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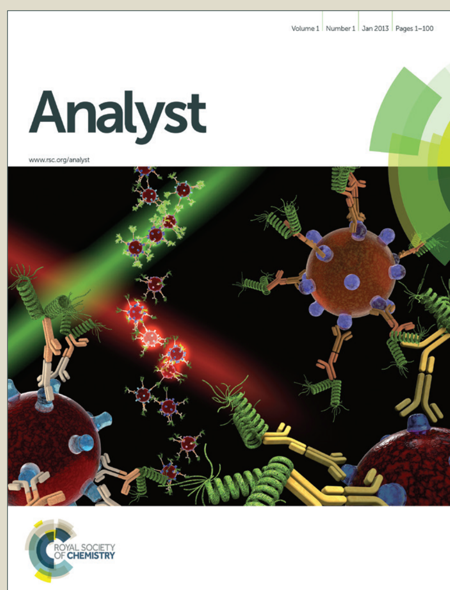

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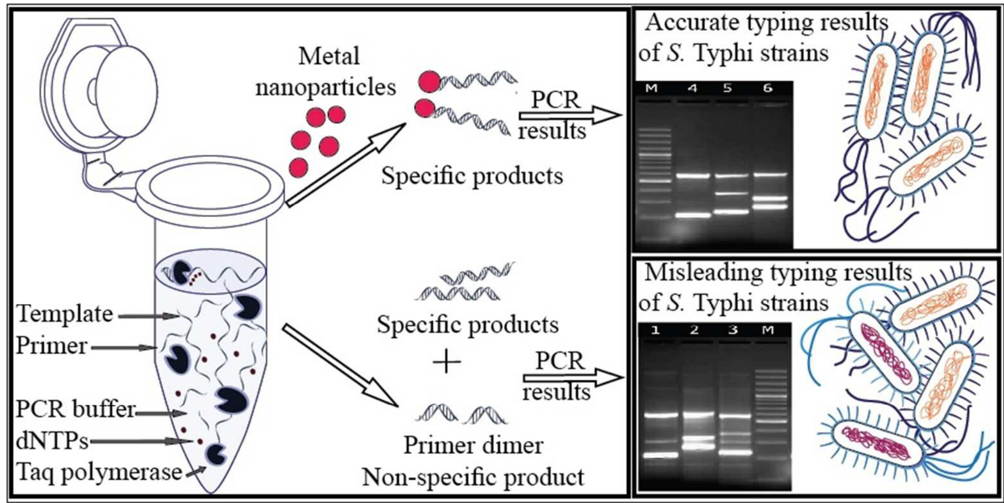

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ARTICLE

Metal Nanoparticles Assisted Polymerase Chain Reaction for Strain Typing of *Salmonella* Typhi

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Salmonella enterica serotype Typhi (*S.* Typhi) is the causative agent of typhoid fever and remains a major health threat in most of the developing countries. The prompt diagnosis of typhoid directly from the patient's blood requires high level of sensitivity and specificity. Some of us were the first to report PCR based diagnosis of typhoid. This approach has since then been reported by many scientists using different genomic targets. Since the number of bacteria circulating in blood of a patient can be as low as 0.3cfu/ml, there is always a room for improvement in diagnostic PCR. In the present study, the role of different types of nanoparticles was investigated to improve the existing PCR based methods for diagnosis and strain typing of *S.* Typhi (targeting Variable Number of Tandem Repeats [VNTR]) by using optimized PCR systems. Three different types of nanoparticles were used i.e., citrate stabilized gold nanoparticles, rhamnolipid stabilized gold and silver nanoparticles, and magnetic iron oxide nanoparticles. The non-specific amplification was significantly reduced in VNTR typing when gold and silver nanoparticles were used in appropriate concentration. More importantly, the addition of nanoparticles decreased the non-specificity to a significant level in the case of multiplex PCR thus further validating the reliability of PCR for the diagnosis of typhoid.

1. Introduction

Salmonella enterica serotype Typhi (*S.* Typhi) is an exclusive human pathogen. The genetic similarity among *Salmonella* strains makes it difficult for epidemiologists to accurately diagnose and track their outbreaks. A highly sensitive typing method is, therefore, required to differentiate between genetically similar but epidemiologically different *Salmonella* strains. For epidemiological studies of such pathogens, the capability to type pathogens strain is a critical tool. A number of methods have been developed for strain typing of *S.* Typhi including molecular techniques such as ribotyping,[1] DNA fingerprinting using random amplification of polymorphic DNA (RAPD),[2] pulsed-field gel electrophoresis (PFGE),[3,4] single nucleotide polymorphism (SNP),[5-7] and variable number of tandem repeats etc.[8,9] The PCR is the most well developed molecular technique and has various potential clinical applications.[10-12] In spite of its wide range utilization in molecular diagnostic, PCR's specificity is not always up to the mark when compared with its sensitivity.[13] Various parameters need to be optimized, [14,15] and various commercially available PCR enhancers have been used to overcome these limitations on a trial-and-error basis.[16,17] In modulating PCR, nanomaterials have recently been found to have special effects. For example various nanomaterials like titania nanoparticles,[18] quantum dots,[19] carbon nanotubes and nanopowders,[20,21] and polyethyleneimine based derivatives,[22] and gold nanoparticles[23, 24] have been evaluated in this regard.

However, there are a number of contradictory reports on whether the addition of nanoparticles to PCR is helpful to improve the PCR efficiency or they may remarkably inhibit the PCR. For instance, Wan and Yeow investigated that Au NPs of larger sizes (~20 nm) can cause complete PCR inhibition at a lower particle concentration than those of smaller sizes (~5 nm).[23] Similarly Vu and co-workers demonstrated that the addition of Au NPs does not tend to increase the PCR specificity rather they favour the amplification of smaller products over the larger ones irrespective of the specificity.[24] Several mechanisms have been proposed to explain the role of nanoparticles in improving the PCR efficiency or its inhibition,[18,24,25,26] but the exact underlying mechanism is still obscure.

We have now investigated the effect of different types of nanoparticles on PCR used for the diagnosis and VNTR based typing of *S.* Typhi. In this study, we have tried to use nanoparticles with the objective to improve the sensitivity of PCR to eliminate one PCR step so that the diagnostic PCR can be completed in one step. For VNTR typing, multiplex PCR assay is used and non-specific amplification in multiplex PCR can cause

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misleading results. The nanoparticles assisted PCR was, therefore, used to check its effect on non-specific amplification. Interestingly, we found that the addition of citrate stabilized gold nanoparticles eliminates the non-specific amplification to a significant level and nanoparticles of different nature were found to have different effect on the PCR efficiency in terms of specificity and sensitivity.

2. Materials and methods

2.1 Preparation of metal nanoparticles

Gold nanoparticles were synthesized using standard citrate reduction method.[27] Magnetic nanoparticles were synthesized *via* the co-precipitation method.[28] Silver nanoparticles (Ag NPs) were synthesized using rhamnolipids as a reducing as well as stabilizing agent by a newly developed method. In this method, an aqueous solution of rhamnolipids (1.0 mL of 2 mg/10 mL stock) was incubated with 1.0 mL silver nitrate (1 mM) solution at pH 12. The color of the solution immediately changed to yellowish brown, indicating the formation of Ag NPs. The resulting silver nanoparticles solution was centrifuged (Microultracentrifuge, Hitachi, GX series) at 20,000 rpm for 1 h followed by thorough rinsing with ultrapure water. The pellets were re-dispersed in ultrapure deionized water for further characterization.

2.2 Preparation of the DNA template

The strains of *S.* Typhi (NIB 25, 38 and As1) used in this study, obtained from NIBGE culture collection, were cultivated in trypticase soy broth (TSB). The overnight grown cultures were centrifuged at 10,000 rpm for 10 min and suspended in T buffer (Tris HCl buffer, 10 mM). The genomic DNA was extracted through phenol/chloroform treatment and ethanol precipitation.[29,30]

2.3 PCR amplification of *fliC* gene for identification of *S.* Typhi

The *fliC* gene was amplified by conventional PCR using gene specific ST1 and ST2 primers,[31] the sequence and product size of each primer is shown in Table 1. The PCR mixture (25 µL) contained 10X PCR buffer (2.5 µL), 1.5 mM $MgCl_2$ (1.5 µL), 0.75 µL dNTPs mixture (dATP, dCTP, dTTP and dGTP, 2.5 mM each), 1.0 µL reverse primer, 1.0 µL forward primer (20 p mol each), 0.2 µLTaq polymerase (5 U/µL), 5.0 µL of template DNA (5 ng/µL). The volume was made up to 25 µL with 10 mM T buffer (pH 8.3). The DNA template was used in serial dilutions (10 fold) to assess the effect of nanoparticles on the amplification of very low concentration of DNA (up to 0.2×10^{-3}µg). Different concentrations of gold (0.124 - 0.49 nM) and silver (0.03 - 0.136 mM) nanoparticles were used to evaluate their effect on PCR sensitivity. The PCR was performed using following conditions: denaturation at 94 °C for 4 min; followed by 30 cycles of 1 min at 94 °C; 45 seconds at 55 °C for annealing and 1 min at 72 °C for extension with a final extension of 10 min at 72 °C. PCR was performed in 0.2 mL reaction tubes in a thermal cycler (Mastercycler gradient, Eppendorf, Germany) under various experimental conditions. The amplified products were separated on 1.5 % agarose gel and stained with ethidium bromide for detection under UV illumination.

2.4 PCR conditions for VNTR typing

The typing of *S.* Typhi strain was performed using TR1, TR2 and TR3 primers based on the sequence of VNTRs present in *S.* Typhi genome.[32] The sequences of the reverse and forward primers and the size of amplified product are given in Table 1. The PCR assay was optimized initially for single primer pairs TR1, TR2 and TR3. Total reaction mixtures of 25 µL contained 10 X Fermentas Taq buffer (2.5 µL), 1.5 mM $MgCl_2$, 0.75 µL dNTPs mixture (dATP, dCTP, dTTP and dGTP, 50 nM each), 0.75 µL reverse primer, 0.75 µL forward primer (20 pM each), 0.2 µL Taq polymerase (2 U/µL), and 5.0 µL of template DNA (5 ng/µL). The volume was made up to 25 µL with 10 mM Tris buffer (pH 8.3). The concentration of various components was changed accordingly to examine their effect on the efficiency of PCR. Different concentrations of gold (0.12 - 0.49nM), silver (0.03 - 0.14 nM), and iron oxide nanoparticles (0.09 - 0.18 µg/µL) were used to optimize their effective concentration. The thermal cycler was programmed to 1 cycle of 5 min at 94 °C, followed by 35 cycles of 30 seconds at 94 °C for denaturing, 30 seconds at 55 °C for primer annealing and 1 min at 72 °C for extension with a final extension of 10 min at 72 °C. For multiplex PCR, the above mentioned conditions were employed except for primers which were added as: TR1 0.75 µL each, TR2 1.5µL each and TR3 0.75 µL each and dNTP's mix 2.0µL. The amplified products were resolved on 1.5 % agarose gel. The gels were stained with ethidium bromide and visualized under UV light. The PCR reaction was also carried out at different annealing temperatures (55 and 52 °C) in the presence and absence of metal nanoparticles.

2.5 Quantification of PCR product

Image J software was used to quantify the amplified PCR product from the gel images generated by loading the equal volume of PCR products. The intensities of each band were expressed as arbitrary units (A. U).

3. Results and Discussion

Some of us have already reported the direct detection of *S.* Typhi from blood of typhoid patients by using two rounds of PCR, i.e., regular and nested, which is required to amplify even 5 bacteria/mL of blood up to a detectable level.[30,33] In order to improve the specificity further, the effect of nanomaterials was investigated using the highly optimized PCR, in terms of amplification yield but have some non-specificity issues. In this study, two different optimized PCR systems (single/uniplex and multiplex) were analyzed for diagnosis and typing of *S.* Typhi based on VNTR markers in the presence and absence of nanoparticles of different types and concentrations.

No aggregation of either of these nanoparticles was observed when they were mixed with the PCR mix because PCR mixture remained clear even after the completion of PCR. This indicated that these nanoparticles could withstand electrostatic shock in the presence of various salts in PCR buffer and therefore did not undergo visually observable aggregation. This is in accordance with previous reports.[18,34 34] It is likely that the single stranded DNA (primers) in the PCR mixture tends to adsorb on the surface of nanoparticles leading to an increase in

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the stability of the nanoparticles in buffer solution and preventing their aggregation especially at the fairly high salt concentration generally used in the buffers.

3.1 Characteristics of metal nanoparticles used in PCR

Gold nanoparticles showed the characteristic absorption band at 520 nm, which clearly corresponds to the surface plasmon resonance of gold nanoparticles of 15-20 nm diameter (Fig. S 1). The concentration of Au NPs was determined on the basis of UV-Vis spectrum data using an extinction coefficient of 4.9×10^8 M^{-1} cm^{-1}.[35] Similarly the Ag NPs were synthesized using rhamnolipids as reducing and stabilizing agent showed the characteristic surface plasmon band at 399 nm. The concentration of silver nanoparticles was determined using UV-Vis absorption data by a method reported by Paramelle *et al* (2014).[36]

The exact diameter of synthesized nanoparticles was determined by transmission electron microscopic (TEM) studies and it was found that the Au NPs were of fairly uniform size and shape with an average diameter of 16-20 nm (Fig. S 2a). The hydrodynamic diameter of gold nanoparticles, determined by Zetasizer Nano ZS, increased when incubated with PCR mixture containing dNTPs, primers and polymerase. For instance as shown in Fig. S 3, the size of Au NPs in PCR buffer was 25 nm, closely corresponding to the size of Au NPs as determined by electron microscopy (15-20 nm). The addition of dNTPs and primers further increased the diameter of nanoparticles to 28 nm due to the adsorption of primers and dNTPs. Subsequently the nanoparticles diameter increased to 31 nm after the addition of polymerase that confirms that the polymerase is also adsorbed on the surface of nanoparticles even in the presence of dNTPs and primers. Similar behaviour was also observed in the case of silver nanoparticles (Fig. S 3).

The TEM characterization of the Ag NPs (Fig. S 2b) showed the presence of NPs with an average diameter of about 10-16 nm. This clearly indicated that the rhamnolipids also stabilized the nanoparticles in addition to their role as a reducing agent. The size of the magnetic nanoparticles was also investigated by TEM and results indicated the presence of 25-30 nm iron oxide magnetic nanoparticles but, due to strong magnetic interactions, the particles appeared aggregated (Fig. S 2c).

3.2 Effect of nanoparticles' concentration on sensitivity of PCR

In order to examine the effect of Au NPs on sensitivity of PCR, different concentration of Au NPs (0.1 - 0.49 nM) was added to the PCR mixture. It is evident from the results that Au NPs affected the PCR in a concentration dependent manner, with slightly increased amplification of the target DNA at 0.248 nM while the concentrations above 0.49 nM effectively inhibited the PCR reaction thus decreasing the yield of amplified product as compared to the control (Fig 1 A). Slightly improved amplification of a hypervariable region of *fliC* gene (495 bp) specific for *S.* Typhi was observed in the presence of 0.248 nM Au NPs as evident from the quantitative analysis graph (Fig. 1 B). Li *et al*. also reported an increase in PCR efficiency in the presence of 0.2 - 0.8 nM of Au NPs,[37] and found inhibitory

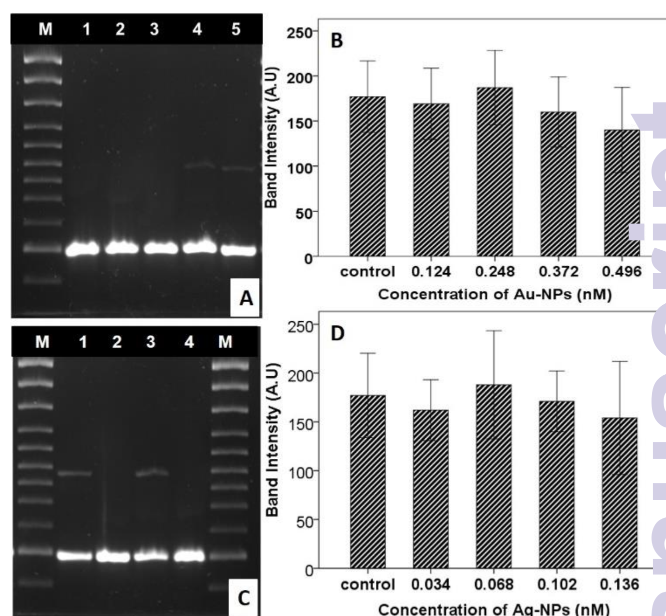

Figure. 1. PCR amplification of fliC gene (495 bp) at different concentration of gold and silver nanoparticles. Lane M: DNA Marker (Fermentas cat# SM0323 showing 3000, 2000, 1500, 1200, 1031, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bp); Lane 1-5: Showing amplification in the presence of 0.124, 0.248, 0.372, and 0.496 nM of Au NPs respectively (A). Quantitative determination of relative amount of PCR yields (B), Lane 1-4: Showing PCR amplification in presence of 0.034, 0.068, 0.102, and 0.136 mM of Ag NPs respectively (C). Quantitative determination of relative amount of PCR yields (D), A. U = arbitrary unit. The error bars are 1 Standard Error of means for three replicates.

effects of Au NPs at higher concentrations. There was not much difference in PCR sensitivity as compared to the control (without nanoparticles), however, these studies led to investigating the effective concentration of Au NPs, which can be further explored for improvement in PCR sensitivity.

In addition to the Au NPs, different concentrations of rhamnolipids stabilized Ag NPs (0.03 - 0.136 nM) were also evaluated for their role in PCR and the results (Fig. 1 C and D) showed little enhancement in PCR yield at 0.068 nM as compared to the control (without the addition of Ag NPs). However, further increase in concentration of Ag NPs decreased the PCR amplification yield as compared to the control. These results clearly indicate that relatively lower concentration of Ag NPs is needed as compared to the Au NPs for almost the same level of enhancement in PCR yield under similar experimental conditions. So it is obvious that both the nature and surface chemistry of metal nanoparticles influence the PCR efficiency differently, as in this case the gold (citrate stabilized) and silver (rhamnolipids stabilized) nanoparticles showed different behaviour under similar PCR conditions. Tong *et al.* also reported that the surface chemistry of nanoparticles played very important role in determining their effectiveness in PCR.

In addition to the sensitivity of PCR, another important issue is the specificity of PCR system. Many attempts have been made to reduce the amplification of non-specific product by optimizing the annealing temperature, magnesium ion

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concentration in reaction mixture and reduction in the number of PCR cycles. However, in some cases non-specific amplification could not be eliminated by these optimizations. Therefore, one of such PCR systems was used as a model during present studies to assess whether the addition of nanoparticles in PCR could improve the specificity of PCR system.

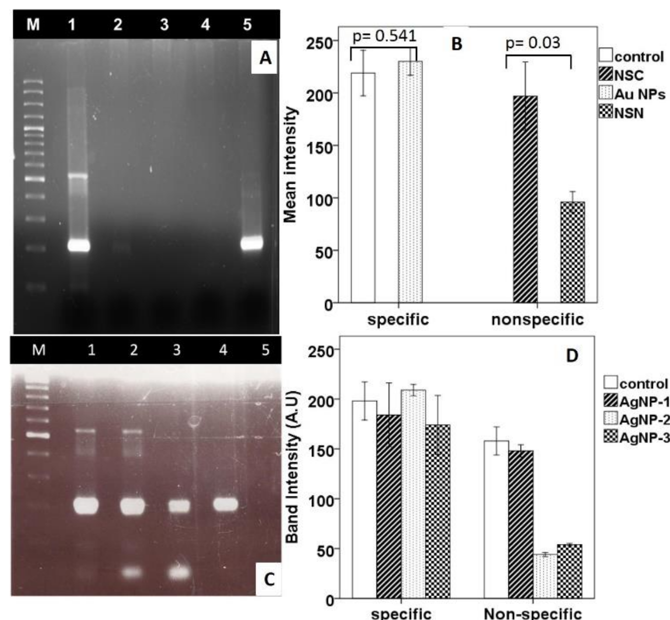

Figure 2. PCR amplification of TR1 region in the absence and presence of gold and silver NPs. Lane M: Fermentas DNA marker Cat# SM0323 showing bands of 3000, 2000, 1500, 1200, 1031, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bp. Lane 1: control (without nanoparticles), Lane 2 and 3: with sodium citrate, Lane 4: negative control (without DNA and nanoparticles), and Lane 5: with 0.248 nM of Au NPs (A). Quantitative estimation of relative amounts of PCR yields; NSC and NSN is nonspecific amplification in control and nanoparticles respectively (B). Lane 1 with control (without nanoparticles), Lane 2 with 0.102 mM Ag NPs (Ag NP-1), Lane 3 with 0.136 mM Ag NPs (Ag-NP-3) and Lane 4 with 0.068 mM Ag NPs (Ag NP-2) and Lane 5 with negative control (without DNA and nanoparticles) (C). Quantitative estimation of relative amounts of PCR yields (D). The error bars are 1 Standard Error of means for three replicates.

Interestingly, reduction in non-specific amplification was observed in the case of Au NPs assisted PCR amplification (Fig. 2 A). Though there was no significant effect on the yield of product, but the non-specificity was reduced (~ 2 fold) to a significant level ($p < 0.05$) as is evident from the quantitative analysis graph (Fig. 2 B). No improvement in PCR was observed using solely the other components like sodium citrate and deionized water (Fig. 2A). The interaction of nanoparticles with the PCR components, including polymerase, dNTPs, primers and PCR products, plays an important role in affecting the PCR. It has been well documented that proteins, DNA, dNTPs and other PCR components can adsorb non-specifically on the surface of gold nanoparticles.[38-41] During PCR, these components adsorb and dissociate from the surface of nanoparticles and may regulate the PCR in multiple ways such as by modulating the active concentration of polymerase and by speeding up the dissociation of PCR product during denaturing step.

Similarly, another round of PCR amplification of TR1 region of *S.* Typhi for VNTR typing indicated that the non-specific amplification could be reduced significantly (~ 4 fold) using Ag NPs (0.068 nM) but it also had no significant increase in the yield of specific amplification in comparison to the control (Fig. 2 C, D). The non-specificity was also reduced to some extent while using 0.102 (Ag NP 1) and 0.136 nM concentrations (Ag NP 3) as compared to control but the yield was lower as compared to the control and even lower than the 0.068 nM concentration of Ag NPs. The nanoparticles based enhancement in the specificity of PCR may be due to the interaction of nanoparticles to the single stranded binding protein (SSB) that specifically binds to the single stranded DNA thus minimizing the mis-pairing between the DNA template and primers. The binding ability of nanoparticles with single and double stranded DNA has also been reported.[42]

The gold nanoparticles may better bind to the single stranded DNA as compared to double stranded DNA due to the availability of nitrogenous bases. This ultimately may limit the non-specific binding of primers and template thus leading to the specific amplification. However, if the concentration of particles is too high then there are fewer chances for the template to encounter the primers as their densities on surface of nanoparticles decrease due to the increase in number of available nanoparticles. This phenomenon either inhibits the PCR or reduces the amplification yield. These results are in consistent to those reported by Chen et al., who observed a decrease in PCR yield with increasing concentration of gold nanoparticles.[43] Moreover, the effect of nanoparticle addition was also monitored in PCR amplification of TR2 for *S.* Typhi strains NIB 25 and 38, respectively (Fig 3). Interestingly, the addition of citrate stabilized Au NPs not only eliminated the non-specific amplification (Fig 3), but yield of target DNA was also increased significantly ($p < 0.05$).

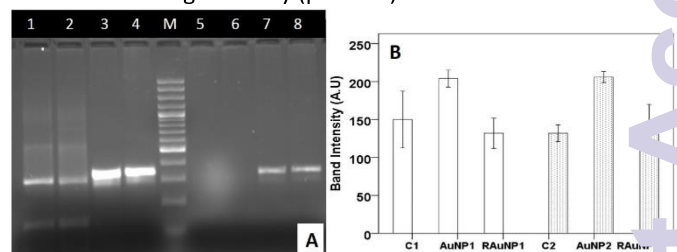

Figure. 3. Effect of nanoparticles on nonspecific amplification of TR2 primers of strains NIB 25 (1) and NIB 38 (2) respectively. Lane 1: Control, C1, NIB 25, Lane 2: Control, C2, NIB 38, in the absence of nanoparticles. Lane 3-4: Citrate stabilized Au NP1 (NIB 25) and Au NP2 (NIB 38), Lane 5-6: magnetic nanoparticles and Lane 7-8: rhamnolipids stabilized AuNP1 (NIB 25) and R AuNP2 (NIB 38). Lane M with DNA marker (Fermentas, cat # SM0323 showing bands of 3000, 2000, 1500, 1200, 1031, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bp). The error bars are 1 Standard Error of means for three replicates.

However, the Au NPs synthesized using rhamnolipids as reducing as well as stabilizing agents were not found to be more effective compared to citrate stabilized Au NPs. But their addition did not completely inhibit the PCR amplification, as

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observed in the case of magnetic nanoparticles (Fig 3). The results are shown only for 0.09 μg/μL as it inhibit the PCR at each concentration used. Recently, Higashi *et al*. (2012) [44] also reported that the addition of magnetic nanoparticles had inhibitory effect on PCR. They suggested that *Taq* DNA polymerase gets captured within the spaces in the clusters of magnetic nanoparticles formed thus reducing its concentration in the reaction mixture.

These results were further confirmed using multiplex PCR reaction and it was observed that the addition of citrate stabilized Au NPs (lane 4-6, Fig. 4) almost completely eliminated the non-specific amplification of the PCR, as compared to the control (lane 1-3, Fig. 4). In multiplex PCR, 2-3 primer pairs are usually used so any non-specific amplification will increase the chances of getting false positive results and may lead to wrong interpretation. In this study, we observed the elimination of non-specific amplification in the presence of nanoparticles thus improving the reliability of PCR significantly.

3.3 Role of gold nanoparticles in improving PCR specificity at low annealing temperature

One of the important parameter in PCR optimization is the adjustment of annealing temperature that determines the efficiency and specificity of PCR. Generally, the high annealing temperature increases the specificity of PCR but it also reduces the efficiency of PCR.[45] The PCR amplification of TR1 for *S.* Typhi strains NIB 25, 38 and As 1 respectively was also examined at 52 °C, in addition to at 55 °C, in order to study the effect of Au NPs on the specificity of PCR as lowered annealing temperature.

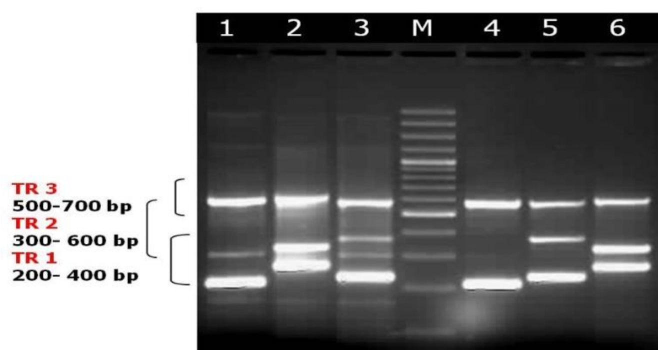

Figure 4. Effect of nanoparticles on nonspecific amplification in multiplex PCR for TR1, TR2 and TR3 for S. Typhi strains NIB 25, 38 and As 1 respectively in absence (Lane 1-3: Control) and presence (Lane 4-6) of citrate stabilized Au NPs, Lane M: Marker (Fermentas, cat # SM0323 showing bands of 3000, 2000, 1500, 1200, 1031, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bp).

Fig. 5 A and B show the agarose gel electrophoreses of the PCR products at annealing temperature of 55 and 52 °C, respectively. It is evident from the results that the addition of Au NPs in lane 4-6 greatly reduced the non-specific amplification as compared to the control in lane 1-3 by PCR at 55 °C (Fig. 5 A). These results indicated the feasibility of using Au NPs to significantly reduce the non-specific amplification under optimum PCR conditions. By lowering the annealing temperature to 52 °C (Fig. 5 B), the non-specific amplification was reduced in the presence of nanoparticles as shown in lane 4-6 (Fig. 5B) as compared to the control (lane 1-3, Fig. 5B). These results suggest that the elimination of non-specific amplification could only be achieved at optimum primer annealing temperature. There are some reports that the non-specificity may also be reduced at lower annealing temperature.[19,36,46]

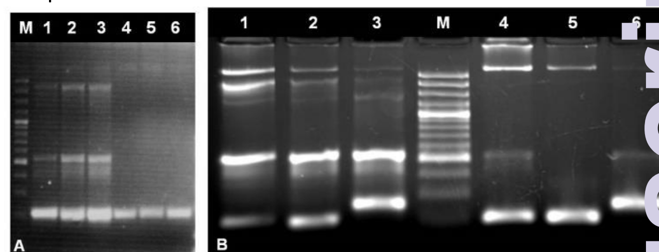

Figure 5. Effect of nanoparticles on PCR amplification of TR1 primers. PCR amplification of TR1 for S. Typhi strains NIB 25, 38 and As 1 respectively in the absence (lane 1-3) and presence (Lane 4-6) of citrate stabilized Au NPs at annealing temperature of 55 °C (A) and 52 °C (B).

The addition of metal nanoparticles to the PCR mixture results in a very complex and dynamic biological-inorganic hybrid system. In contrast to the previous reports,[18,47] about the yield enhancement of PCR in the presence of nanoparticles, we found no such evidence in any PCR reaction using highly optimized system where comparable amplification was obtained in the absence of nanoparticles. Non-specific amplification from DNA templates was observed in the case of only a few *S.* Typhi isolates in the absence of nanoparticles. These DNA templates were, however, amplified with high specificity in the presence of nanoparticles. The enhancement in PCR was thus qualitative not quantitative, and to the best of our knowledge, this has not yet been documented for the typing of *Salmonella*. This aspect validates our study as a novel and an important observation for the detection of *S.* Typhi.

Despite of many reports about the improvement of PCR efficiency in terms of specificity and yield, the exact mechanism explaining this improvement is still unclear, however, a few hypotheses have been proposed in this regard.[46,48,49] The gold and silver nanoparticles used during this study improved the specificity of PCR, however, it does not imply that they will also be effective for other DNA templates with the same efficiency. The role of nanoparticles as inhibitor or enhancer for particular PCR is thus highly unpredictable. Moreover, different types of nanoparticles may show different effects on the same DNA template and similarly even the same type of nanoparticles may differentially affect the amplification of different DNA templates creating a lot of room in this field to be investigated.

Table 1. Oligonucleotide primers used for PCR amplification.

Target gene	Primers		Sequence (5′ – 3′)	Amplicon size (bp)	Reference
VNTRs Loci	TR1	F	AGA ACC AGC AAT GCG CCA ACG A	200-400	Liu *et al.* (2003)
		R	CAA GAA GTG CGC ATA CTA CAC C		
VNTRs Loci	TR2	F	CCC TGT TTT TCG TGC TGA TAC G	300-600	Liu *et al.* (2003)
		R	CAG AGG ATA TCG CAA CAA TCG G		
VNTRs Loci	TR3	F	CGA AGG CGG AAA AAA CGT CCT G	500-700	Liu *et al.* (2003)
		R	TGC GAT TGG TGT CGT TTC TAC C		
fliC	ST1	F	TATGCCGCTACATATGATGAG	495	Song *et al.* (2003)
	ST2	R	TTAACGCAGTAAAGAGAG		

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4. Conclusion

In conclusion, we demonstrated that the use of an appropriate concentration of metal nanoparticles, the amplification of target DNA template can be improved in terms of specificity. But this improvement strongly depends on the type of metal nanoparticles used, as in the case of magnetic nanoparticles we found no or very low amplification of the DNA under similar PCR conditions. Moreover, the non-specific amplification was eliminated significantly in molecular typing of *S.* Typhi by PCR assay based on VNTRs. In future, this strategy will be extended to further improve the sensitivity and specificity of PCR assays both for typing and diagnosis of *S.* Typhi at low annealing temperature that may help to shorten two rounds of PCR needed for *S.* Typhi diagnosis to a single step. This strategy holds a promise not only for the diagnosis and typing of *S.* Typhi but also for other pathogenic microbes. Further investigations are needed to explore the exact mechanism and effect of metal nanoparticles on PCR based DNA amplification and diagnostics.

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