i>	Eye Infection and Digestive Infection

## 6. Microbial and Pathological Characteristics of Identified Species

### 6.1. Staphylococcus

Historically classified as commensal residents of the human skin, a group of staphylococci known as coagulase-negative staphylococci (CNS) have undergone a paradigm shift in their perceived role, now recognized as significant nosocomial pathogens [25]. A key differentiating factor between CNS and their highly virulent relative, *Staphylococcus aureus*, is the absence of coagulase production. This enzyme plays a crucial role in blood clot formation. While this characteristic initially led to the notion of CNS being innocuous, it now underscores their opportunistic potential. In healthcare environments, the widespread use of antibiotics disrupts the natural balance of the human microbiome. This creates a window of opportunity for CNS to readily colonize implanted medical devices, such as catheters. This colonization can subsequently contribute to a range of healthcare-associated infections, with bloodstream infections (bacteraemia) being a prominent concern. Notably, CNS have emerged as leading global causes of bacteraemia, with a growing number of strains exhibiting multidrug resistance, further complicating treatment strategies. While CNS infections typically manifest as less severe and subacute compared to those caused by *S. aureus*, their high prevalence and the associated healthcare burden necessitate the development of effective control strategies. A comprehensive understanding of the mechanisms employed by CNS for pathogenesis and the development of targeted therapeutic approaches remain crucial steps towards mitigating their impact and restoring equilibrium within the human microbial landscape [25].

### 6.2. Enterococcus

*Enterococcus* species, particularly *Enterococcus faecalis* and *Enterococcus faecium*, are ubiquitous Gram-positive cocci found within the intestinal tracts of terrestrial animals. While they play a vital role in maintaining gut health by contributing to digestion and nutrient absorption, they also possess a concerning ability to transition from harmless commensals to opportunistic pathogens. This dual nature highlights the complex relationship between enterococci and their hosts. The pathogenic potential of enterococci was first recognized in the late 19th century by MacCallum and Hastings. Since then, enterococci have emerged as a major concern in healthcare settings, ranking as the third most common cause of nosocomial infections. These infections encompass a diverse range, including urinary tract infections, bloodstream infections (bacteraemia), and potentially life-threatening endocarditis [26].

The alarming prevalence of enterococcal infections can be attributed, in part, to their remarkable adaptability. Notably, enterococci readily capitalize on disruptions caused by antibiotic use in hospitalized patients. These disruptions to the gut microbiome create a vulnerable environment where enterococci can flourish and exploit their pathogenic potential. Similar to

opportunistic predators within a weakened ecosystem, enterococci can leverage their arsenal of virulence factors to establish infections. This arsenal includes robust intrinsic and acquired mechanisms of antibiotic resistance, the ability to adhere to and invade host tissues, and the production of toxins that damage host cells. This review delves deeper into the specific mechanisms employed by enterococci for pathogenesis, highlighting recent advancements in our understanding of these processes. Such knowledge is crucial for the development of effective control strategies against these adaptable nosocomial pathogens. Ultimately, the goal is to restore the delicate balance within the human gut microbiome and safeguard public health from the growing threat of enterococcal infections [26].

### 6.3. Streptococcus

Streptococcal infections remain a significant global health burden, posing a major challenge for contemporary medicine. The World Health Organization (WHO) highlights *Streptococcus pneumoniae* as the leading cause of pneumonia worldwide. Despite the increasing use of pneumococcal vaccines, the incidence of diseases caused by this pathogen hasn't shown a significant decrease. This concerning trend can be attributed to the emergence and spread of non-vaccine serotypes of *S. pneumoniae*. Notably, the pathogenic potential isn't limited to established pathogens. Even bacterial species previously considered commensal flora (harmless inhabitants) in humans or animals can exhibit virulence under certain conditions. Additionally, new species with pathogenic capabilities are being discovered [27]. This work presents a comprehensive analysis of streptococcal infections, drawing upon case studies and existing scientific literature. By incorporating recent reports, it goes beyond existing knowledge and delves into the limitations of current preventive strategies, particularly the application of pneumococcal vaccines against the rising threat of non-vaccine serotypes. The aim is to provide novel insights into this complex public health issue [28].

### 6.4. Bacillus

The genus *Bacillus* encompasses a diverse group of bacteria with a wide range of characteristics and potential impacts. *Bacillus flexus*, for example, thrives in aerobic environments (with oxygen) and is known for forming creamy colonies on culture plates. In contrast, *Bacillus licheniformis* is primarily found in soil and exhibits Gram-positive cell wall structure, a characteristic shared with *Bacillus halodurans*, another soil-dwelling, rod-shaped bacterium [29]. *Bacillus paralicheniformis* adds another layer of diversity, demonstrating facultative anaerobic capabilities, meaning it can grow with or without oxygen [30]. Like other *Bacillus* species, it forms rod-shaped cells and endospores, which are dormant structures that allow the bacteria to survive harsh conditions. One notable feature shared by many *Bacillus* species is the production of extracellular substances, including enzymes, toxins, and pigments. These substances can have various roles, with enzymes aiding in the breakdown of complex molecules, toxins potentially causing harm to other organisms, and pigments playing a part in cellular functions or communication [31]. However,

within this diverse genus, *Bacillus* SPS stands out for its specific toxin production. This species produces two distinct enterotoxins, which are protein toxins known to cause gastrointestinal distress. These enterotoxins are responsible for symptoms like diarrhoea, vomiting, and emetic effects (inducing nausea and vomiting). This highlights the importance of identifying specific *Bacillus* species, as some can pose a health risk due to toxin production [32].

## 7. Inhibitory Potential

An inhibition test was conducted using the cup-plate method to evaluate the potential antimicrobial activity of the twelve bacterial

isolates against four standard bacterial cultures. Interestingly, the isolates derived from nutrient agar (NA) (n=6) did not exhibit any inhibitory zones against the test organisms. In contrast, all six isolates obtained from potato dextrose agar (PDA) which is now named as DR1-DR6 has displayed zones of inhibition, indicating potential antimicrobial properties. These findings suggest that the bacterial population isolated from PDA have capability of producing antimicrobial compounds (table 6). Focusing on the isolates derived from potato dextrose agar (PDA), the inhibition test using the cup-plate method revealed promising results against *Escherichia coli* (figure 2).

Table 6.  
Measurements for zone of inhibition by isolated sample against *E. coli*

Isolated Samples	Zone of inhibition (in cm)
Negative- control	No Zone of Inhibition
DR-1	4.5
DR-2	1.6
DR-3	1.8
DR-4	1.5
DR-5	2.0
DR-6	2.0

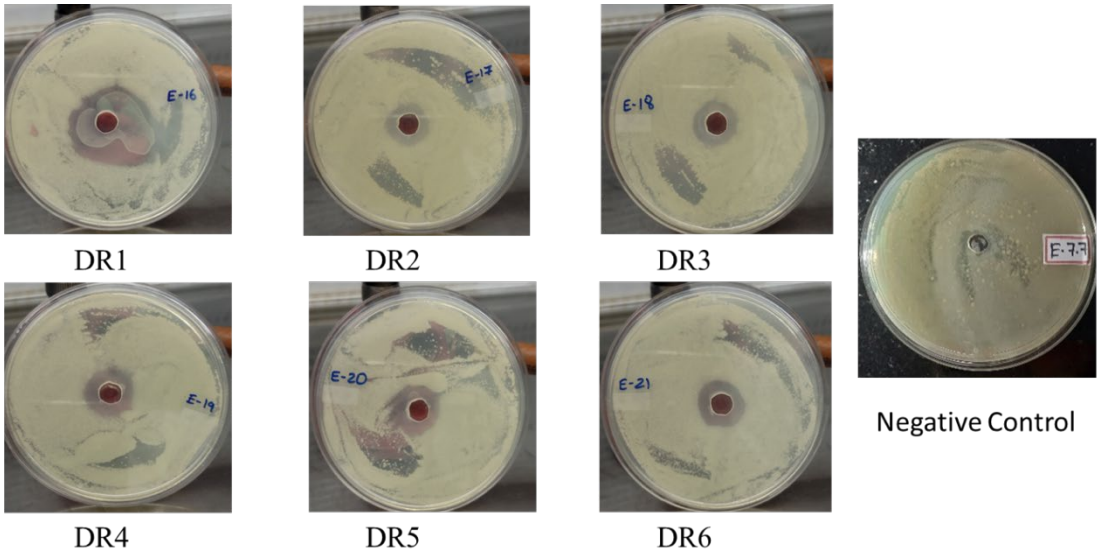


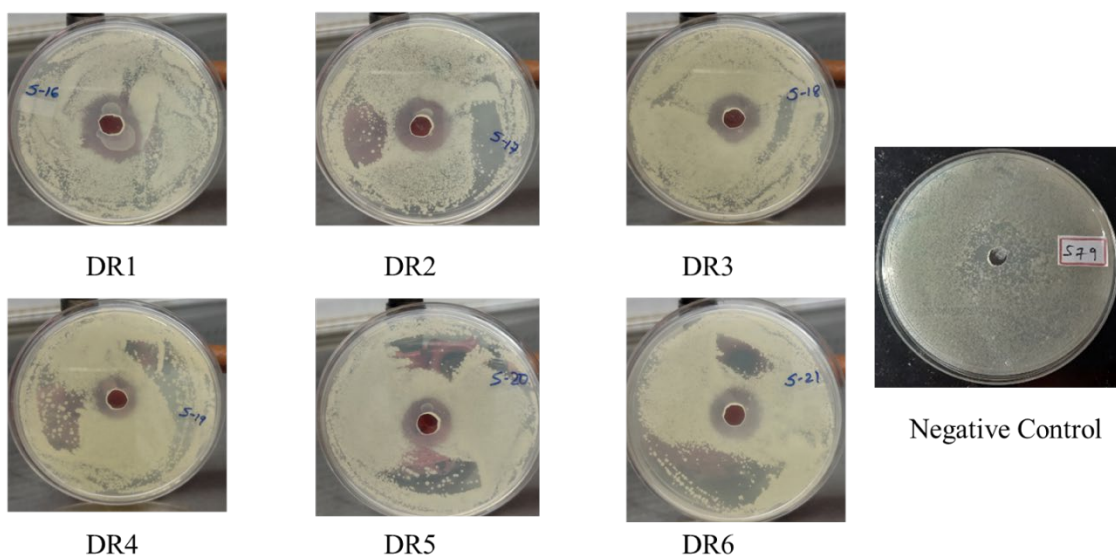
Fig. 2. Results of zone of inhibition by isolated sample against *Escherichia coli*

Focusing on the isolates derived from potato dextrose agar (PDA) (table 7), the inhibition test using the cup-plate method revealed promising results against *Staphylococcus aureus* (figure 3).

Table 7.

Measurements for zone of inhibition by isolated sample against *S. aureus*

Isolated Samples	Zone of inhibition (in <i>cm</i> )
Negative- control	No Zone of Inhibition
DR-1	3.0
DR-2	2.0
DR-3	2.0
DR-4	3.4
DR-5	2.0
DR-6	1.8

Fig. 3. Results of zone of inhibition by isolated sample against *S. aureus*

Focusing on the isolates derived from potato dextrose agar (PDA) (table 8), the inhibition test using the cup-plate method revealed promising results against *B. subtilis* (figure 4).

Table 8.

Measurements for zone of inhibition by isolated sample against *B. subtilis*

Isolated Samples	Zone of inhibition (in <i>cm</i> )
Negative- control	No Zone of Inhibition
DR-1	2.6
DR-2	1.7
DR-3	1.8
DR-4	1.9
DR-5	1.8
DR-6	2.0



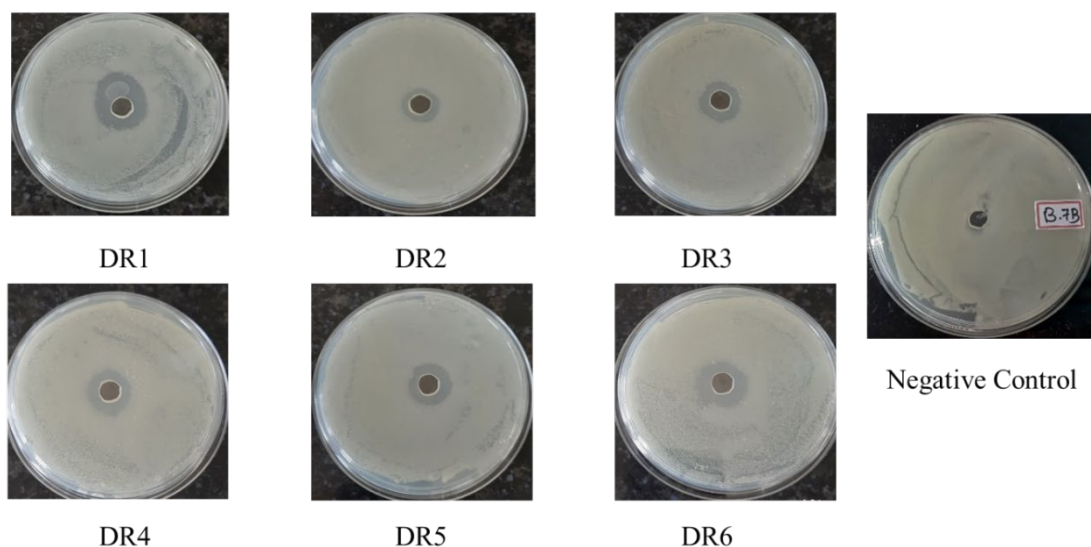


Fig. 4. Results of zone of inhibition by isolated sample against *B. subtilis*

Focusing on the isolates derived from potato dextrose agar (PDA) (table 9), the inhibition test using the cup-plate method

revealed promising results against *Klebsiella pneumoniae* (figure 5).

Table 9.

Measurements for zone of inhibition by isolated sample against *K. pneumoniae*

Isolated Samples	Zone of inhibition (in cm)
Negative- control	No Zone of Inhibition
DR-1	3.8
DR-2	1.3
DR-3	1.4
DR-4	3.4
DR-5	2.0
DR-6	3.6

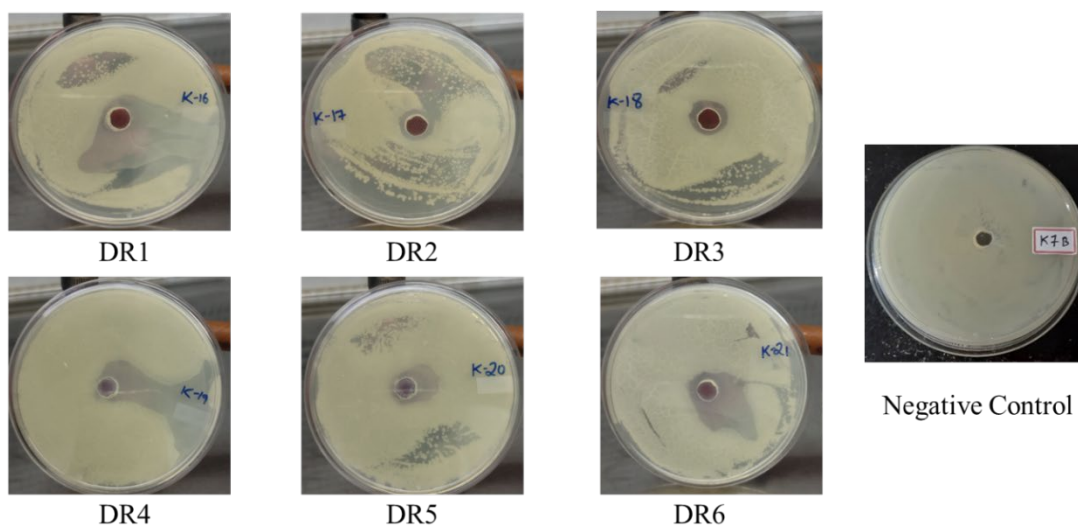


Fig. 5. Results of zone of inhibition by isolated sample against *K. pneumoniae*



## 8. Interaction of antibacterial stains with sample slurry

To assess the inhibitory effects of the six antibacterial species, it was performed a zone of inhibition assay with slurry

sample, the result of inhibitory testing is as mention bellow here we can clearly see the inhibition in growth of bacteria (table 10 & figure 6, 7).

Table 10.

Combined analysis of Inhibition test (in cm)

	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Klebsiella pneumoniae</i>	<i>Bacillus subtilis</i>
Negative	0.0	0.0	0.0	0.0
DR-1	4.5	3.0	3.8	2.6
DR-2	1.6	2.0	1.3	1.7
DR-3	1.8	2.0	1.4	1.8
DR-4	1.5	3.4	3.4	1.9
DR-5	2.0	2.0	2.0	1.8
DR-6	2.0	1.8	3.6	2.0

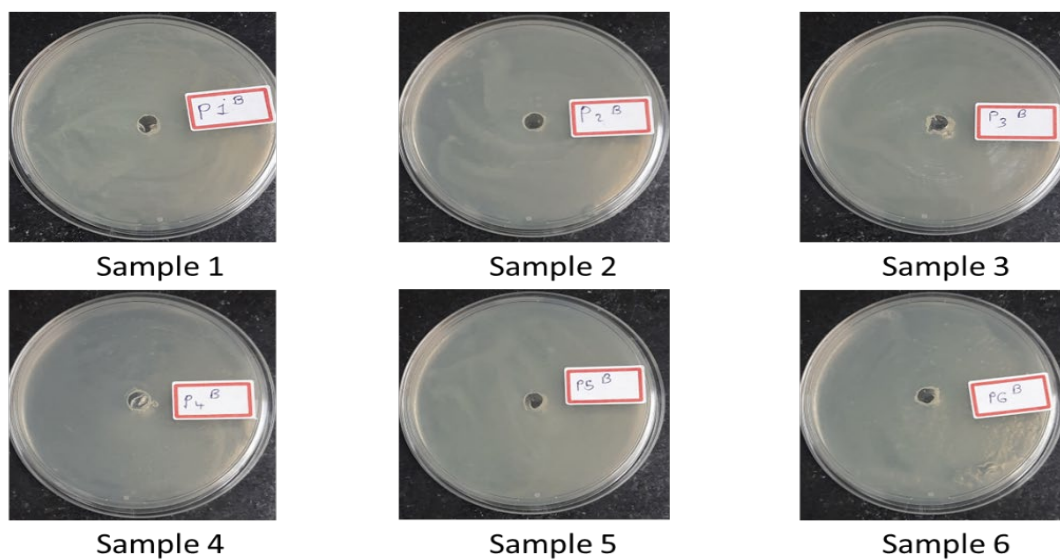
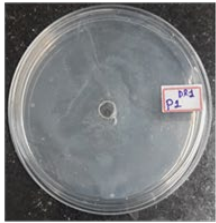
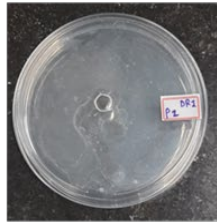


Fig. 6. Results of negative control of sample slurry

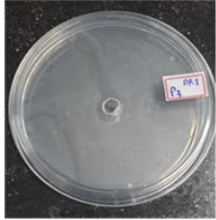
### DR 1 sample inhibiting sample slurry



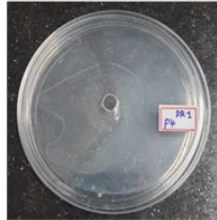
Slurry sample-1



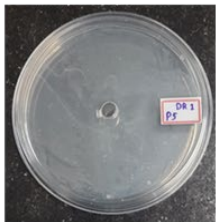
Slurry sample-2



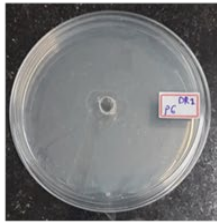
Slurry sample-3



Slurry sample-4

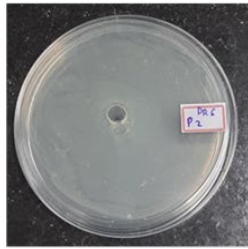


Slurry sample-5

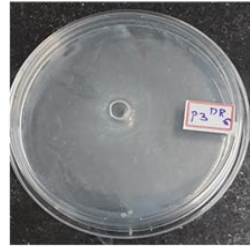


Slurry sample-6

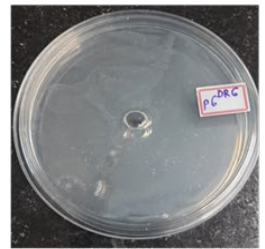
### DR 6 sample inhibiting sample slurry



Slurry sample-2

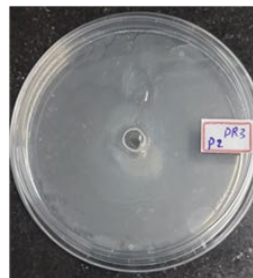


Slurry sample-3

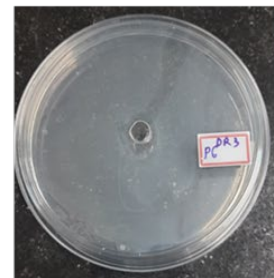


Slurry sample-6

### DR 3 sample inhibiting sample slurry



Slurry sample-2



Slurry sample-6

Fig. 7. Results of Interaction of 6 inhibiting strains with sample slurry

## 9. Discussion

The inhibition test results presented in this study strongly suggest the exceptional potential of the DR-1 sample. DR-1 demonstrated a clear advantage over the other samples, exhibiting the strongest inhibitory effect against all four prepared stock samples. More importantly, its effectiveness extended beyond these controlled conditions, as DR-1 also inhibited the growth of bacteria in all six types of initial slurry samples tested. This comprehensive analysis provides compelling evidence that the isolated bacterial strain, DR-1, possesses powerful broad-spectrum antibacterial properties. Notably, DR-1's efficacy against the real-world slurry samples underscores its potential for significant real-world applications, particularly in minimizing bacterial growth and reducing slurry rejection rates.

Based on these promising findings, DR-1 emerges as the most favourable candidate for further investigation. Future studies

should focus on elucidating the specific mechanisms behind DR-1's antibacterial activity. A crucial aspect of this research will involve extracting the secondary metabolites responsible for these potent properties. Once isolated and characterized, these metabolites can be formulated into a bio-antibiotic dosage form suitable for application in slurry. The incorporation of a bio-antibiotic derived from DR-1 offers a sustainable and potentially more environmentally friendly approach to slurry management. This approach holds the promise of minimizing rejection rates while simultaneously maintaining the desired physical and chemical properties of the slurry.

To gain a deeper understanding of the initial results and identify the other promising bacterial strains, all six samples that displayed some level of antibacterial activity will undergo further analysis through biochemical testing by accredited biological testing laboratory. This testing will provide crucial information on the identity of the bacteria responsible for the observed effects, allowing for a more targeted approach in future studies (table 11).

Table 11.

Result of Identified species by biochemical test

Isolates name	Identified species
DR-1	<i>Enterococcus hirae</i> .
DR-2	<i>Enterococcus faecium</i> .
DR-3	<i>Micrococcus luteus</i> .
DR-4	<i>Staphylococcus epidermidis</i> .
DR-5	<i>Streptococcus sp.</i>
DR-6	<i>Klebsiella pneumoniae</i> .

## 10. Conclusions

Investment casting process is considered to be one of the very complex processes that requires knowledge of diversified domains such as manufacturing, and metallurgy. However, microbiological aspects in investment casting process are still not explored especially for the sub-process related to ceramic slurry making. It was observed that making of ceramic slurry mostly comprises ethyl silicate or colloidal silica that are prone to generate some microorganisms in the ceramic slurry. However, specific types of microorganisms present in slurry is still not explored. Furthermore, control of these microorganisms is still unexplored in previous research conducted in this direction.

In the present work, specific set of experiments have been conducted on ceramic slurry that is used in manufacturing of investment castings to identify presence of various microorganisms. Presence of diversified 12 bacterial colonies have been observed, and their effects on human are also relatively severe especially working with it in longer run. This will require specific attention, and need to have control on growth of these colonies. Furthermore, specific trials including zone of inhibition have been carried out. It was observed that *Enterococcus hirae* has potential to control growth identified bacterial colonies, and can further be developed as secondary metabolites that can be utilized for development of antibiotics in future. Effect of proposed secondary metabolites on other aspects of mechanical properties of slurry can also be explored in future along with specific controlling measures (e.g. quantity as well as specific interval for addition of antibiotics).

These identified microorganisms will be passed through genetic analysis to assess whether DNA of identified microorganism are matched with available database of microorganisms or not. In case, genetic sequencing of these identified microorganisms are then available antibiotics based on these microorganisms will be used to make antibiotics otherwise consortia of these identified microorganisms will be used for development of antibiotics. The developed antibiotics will be tested and evaluated for their performance in real industrial environment, and antibiotics will be fine-tuned to achieve better results in case of reasonable performance to prevent the growth of microorganisms in slurry used in investment casting.

## Data Availability Statement

The authors confirm that the majority of the data supporting the findings of this study are available within the article. Some of the

data that support the findings of this study are available from the corresponding author, [Amit Sata], upon reasonable request.

## References

- [1] Nikunj, M. & Sata, A. (2023). Systematic development of cumulative complexity index for investment casting. *Journal of Advanced Manufacturing Systems*. 22(02), 323-338. <https://doi.org/10.1142/S0219686723500166>.
- [2] Nikunj, M. & Sata, M. (2023). Development of a novel complexity index for investment casting. *International Journal of Metalcasting*. 18, 2165-2180. <https://doi.org/10.1007/s40962-023-01151-1>.
- [3] Chirag, M. (2011). *Thermo-physical properties measurement and steel-ceramic shell interactions in investment casting*. Master's Thesis, Missouri University of Science and Technology.
- [4] Vyas, A.V., Prajapati, H.P. & Sutaria, M.P. (2023). Comparative evaluation on oxidation resistance of reactive AZ91D magnesium alloy with various face-coats in investment casting. *International Journal of Metalcasting*. 18, 180-1810. <https://doi.org/10.1007/s40962-023-01153-z>.
- [5] Sanjay, K. & Karunakar, D.B. (2021). Characterization and properties of ceramic shells in investment casting process. *International Journal of Metalcasting*. 15, 98-107. <https://doi.org/10.1007/s40962-020-00421-6>.
- [6] Lu, Yan, Haiyang Shi, Kai Lü, Jie Li, Yanfen Li, & Weidong Chen. (2023). Properties and fracture mechanism of composite plaster mold covered with multiple adhesion layer for investment casting. *International Journal of Metalcasting*. 18, 1770-1782. <https://doi.org/10.1007/s40962-023-01152-0>.
- [7] Song, Q., Zha, X., Ou, M., Lu, C., Li, J. & Ma, Y. (2023). High-temperature flexural strength of aluminosilicate ceramic shells for the investment casting of nickel-based superalloy. *International Journal of Metalcasting*. 18, 962-974. <https://doi.org/10.1007/s40962-023-01061-2>.
- [8] Lü, K., Duan, Z., Liu, X., Li, Y. & Du, Z. (2020). Effect of dispersant on fiber-reinforced shell for investment casting. *International Journal of Metalcasting*. 14, 1005-1012. <https://doi.org/10.1007/s40962-020-00409-2>.
- [9] Lopes, V., Puga, H., Barbosa, J. & Teixeira, J.C. (2020). Effect of yttria mould coating on the investment casting of AZ91D-1 wt% CaO magnesium alloy. *International Journal of Metalcasting*. 14, 98-107. <https://doi.org/10.1007/s40962-019-00339-8>.

- [10] Nwaogu, U. C. & Tiedje, N. S. (2011). Foundry coating technology: A review. *Materials Sciences and Applications*. 2(08), 1143-1160. DOI: 10.4236/msa.2011.28155.
- [11] Romberger, J.A., Payne, C. C. (1993). Patent No. 5230833. United States Patent on Low Sodium Low Metals Silica Polishing Slurries.
- [12] Li, J., Zhu, Y., Wu, X. & Hoffmann, M.R. (2020). Rapid detection methods for bacterial pathogens in ambient waters at the point of sample collection: a brief review. *Clinical Infectious Diseases*. 71(Supplement 2), S84-S90.
- [13] Bonnet, M., Lagier, J. C., Raoult, D. & Khelaifia, S. (2020). Bacterial culture through selective and non-selective conditions: the evolution of culture media in clinical microbiology. *New microbes and new infections*. 34, 100622, 1-11. <https://doi.org/10.1016/j.nmni.2019.100622>.
- [14] Mészáros, É. (2024). *How to culture bacteria*. Retrieved February 15, 2025, from <https://www.integrabiosciences.com/china/en/blog/article/how-culture-bacteria>.
- [15] Basha, M. (2020). Centrifugation. In *Analytical Techniques in Biochemistry* (pp.13-21). Springer.
- [16] Ali, S., Sarfraz, M.H., Muhjudin, R. & Sean, W.A. (2018). Screening And Selection of industrially important microorganisms. A Review. *Journal of Pharmaceutical and Medical Research*. 42-47.
- [17] Qayyum, S., Basharat, S., Mian, A. H., Qayum, S., Ali, M., Changsheng, & Sultan, F. (2020). Isolation, identification and antibacterial study of pigmented bacteria. *Applied Nanoscience*. 10, 4495-4503.
- [18] Jufri, R.F. (2020). Microbial isolation. *Journal La Lifesci*. 1(1), 18-23.
- [19] Kumar, A., Murthy, L.N. & Jeyakumari, A. (2018). *Plating techniques in isolation of micro-organisms*. Retrieved February 19, 2025, from <https://drs.cift.res.in/server/api/core/bitstreams/e138783f-966e-4816-b29a-d8565fb3078e/content>.
- [20] Kumar, D., Kumar, V., Jangra, P. & Singh, S. (2016). Leucas cephalotes (Spreng): photochemical investigation and antimicrobial activity via cylinder-plate method or cup-plate method. *International Journal of Pharmaceutical Science and Research*. 1(4), 28-32.
- [21] Yu, M., Zhang, L., Qian, G., Shi, H. & Yu, S. (2022). Fe-MBL-modified Fe<sub>3</sub>O<sub>4</sub> magnetic bead enrichment and fixation in Gram stain for rapid detection of low-concentration bacteria. *Microchimica Acta*. 189(4), 169, 1-10. <https://doi.org/10.1007/s00604-022-05277-7>.
- [22] Moyes, R.B., Reynolds, J. & Breakwell, D.P. (2009). Differential staining of bacteria: gram stain. *Current Protocols in Microbiology*. 15(1), A-3C. <https://doi.org/10.1002/9780471729259.mca03cs15>.
- [23] Ogofure, A.G., Beshiru, A. & Igbinsosa, E.O. (2022). Evaluation of different agar media for the antibiotic susceptibility testing of some selected bacterial pathogens. *University of Lagos Journal of Basic Medical Sciences*. 8(1-2), 34-41.
- [24] Barnard, R.T. (2019). The zone of inhibition. *Clinical chemistry*. 65(6), 819-819. <https://doi.org/10.1373/clinchem.2018.299800>.
- [25] Frank, K.L., del Pozo, J.L. & Patel, R. (2008). From clinical microbiology to infection pathogenesis: how daring to be different works for staphylococcus lugdunensis. *Clinical Microbiology Reviews*. 21(1), 111-133. <https://doi.org/10.1128/cmr.00036-07>.
- [26] Fiore, E., van Tyne, D. & Gilmore, M.S. (2019). *Pathogenicity of enterococci. Gram-Positive Pathogens*. Retrieved November 26, 2024, from <https://doi.org/10.1128/9781683670131.ch24>.
- [27] Krzyściak, W., Pluskwa, K.K., Jurczak, A. & Kościelniak, D. (2013). The pathogenicity of the streptococcus genus. *European Journal of Clinical Microbiology & Infectious Diseases*. 32, 1361-1376. <https://doi.org/10.1007/s10096-013-1914-9>.
- [28] Andrewes, F.W. & Horder, T.J. (1906). A study of the streptococci pathogenic for man. *The Lancet*. 168(4334), 775-783. [https://doi.org/10.1016/s0140-6736\(01\)13797-9](https://doi.org/10.1016/s0140-6736(01)13797-9).
- [29] Wang, Xi, & Hua Zhao. (2013). Isolation and Characterization of a Bacillus Flexus Strain Used in Alkaline Wastewater Treatment. *Advanced Materials Research*. 750-752, 1381-1384. <https://doi.org/10.4028/www.scientific.net/amr.750-752.1381>.
- [30] Parte, A.C., Carbasse, J.S., Meier-Kolthoff, J.P., Reimer, L.C. & Göker, M. (2020). List of prokaryotic names with standing in nomenclature (LPSN) moves to the DSMZ. *International Journal of Systematic and Evolutionary Microbiology*. 70(11), 5607-5612. <https://doi.org/10.1099/ijsem.0.004332>.
- [31] Takami, H. & Horikoshi, K. (1999). Reidentification of facultatively alkaliphilic bacillus sp. C-125 to bacillus halodurans. *Bioscience, Biotechnology, and Biochemistry*. 63(5), 943-945. <https://doi.org/10.1271/bbb.63.943>.
- [32] Dunlap, C.A., Soon-Wo Kwon, Rooney, A.P. & Soo-Jin Kim. (2015). Bacillus paralicheniformis sp. nov., isolated from fermented soybean paste. *International Journal of Systematic and Evolutionary Microbiology*. 65(Pt 10), 3487-3492. <https://doi.org/10.1099/ijsem.0.000441>.