### Plan of work:

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### Plan of work (Details)

- **Isolation of pathogen and establishment of Koch’s postulates.**
  - Isolation of disease causing bacteria from different locations of Gujarat.
  - Inoculation of healthy plants and establishment of the pathogenicity.

- **Pathological, biochemical and molecular characterisation of the pathogen.**
  - Pathological characterisation of the isolates on the basis of host reactions and cross inoculation study of respective 10 host plants.
  - Characterisation of the isolates through standard biochemical differentiation study.
Plan of work

- Isolation of bacterial genomic DNA and RFLP pattern of ITS gene and their comparison with other plant pathogenic *Xanthomonads*. Other molecular characterization study with ISSR (5 primers) and Rep, Eric-Box PCR marker (with 43 isolated bacteria and other 13 diff sp). MLSA analysis with 4 different housekeeping genes like *dnaK*, *gyrB*, *fyuA* and *rpoD*.
- Total bacterial protein profiling study (SDS-PAGE).

**Studies on host range and nature of predisposition.**
- Host range study (nearly 150 plants will be tested for host reaction).
- Effect of different temperature on pathogenesis and gum oozing (*in vitro*).
- Bacterial growth study at different temperature (*in vitro*).
- In-planta bacterial growth at different temperature (*in vitro*).
- Inoculation access study (host-pathogen association time required for successful pathogenesis *i.e.* gum oozing)

**Studies on cell degrading enzymes *in vitro*.**
- Quantification of different cell degrading enzymes (Pel, PG, Cel) at different temperatures (*in vitro*).

**Studies on toxin production, if any.**
- Isolation and characterization of toxin, if any.

**Effect of pathogen infection on host gas exchanger parameters.**
- Changes in gas exchange parameters (photosynthesis) and chlorophyll content after leaf inoculation.

**Leaf histo-chemical changes studies.**
- Changes of leaf biochemical components after infection (Total Phenol, OD Phenol, Total protein, Reducing sugar, Total sugar etc.)
**Methodology:**

1. Infected bark samples will be collected and micro-organisms associated with the same will be isolated after surface sterilization of the materials. Bacterial organisms will be targeted using nutrient agar as growth medium. All the isolated colonies will be purified and tested for pathogenecity upon re-inoculation. Organism(s) found positive towards symptoms developments will be further characterized (Agrios, 1997).

2. Inoculation technique for the pathogen will be developed and host-pathogen interactions on different host will be tested (Agrios, 1997; Janse, 2005).

3. After identification of pathogen it will be introduced in different hosts to determine the host range for the pathogen. Effect of different external factors like temperature, humidity, etc. will be studied on pathogenicity (Bradbury, 1984).

4. Standard biochemical/physiological tests such as levan production, catelase, oxidase, indole production, etc will be performed (Vauterin et al., 1995).

5. For molecular characterization ISSR, RFLP of ITS and rep-PCR will be done (Abdo-Hasan et al., 2008; Jaccard, 1908; Nei and Kumar, 2000; Rademaker et al., 2005; Rolf, 1997; Fatima et al., 2012).

6. A comparison analysis will be done with four housekeeping gene (gyrB, rpoD, fyuA and dnaK) sequences based on MLSA using different software like, Mega 4.0.2, DnaSP V.5 and BioEdit 0.9.0 (Tamura et al., 2007; Hall, 1999 and Librado and Rozas, 2009). However protein sequences analysis of these genes will be done using same softwares.

7. Total protein isolation from all isolates and analysis will be done by SDS-PAGE method (Vauterin et al., 1993).

8. Cell degrading enzymes such as cellulases, polygalacturonase, pectin methyl esterase, protease, etc will be tested in vitro. The organisms will be grown under laboratory conditions and exogenous
enzymes will be assayed following standard protocols (Chapon et al., 2001; Elizabete et al., 2008).

9. Laboratory grown culture filtrate will be heated to destroy the pathogen and enzymes. Then suspensions will be tested for toxic reaction on the host. If found positive, chemical fractionation methods will be employed to purify toxin (Beckman, 2003).

10. Fully expended leaves will be inoculated with pathogen and biochemical changes (total sugar, reducing sugar, total protein, total phenol and OD phenol) will be measure after inoculation (Janse, 2005).

11. Host plant will be injected by the pathogen and sequential changes in leaf chlorophyll content, photosynthesis, respiration; etc will be recorded using infrared gas analyser (IRGA) (Janse, 2005).